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FROM THE EDITORS' DESK:

The academic journal "Aureole" started its journey in the year 2009, thanks to the tireless efforts of some teachers of Barasat Government College. Three consecutive volumes were published, maintaining high academic standards. We apologize to our readers for not being able to publish the journal in 2012, due to some technical difficulties. However, we are back on track and our newly constituted Editorial Board this year has promised a new look for the journal by incorporating a wide range of articles — from social sciences to some of the most advanced fields of physical sciences.

It is always a pleasure to review interesting articles. We request our readers to give wide publicity to this academic journal. We pledge to accept only quality articles on research and general topics covering a wide range. We earnestly look forward to such motivating articles for the benefit of the academic community.

It is also very inspiring to note that the journal has been accorded a special place in the academic domain and we strongly believe that it will further expand itself. Heartiest thanks are due to the Honorable Members of the Advisory Board and all the Scholarly Referees for their valuable support which were indeed indispensible for the publication of this issue.

Despite our sincere efforts, some mistakes may creep in - we would obviously remain liable for such errors. Any comments or suggestions are solicited.

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Functional *in vitro* Analysis of Thyroid Peroxidase (TPO) Gene Mutations in Hypothyroid Patients in West Bengal

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Abstract

Thyroid Peroxidase (TPO) is the key enzyme in the biosynthesis of thyroid hormones. We aimed to identify the spectrum of mutations in the TPO gene leading to hypothyroidism in the population of West Bengal to establish the genetic etiology of the disease. 200 hypothyroid patients (case) and their corresponding sex and age matched 200 normal individuals (control) were screened depending on their clinical manifestations. Peripheral blood samples were collected. Genomic DNA was isolated from the blood. The human TPO gene (Exon 8 to Exon 14) was amplified by PCR. The PCR products were subjected for sequencing to identify mutations. Different single nucleotide changes like mutations in the exon 8(Thr449Pro), exon 10 (Glu641Lys; Asp668Asn), exon 11(Thr725Pro) were found. Mutations in the tpo have been assayed *in vitro*. Mutant and wild-type activities have been compared. Three mutants were enzymatically inactive in the guaiacol and iodide assay. This is a strong indication that the mutations are present at crucial positions of the TPO gene, resulting in inactivated TPO. The results of this study may help to develop a genetic screening protocol for goiter and hypothyroidism in the population of West Bengal.

Key words: Thyroid peroxidase, hypothyroidism, mutation, *in vitro* assay, transfection.

Introduction:

TPO is a membrane-bound glycoprotein (102 kDa), found as a dimer [1]. Each monomer consists of 933 amino acid residues and contains a peroxidase domain, three additional extracellular domains, a transmembrane helix and a short C-terminal intracellular tail [2]. The human TPO gene is located on chromosome 2p25 and spans approximately 150 Kb, containing 17 exons [3]. Mutations in TPO gene can lead to severe defects in thyroid hormone production, due to total iodide organification defects (TIOD) or partial iodide organification defects (PIOD) [4]. Therefore, it is important to screen the percentage of people having gene mutations among the clinically identified hypothyroid

patients. The present investigation is aimed to screen the mutations in the TPO gene leading to hypothyroidism in the population of West Bengal to establish the genetic etiology of the disease.

Methodology:

Collection of study samples:

200 hypothyroid patients (case) and their corresponding sex and age matched 200 normal individuals (control) were screened depending on their clinical manifestations, detailed familial history from the Institute of Post Graduate Medical Education & Research (IPGME & R), Kolkata. Peripheral blood samples were collected on the basis of prior consent given by patients/normal individuals, families and parents on behalf of minor children. The highest biomedical ethics had been enforced in this study.

Patients (Case): Subjects presenting over a period of 1 year to Endocrine OPD with hypothyroidism. Personal history includes the duration of disease, Age, Sex, Drug History, Menarche, Menstrual cycle abnormality, Abortions, Thyroid swelling, Lethargy, Weight gain/Weight loss, Cold intolerance and Constipation.

Normal (Control): Age and sex-matched subjects with no goitre, no clinical evidence of hypothyroidism and normal levels of serum T3, free T4, TSH, anti-TPO antibody.

Genomic DNA isolation from patients:

Peripheral blood samples were collected from the case and control individuals. Genomic DNA was isolated from the blood leucocytes by using QIAamp Blood Kit (QIAGEN, Hilden, Germany).

PCR (Polymerase Chain Reaction):

The human TPO gene (Exon 8 to Exon 14) was amplified by PCR. PCR was performed in a thermocycler (Applied Biosystems, Model No. 9902) using specific primers for each of the exons (Table.1). The reaction mixture (25 µl) contained 40-100 ng of genomic DNA, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 0.4 µM of each primer, and 0.5 unit of Taq DNA polymerase (Applied Biosystems). Denaturation at 95°C for 30 seconds, annealing at 55-60°C for 30 seconds, and extension at 72°C for 30 seconds x 44 cycles were performed.

Table 1. Primer sequence used to screen the different Exons of TPO gene.

Exons	Primers	Sequence	Product size (bp)
Exon 8	Forward	5'-ccctacgtaacaaacctgcac-3'	474
	Reverse	5'-ggctgtcaaggaagatgctc-3'	
Exon 9	Forward	5'-cgttgcttagaaggcctcag-3'	444
	Reverse	5'-cttgcagtgagctgagatcg-3'	
Exon 10	Forward	5'-acaacctgaccaggcttacg-3'	485
	Reverse	5'-caggactctgccctgctg-3'	
Exon 11	Forward	5'-ctgccctgagggtgtaagg-3'	446
	Reverse	5'-gagaggctggcagcacacag-3'	

Exons	Primers	Sequence	Product size (bp)
Exon 12	Forward	5'-ctatccccagattgctcctg-3'	449
	Reverse	5'-gctcagtgagtgaccacagc-3'	
Exon 13	Forward	5'-gtgtgcttcgagggtctctg-3'	485
	Reverse	5'-ccctagaccaggtgggatg-3'	
Exon 14	Forward	5'-ccatgtccagaggaaaggag-3'	238
	Reverse	5'-cagactcaggcaggacaacc-3'	

Agarose gel electrophoresis & Gel Extraction:

The PCR product was analyzed in 2 % agarose gel electrophoresis for verifying the size of the PCR product. PCR fragments were purified from agarose gel using Gel Extraction kit (Genei Bangalore).

DNA Sequencing:

The PCR products were sequenced by using Big Dye Terminator kit v3.1 (Applied Biosystems) in ABI Prism 377 DNA Sequencer (PE Applied Biosystems). For sequencing the purified PCR fragments were used for PCR again and the reaction mixture (10 µl) contained 150 ng DNA, 5 µM primer (either forward or reverse), 0.5µl BD, 1.9µl 5x BD buffer. Denaturation at 96°C for 10 seconds, annealing at 50-55°C for 10 seconds, and extension at 60°C for 4 min x 25 cycles were performed. 10 µl PCR mix was added with solution I (10µl mq H_2O, 2µl 125 mM EDTA, P^H8.0) and solution II (50 µl ehanol, 2µl Na-acetate, P^H4.6). The mixture was kept in dark for 25 min and centrifuged at room temperature (13000 rpm for 30 min) and sample was dried in speed vac. Then 12µl Hi-Di (formamide) was added in sample and stored at dark for 15 min, incubated at 96°C dry bath and allowed the sample for snap chill in ice. Then the sample was loaded in DNA Sequencer.

Alignment of DNA sequences:

Sequences Alignment between sequences of Case and Control individuals was performed to find the best-matching piecewise (local) or global alignments of two query sequences using clustalW programme.

Functional *in vitro* analysis of TPO mutations:

Clinically relevant mutations in the TPO gene, identified in this study, including non-synonymous SNPs in the coding region, deletions, insertions have been assayed *in vitro*. Essentially, a cDNA clone for TPO (supplied from Invitrogen) in a mammalian expression vector has been mutated by site-directed mutagenesis to create clones representing patient mutations. The clones have been transfected into COS 7 cell lines and assayed for efficacy of TPO enzyme activity and expression levels. Mutant and wild-type activities have been compared.

Site directed mutagenesis:

We used Invitrogen GeneArt® Site-Directed Mutagenesis System for this purpose for eg. Glu 641 Lys, Asp 668 Asn and Thr 725 Pro these 3 mutations were incorporated into wild TPO cDNA through this method.

Preparation of recombinant DNA:

We used pcDNA3.1 (vector) for TPO cDNA cloning. Double restriction enzyme (EcoRI, KpnI) digestions were performed by incubating TPO cDNA and vector molecules with an appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier, and at the optimal temperature. The cut DNA fragment and vector are covalently joined together by DNA ligase.

Tranformation in bacteria:

Transformation is the method of introducing foreign DNA into bacterial cells. The recombinant plasmids (TPO cDNA + plasmid) were amplified in DH5 alpha competent cells. The uptake of recombinant plasmids by DH5 alpha cell was carried out in ice-cold $CaCl_2$ (0-5°C) and subsequent heat shock (42°C for about 90 sec). Selection of DH5 alpha competent cells containing recombinant plasmids (TPO cDNA + plasmid) was done by ampicillin treatment.

Isolation and purification of plasmid: Plasmid was purified by use of the plasmid isolation kit (PureLink™ Quick Plasmid Miniprep Kit, Invitrogen).

Transfection in cell line:

Transfection is the process of deliberately introducing nucleic acids into cells. We used Invitrogen Lipofectamine® Reagent for transfection. COS 7 cells were grown in 3.8-cm dishes in DMEM supplemented with 50 mL/L bovine calf serum and 100 kIU/L penicillin–streptomycin in 5% CO2 atmosphere at 37°C. When the cells reached ~90% confluence, they were transfected with 1 µg of recombinant plasmid DNA (wild-type and mutant) per 3.8 cm dish with Lipofectamine. After incubation for 48 h, the cells were harvested for activity study.

Peroxidase activity measurement:

Cells were harvested with trypsin EDTA treatment and protein concentration was determined on a 100µl aliquot using the Bio-Rad protein assay (BioRad,Munchen, Germany). The cells were pelleted and subsequently suspended in 0.1% deoxycholate containing 1% aprotinin and incubated for 10 min at 4°C. The extract was microcentrifuged for 5 min, and the supernatant removed to measure enzymatic activity using the guaiacol and I_3^- assay. For the guaiacol assay, 50 µl of the supernatant was assayed in a final volume of 750 µl, containing 35mM guaiacol and 0.5mM H_2O_2 in 0.1 M Tris-HCl, pH 8.6. The absorbance at 470 nm was followed and activity was expressed as $\Delta A \cdot min^{-1} \cdot mg\ protein^{-1}$. For the I_3^- assay, 25 µl of the supernatant was used in a final volume of 750 µl containing 0.1 M potassium phosphate, pH 7.5, 50 mM potassium iodide, and 0.25mM H_2O_2. The absorbance at 353nm was followed, and activity was expressed as $\Delta A \cdot min^{-1} \cdot mg\ protein^{-1}$. Spectrophotometric analysis was done on a Shimadzu UV-200.

Results:

Study population:

The case and control groups were well balanced in terms of age and gender. There is no significant difference between two groups (Table. 2).

Table2. Study population			
	Case (n=200)	Control (n=200)	p- value
Sex			
Male	29 (14.5 %)	32 (16%)	0.88
female	171 (85.5%)	168 (84%)	0.80
Age (Mean ± SD)	29.85± 19.51	30.38 ± 12.90	

Phenotype:

Case individuals exhibited varied clinical manifestations. The associated clinical manifestations of hypothyroidism were goiter (65.5%), Lethargy (59%), Muscle Cramp (48.5%), Loss of Memory (53%), Hoarseness of voice (40.5%), Weight Loss (8% in male and 30% in female), Constipation (51.5%) etc. (Table 3).

Table 3. Clinical manifestations of case at initial presentation.

Clinical manifestations	Case (n=200)	%	Control (n=200)	%	p-value
*Family History					
Present	93	46.5	6	3	<0.0001
Absent	107	53.5	194	97	
*Goiter					
Present	131	65.5	-	-	
*Weight Gain					
Yes	96	48	68	34	0.006
No	104	52	132	66	
Loss of memory					
Yes	106	53	97	48.5	0.42
No	94	47	103	51.5	
*Lethargy					
Yes	118	59	94	31	0.02
No	82	41	106	69	
*Muscle cramp					
Yes	97	48.5	84	37	0.2
No	103	51.5	116	63	
*Cold intolerance					
Yes	121	60.5	112	56	0.36
No	79	39.5	88	44	
*Constipation					
Yes	103	51.5	82	41	0.035
No	97	48.5	118	59	

* At diagnosis

Mutations in the coding region:

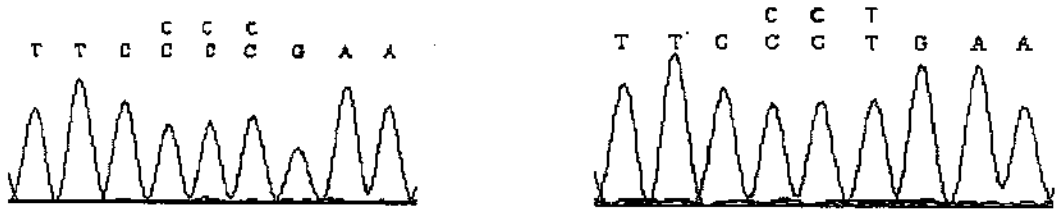
Mutation screening of TPO gene showed different single nucleotide changes, which includes synonymous and nonsynonymous changes (Table. 4 and 5).

Table 4. Nucleotide variants of TPO.

sample ID	Sex	Age (Yrs)	Exon	Nucleotide position	Codon Position	Nucleoide Triplet change	Amino acid change	Domain
6	M	64	11	2007	669	TGG>TGA	Trp>stop codon	catalytic
6	M	64	11	2017	673	GAG>CA-	Deletion	catalytic
14	M	20	11	2013	671	TGG>TGC	Trp>Cys	catalytic
14	M	20	11	2015	672	TGG>TAA	Trp>stop codon	catalytic
8	F	6	8	1346	449	ACC>AAA	Thr>Lys	catalytic
8	F	6	8	1348	450	CTG>AGA	Leu>Arg	catalytic
3	M	11	8	1345	449	ACC>CCC	Thr>Pro	catalytic
3	M	11	8	1349	450	CTG>CCG	Leu>Pro	catalytic
3	M	11	8	1392	464	CAG>CAT	Gln>His	catalytic
58	F	49	8	1340	447	ATC>AGC	Ile>Ser	catalytic
13	F	57	8	1348	450	CTG>TCG	Leu>Ser	catalytic
13	F	57	8	1391	464	CAG>CTT	Gln>Leu	catalytic
13	F	57	8	1465	489	GCC>TCC	Ala>Ser	catalytic
47	F	17	10	1777	593	GAG>TTC	Glu>Phe ala	catalytic
47	F	17	10	1793	598	TGC>CCC	Cys> Pro	catalytic
47	F	17	10	1853	618	GTG>GAG	Val>Glu	catalytic
4	M	43	10	1806	602	CGC>TGT	Arg>Cys	catalytic
49	F	2.6	10	1776	592	AAT>AAG	Asn>Lys	catalytic

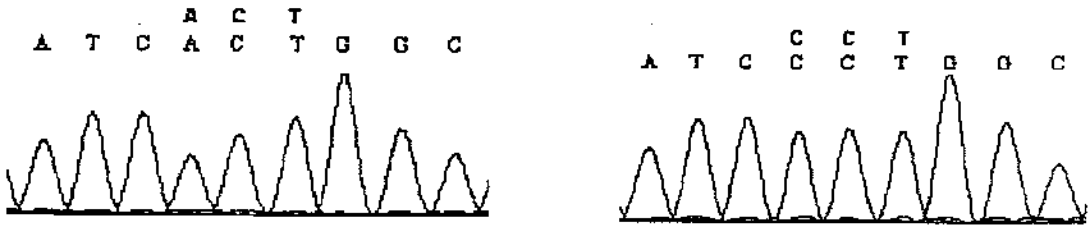
Table 5. Some important nonsynonymous and synonymous changes in exon 10 and exon11 in patients.

Sample ID	Exon	Codon position	Codon change	Amino acid change
10	10	641	GAA>AAA	Glu>Lys
11	10	641	GAA>AAA	Glu>Lys
12	10	641	GAA>AAA	Glu>Lys
13	10	641	GAA>AAA	Glu>Lys
159	10	641	GAA>AAA	Glu>Lys
10	10	668	GAC>AAC	Asp>Asn
147	11	725	ACT>CCT	Thr>Pro
6	11	712	CCC>CCT	Pro>Pro
13	11	712	CCC>CCT	Pro>Pro



Normal nucleotide sequences Mutant nucleotide sequences

Fig.1: Synonymous nucleotide changes in normal and patient (Pro712Pro)



Normal nucleotide sequences Mutant nucleotide sequences

Fig.2: Nonsynonymous nucleotide changes in normal and patient (Thr725Pro)

Allele and Genotype distribution:

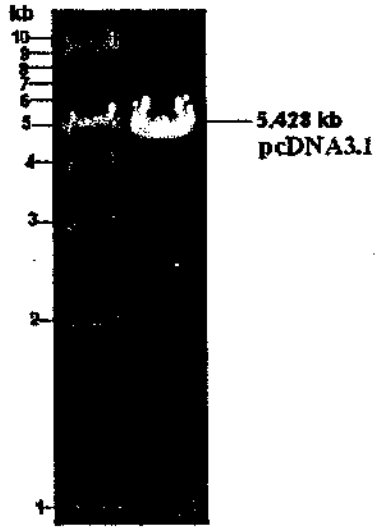
Chi-square test was used to compare the genotype and allele frequencies between cases and controls (Table 6).

Table 6. Allele and Genotype distribution of Thr725Pro

SNP	Allele	Allele frequency Case	Allele frequency Control	Odds Ratio (95%CI)	P value	Genotype	Case, *n* (%) (N=200)	Control, *n* (%) (N=200)	Odds ratio (95% CI)	P value
Thr725Pro (rs732609)	A C	0.54 0.46	0.62 0.38	1.39 (1.05-1.84)	0.022*	AA AC CC	78 (39) 59 (29.5) 63 (31.5)	80 (40) 88 (44) 32 (16)	Reference AA vs. AC: 0.69 (0.44-1.08) AA vs. CC: 2.02 (1.19-3.42)	0.133 0.013*

*P-Value < 0.05 is considered to be statistically significant.

Gel electrophoresis of Pc DNA 3.1 Vector with TPO cDNA clone: Pc DNA 3.1 Vector with TPO cDNA clone gave electrophoretic band at 8kb size (Fig.3a and b).



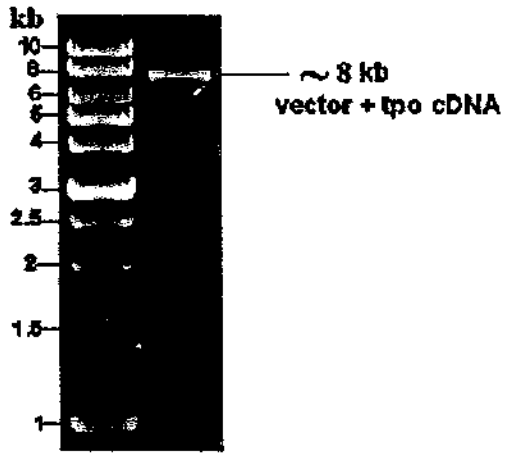


Fig.3a: Pc DNA 3.1 Vector

Fig3b: Pc DNA 3.1 Vector with TPO cDNA clone

Peroxidase activity:

To measure wild-type and mutant enzyme activity, both the I⁻ and guaiacol assays were carried out. Wildtype recombinant TPO showed enzymatic activity in both assays. mutant TPO showed relatively nonenzymatic reaction rate (Table 7).

Table 7. Guaiacol and iodide oxidation activity of expressed human TPO protein

Mutation	**Guaiacol oxidation**	**Iodide oxidation**
Wild type	0.76 ± 0.07	0.90 ± 0.05
Glu 641 Lys	ND	*
Asp 668 Asn	0.63 ± 0.07	0.69 ± 0.27
Thr 725 Pro	ND	*

*= Comparable with nonenzymatic reaction rate (0.51 ± 0.04). ND=No detectable activity (< 10% of wild-type expressed TPO).

Discussion :

Mutations in the TPO gene of patients with hypothyroidism, resulting in Total or Partial Iodide Organification Defects (TIOD and PIOD) have been reported by several groups in the world. A large volume of work has been performed by countries like Netherlands [5], Germany [6], Portugal [7], Italy [8], Japan [9], Argentina [10], Turkey [11] as well as China [12] . These results showed variants of nucleotide sequences in TPO, some of which are common with our result. TPO enzyme activity depends on both proper folding and membrane insertion, and an intact catalytic site. An amino acid substitution caused by a missense or a nonsense mutation either induces a three-dimensional change or disrupts the glycosylation consensus sequence leading to impaired folding. Thus, the misfolded mutant TPO being trapped in the endoplasmic reticulum, its expression at the apical membrane is impaired [13]. On the other hand, these findings also indicate that exons 8-14 harbor mutational hot spots [7]. Thus, mutations in these regions are expected to have major effects on TPO activity resulting in severe organification defect and severe hypothyroidism [13]. Earlier studies reported that exons 8-14 encode the catalytic center of the TPO protein (hemebinding region) which is crucial for the enzymatic activity [14], so we selected exons 8-14 for detection of mutations in our study population. We identified different TPO gene mutations which were previously reported. Our results suggest in Thr 725 Pro, C is the risk allele ($p = 0.022$; odds ratio = 1.39; 95% CI: 1.05-1.84) and CC is a risk genotype ($p = 0.013$; odds ratio = 2.02; 95% CI: 1.19-3.42) towards the development of hypothyroidism. The missense change of Thr to Pro may interrupts the secondary structure of TPO protein. As we know Threonine (Thr) is the phosphorylation site of the protein which is important for the activation of the protein [15]. In Glu 641 Lys, negative charged amino acid (glutamic acid) is replaced by positive charged amino acid (lysine). Therefore, mutations in this amino acid may cause change in the activity of TPO enzyme which ultimately may reduce the functional efficacy of the enzyme. In this present study we found many polymorphism in TPO gene. We also verified the effect of these mutation through *in vitro* assay. Three mutants were enzymatically inactive in both the guaiacol and iodide assay. This is a strong indication that the mutations are present at crucial positions of the TPO gene, resulting in inactivated TPO. Thus, the above changes of amino may exert its effect on the structure and functional activity of TPO protein which is the key enzyme in the biosynthesis of thyroid hormones.

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Graphene & its composites as "Supercaps"

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Abstract

The unique 2D structure and outstanding intrinsic physical properties, such as extraordinarily high electrical conductivity and large surface area, make graphene-based systems a challenging material for potential applications in supercapacitors. In this short review, the progress made so far for their applications in supercapacitors has been discussed. These materials are found to posses some novel characteristics and superior energy storage & release mechanisms. Several key issues for improving the structure of graphene-based materials for achieving better capacitor performance, along with the recent outlook for the field have been highlighted.

Keywords: *graphene, nanocomposites, specific capacitance, supercapacitor.*

Scientists all over the world have spent decades trying to create a "perfect battery"—a battery with great energy density or, at least, one that doesn't take too long to charge. Such fabrication will amazingly revolutionize our life than they are today. One definite approach for improving the battery is to forget about the battery but improve the competence of capacitors. A capacitor, like a battery, is a device that stores electrical energy and they charge and discharge energy an order of magnitude faster than batteries. But capacitors have a big limitation — they're even less energy dense than batteries. Now the question arises how could we make a dense capacitor, one that stored a lot of energy but also charged very quickly?

Over the past few years, researchers at several companies and institutions around the world have been toiling hard to achieve this goal of the fabricating a perfect "supercap/supercapacitor". Presently available commercialized electro-active materials are limited by the low power density that arises from the poor electrical conductivity restricting fast electron transport, and by the lack of a pure cycling stability owing to the easily damaged structure of the materials during the redox process. Hence, to resolve these problems, carbon-based materials with high electrical conductivity and large specific surface area are usually opted as the backbone materials to combine with these active materials for designing pseudo-capacitor electrodes. One of such promising material

stands with "graphene"—a much-celebrated substance composed of a one-atom thick 2D layer of carbon [1].

Theoretical calculations show that graphene has been considered to be an ideal supercapacitor electrode material due to its extremely large surface area, extraordinarily high electrical conductivity, and strong mechanical strength [2]. However, practically, we are still away from the ultimate goal to turn this promising material into a real fruitful material. The most essential problem lies in how to prepare it in large-scale and in a most cost-effective way [3]. It's well known that chemically exfoliated graphene based supercapacitors that have been so far the cheapest material. The specific capacitance of the chemically exfoliated graphene in the aqueous electrolyte is comparable to that obtained with activated carbons and superior to that of carbon nanotubes. The supercapacitor characteristics are directly related to the quality of the graphene, specifically, the number of layers and the associated surface area, which are, however, often hard to control by chemical synthesis. To achieve better results, very recently, Laser Scribed Graphene (LSG) electrodes have been fabricated which exhibit ultrahigh energy density values in different electrolytes while maintaining the high power density and excellent cycle stability [4]. The open network structure of the electrodes helps to minimize the diffusion path of electrolyte ions, which is very crucial for charging the device. This can be accounted for by the easily accessible flat graphene sheets, whereas most of the surface area of activated carbon resides in very small pores that limit the diffusion of ions. However, producing graphene-based supercapacitor at large scale by such expensive method has proved elusive.

The other forms of 2-D carbon nanosheets such as graphene oxide (GO) and reduced graphene oxide (rGO) also have been used for the supercapacitor applications [2,5]. Chemically reduced GO (rGO) is attractive as supercapacitor electrode because of its hydrophilic feature, which is not only good for further compositing with metal oxides but also for wettability in the aqueous electrolyte. In addition, rGO still retains high electronic conductivity. Ruoff et al. have produced the activated rGO with a specific surface area of 3100 $m^2 g^{-1}$. The electrode made of such activated rGO exhibited a specific capacitance of 166 F g^{-1} at a current density of 5.7 A g^{-1} [6]. Moreover, it is rich in surface functioned groups such as alcohol, epoxide, carbonyl and carboxylic acid which make it possible to introduce additional functional groups on rGO by further modification [7]. These functional groups may serve as the redox centers, which can contribute to pseudo-capacitance.

Therefore, its quite clear at this stage that the only option stands as — is to use of graphene-composites to modify the supercapacitive behaviour of graphene or r-GO. Graphene-composites include graphene modification with a vast range of materials including metal oxides, semiconductors, polymer nanostructures, etc [8]. Until now, two popular methods—the chemical exfoliation of graphite into graphene oxide followed by controllable reduction to make reduced graphene materials or the in-situ reaction (with transition metal oxides or conducting polymer precursor) to fabricate graphene based composite materials—have been widely investigated and deemed as the most promising for producing supercapacitor electrodes. Another challenge

lies in the fact that the graphene materials tend to re-stack easy, causing declination of its physical properties and processability. Some efficient methods, including the functionalization of graphene or the addition of spacers between the graphene layers, are required to solve this problem [9].

Conjugated polymers are popular electroactive materials and are well renowned as existing supercapacitor material [10]. Thus various techniques are employed to grow conjugated polymer nanostructures on the graphene surface. Epitaxial growth of PANI over r-GO has been achieved by mutual oxidation-reduction of GO and aniline [11]. However, because of electrochemical instability of GO, GO–PANI nanocomposites cannot take advantage of the best potentials of GO, which would be ideal for applications in supercapacitor electrodes; only a small amount of insulating GO has been used in such composites because excess GO would reduce the conductivity of the composite. Thus, graphene nanosheets (GNS) are more favorable than GO to be doped with PANI nanostructures. The measured specific capacitance of such graphene–PANI electrode reached as high as 489 F/g. Importantly, after 500 cycles there is still more than 475 F/g remaining at 400 mA/g (above 96% of the original value), illustrating this electrode possesses good cycling stability and lifetime [12]. Similar fabrications using other conjugated polymers like polypyrrole (PPy) to form graphene–PPy composite electrodes exhibited an ultrahigh specific capacitance of 1510 F/g, an area capacitance of 151mF/cm^2 , and a volume capacitance of 151 F/cm^3 at 10 mV/s due to highly porous structure of the composite electrode compared to that of the virgin PPy electrodes [13].

However, instability as well as wettability of these organic composites limits their use in practical fields. So efforts are always made to fabricate inorganic-based nanocomposites to overcome this problem. Many metal oxide nanoparticles/nanostructures of MnO_2, NiO, Co_3O_4, TiO_2, RuO_2, etc are found to be exhibit powerful capacitive behaviour [14], but the property abruptly decreases due to agglomeration. The unique planar structure of the graphene family materials makes good supports for such metal oxides thereby preventing agglomeration besides increasing the effective surface area. As compared with 1-D carbon nanostructures, 2-D carbon nanostructures are even easier and more flexible to integrate with these metal oxides. The formation of reduced graphene (rGO) –metal oxide or graphene–metal oxide composites can be realized via various methods including electrodeposition [15], hydrothermal processing[16], co-precipitation [17], spray pyrolyzing[18] and so on. Yu's group has developed a general strategy to prepare rGO–metal oxide core–shell structures, which could also be used to fabricate supercapacitor electrodes [19]. So far rGO has been coupled with various metal oxides such as TiO_2, NiO, Co_3O_4 and MnO_2 as the supercapacitor electrodes [20-23]. Table 1 lists the representatives of the metal oxide–rGO composite electrodes [14]. Recent reports on Co_3O_4–graphene nanofoam electrode showed a specific capacitance of 765 F g^{-1} with good reproducibility [24]. The main emphasis being on the synergistic effects of the composite on the performance of supercapacitors in terms of specific capacitance, energy density, power density, rate capability and cyclic stability.

Table 1 Metal oxide–rGO composite electrodes showing supercapacitance behaviour:

Synthesis method	Metal oxide–rGO composite	Specific capacitance	References
Electrodepositing	NiO porous film on rGO sheet	432 F g^{-1} at 2 A g^{-1} for NiO, 636 mF cm^{-2} at 0.5 mA cm^{-2}	G. Ning, Z. Fan, G. Wang, J. Gao, W. Qian, F. Wei, *Chem. Commun.***2011**, 5976 .
	NiO nanoparticles–rGO	569 F g^{-1} at 5 A g^{-1} for NiO	A. Yu, I. Roes, A. Davies, Z. Chen, *Appl. Phys. Lett.* 2010, 96, 253105 .
	MnO_2 nanoparticle–rGO	476 F g^{-1} at 1 A g^{-1} for MnO_2	J. J. Yoo, K. Balakrishnan, J. Huang, V. Meunier, B. G. Sumpter, A. Srivastava , M. Conway, A. L. M. Reddy, J. Yu, R. Vajtai , P. M. Ajayan , *Nano Lett.* **2011**, 11, 1423 .
Hydrothermal method	MnO_2 nanowire–rGO	211.2 F g^{-1} at 0.15 A g^{-1} for composite	D. W. Wang, F. Li, Z. S. Wu , W. Ren , H. M.
	Flower-like NiO–rGO sheet	346 F g^{-1} for composite	Cheng, *Electrochem. Commun.* **2009**, 11, 1729. C. T. Hsieh, H. Teng, *Carbon* **2002**, 40, 667
	Co_3O_4 nanoparticle–rGO	415 F g^{-1} at 3 A g^{-1}	
Co-precipitation	Monolayer NiO–rGO	525 F g^{-1} at 0.2 A g^{-1}	M. Zhi, C. Xiang, J. Li, M. Li and N. Wu *Nanoscale*, **2013**, 5,72.
	MnO_2 nanowire–rGO	176 F g^{-1} at 5 mV s^{-1}	C. T. Hsieh, S. M. Hsu , J. Y. Lin, H. Teng, *J. Phys. Chem. C* 2011, 115 , 12367 .

In order to improve one of the several aspects– the supercapacitor energy density – a great deal of recent research effort has been directed toward improving specific capacitance or methods to provide the maximum operation voltage to the cell system. Z. Fan *et. al*, have reported an asymmetric supercapacitor based on a nickel hydroxide - graphene composite, used as the positive electrode, and porous graphene, used as the negative electrode. The hybrid material has a high specific capacitance (1735 F g^{-1}) and a high rate capability compared to pure nickel hydroxide [25].

For further modifications, nowadays, asymmetric supercapacitors [26], combinations of Faradic electrode (as energy source) and the capacitive electrode (as power source) are employed to increase the operation voltage, which leads to an obvious improvement of the energy density of high-power ECs (electrochemical capacitors) so that it approaches that of batteries. A novel kind of asymmetric supercapacitor using a graphene–MnO_2 composite as the positive electrode and activated carbon nanofibers (ACN) as the negative electrode in a neutral aqueous Na_2SO_4 electrolyte was also developed by Wei and co-workers [27]. Extensive research is still in progress to understand their behaviors [28].

As far as pseudo-supercapacitor applications are concerned, it has been demonstrated that the improvement of the electrochemical performance of the graphene-based composite electrodes is mainly attributed to the following reasons. Firstly, graphene in composites can act as supports for the deposition of active component(s) at the nanoscale dimensions, and thus can effectively

increase the specific surface area of these active components. Secondly, graphene can provide the electronic conductive channels, and the excellent interfacial contact between the active components for fast transportation of electrons throughout the whole electrode matrix. Thirdly, graphene nanosheets have unique structural and mechanical properties, which can restrict the mechanical deformation of the active component during the redox process thereby avoiding easy destruction of the electrode material and leading to better stability of the composite.

Thus graphene-based materials have promising potential for application in supercapacitors and other renewable & sustainable energy devices. The key issue is to fully utilize excellent intrinsic properties of graphene, especially its large surface area and high conductivity, and to improve the synergistic effect of the graphene substrate with the other active components. Thus, it is evident from the present scenario that for the purpose of designing a "perfect battery", the designing and syntheses of new nanostructures and architectures based on graphene will be an important task in the near future.

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Application of Bargellini Condensation Reaction: An Approach to Overcome the Heliannuol C Synthesis

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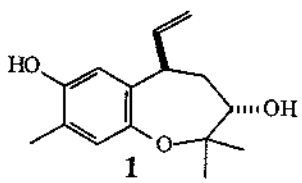
Abstracts:

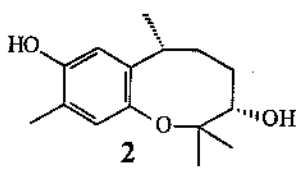
This review describes the total synthesise of the allelochemical heliannuol C employing different methodologies to generate the central benzoxepane ring system.

Keywords: Allelochemicals, Ring Closing Metathesis, Epoxidation, Claisen rearrangement, Bargellini condensation reaction, Dieckmann cyclisation.

Heliannnuol C (**1**), represents a new class of compounds among the heliannuol family of allelopathic sesquiterpenes isolated [1] from cultivar sunflower *Helianthus annus*. The other primary constituents are heliannuols A, B, D and E (**2–5**) and these have been implicated in the powerful allelopathic activity displayed by sunflowers [2]. Subsequently, other oxidized variants of some of these primary compounds have also been isolated from these flowers [3].

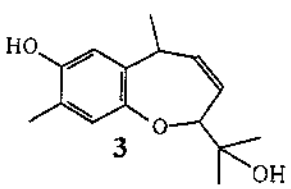


1



2

Allelopathy, involving plant–plant and plant–microorganism interactions, has been proposed as an alternative weed management policy [4]. The growing concern on the natural ecological balance and the arguments against indiscriminate use of synthetic pesticides have provided an impetus for exploration of allelochemicals for effective weed control devoid of any hazardous side effects. In this context, the heliannuols, due to their novel structural features and associated bio-activity, have served as attractive targets for synthesis in many laboratories [5].



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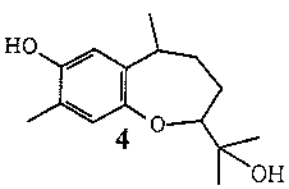

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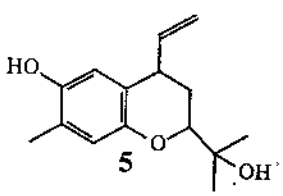
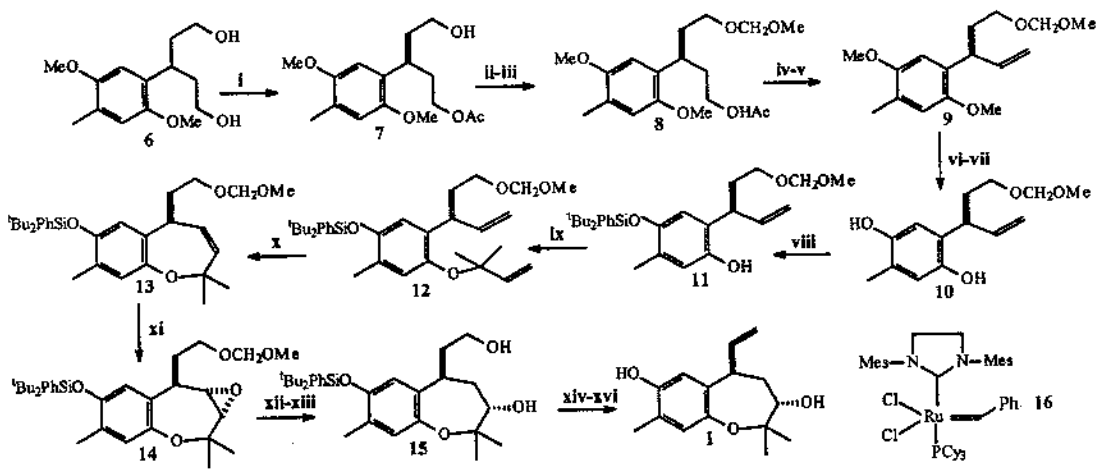


5

Heliannuol C (**1**) and heliannuol A (**2**) are the most biologically active allelochemicals of this family. Heliannuol C (**1**) contains a benzoxepane ring system. The structure of **1** was determined from extensive spectral studies. The two stereogenic centers in the oxepane ring of **1** are *trans* oriented as against the *cis* substituted heliannuol-A (**2**).

The first enantioselective synthesis of heliannuol **C** (**1**) was disclosed by Shishido *et al* [6]. They have applied a ring closing metathesis for the construction of benzoxepane ring of (**1**). Treatment of the prochiral diol (**6**) with immobilized lipase PS on diatomite in the presence of vinyl acetate furnished the chiral monoacetate (**7**). The configuration at the asymmetric center was assigned as *S* from correlation. The free hydroxyl group of (**8**) was protected (MOM) and the acetoxyethyl moiety was converted to the vinyl group to furnish the alkene (**9**) via reduction followed by dehydration. Sequential oxidation-reduction of the aromatic ring followed by site selective protection of the resultant hydroquinone (**10**) afforded the phenol (**11**). Treatment of the phenol with *t*-butyl-2-methyl-3-butene-2-yl carbonate in the presence of tetrakis (triphenylphosphine) palladium in THF furnished the diene (**12**). This diene (**12**) underwent facile RCM reaction with Grubbs' catalyst (2nd generation, **16**) to furnish the benzoxepene (**13**). Diastereoselective epoxidation of (**13**) followed by regioselective reduction of the resultant epoxide (**14**) furnished the alcohol (**15**). Removal of the MOM protecting group, dehydration of the resulting alcohol, followed by desilylation finally afforded (–)-helianoul C (**1**) (**Scheme 1**). The authors assigned the absolute stereochemistry for the two stereogenic centers as (8*R*, 10*S*).

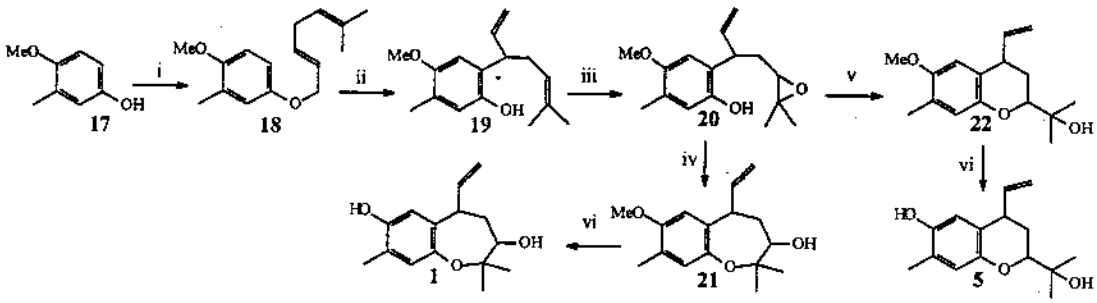


Scheme- 1.

Reagents and conditions: **i.** Immobilized lipase PS, Diatomite, CH_2=CHOAc, Et_2O, rt, 13h. **ii.** $MeOCH_2Cl$, i-Pr_2NEt, rt, 18h, 95%. **iii.** $LiAlH_4$, THF, rt, 10min, 96%. **iv.** *o*-nitrophenylselenocyanate, *n*-Bu_3P, THF, 15 min, **v.** 35% H_2O_2, THF, rt, 3.5h, 91%. **vi.** $(NH_3)_2Ce(NO_3)_6$, CH_3CN, H_2O, rt, 20 nim. **vii.** $Na_2S_2O_4$, THF, H_2O, 10 min, 95%. **viii.** *t*-$Bu_2PhSiCl$, imidazole, 4-DMAP, CH_2Cl_2. rt, 1h, 80%. **ix.** *i*-$BuOCOCMe_2CH$=CH_2, $(Ph_3P)_4Pd$, THF, rt, 14h, 85%. **x.** Grubbs Catalyst (Structure-

16), CH_2Cl_2, rt, 16.5h, 98%. **xi.** CH_3COCF_3, Oxone, CH_3CN, rt, 2.5h, 78%. **xii.** $LiAlH_4$, THF, 40°C, 20 min, 89%. **xiii.** 6N-HCl, THF, rt, 3h, 84%. **xiv.** *o*-nitrophenylselenocyanate, *n*-Bu_3P, THF, 20 min, rt. **xv.** 35% H_2O_2, THF, rt, 3.5h, 88%. **xvi.** *n*-Bu_4NF, THF, rt, 15 min, quant.

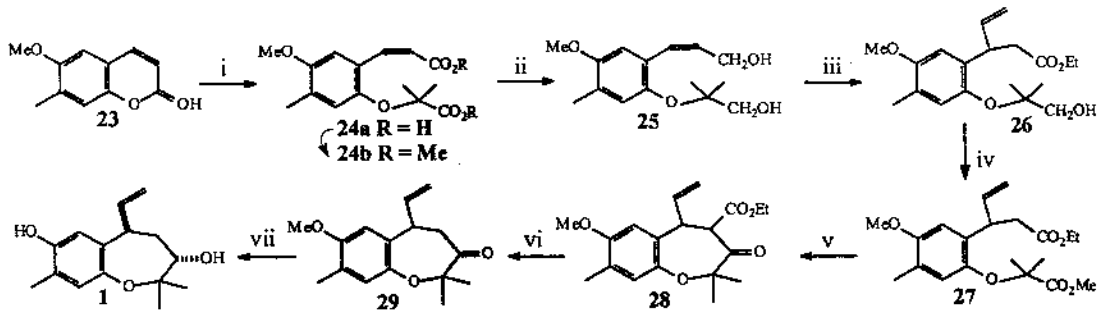
Vyvyan *et al* [7], have disclosed a synthesis of (**1**) employing aromatic Claisen rearrangement to install the vinylpentenyl segment followed by a biomimetic type epoxide cyclisation. Etherification of 2-methyl quinol monomethyl ether (**17**) with 6-methyl 2, 5-heptadiene-1-ol furnished the diene ether (**18**) which on a Claisen rearrangement produced the dienephenol (**19**). Selective epoxidation of the more substituted olefin furnished the epoxide (**20**) as a mixture of diastereomers. The required *endo* cyclisation of this epoxide (**20**) was achieved through treatment with $SnCl_4$ to yield a separable mixture of heliannuol C methyl ether (**21**) and its epimer. Finally demethylation of (**21**) furnished heliannuol C (**1**). Interestingly, exo-cyclisation of the epoxides (**20**) followed by demethylation affords heliannuol E (**5**) (**Scheme 2**).



Scheme -2.

Reagents and conditions: i. $Me_2C{=}CHCH_2CH{=}CHCH_2OH$, DIAD, PPh_3, THF, -5°C, rt, 70-98 %. ii. Et_2AlCl, -78°C, Hexanes, 81%. iii. *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0°C, 100%. **iv.** $SnCl_4$, THF, rt, 42-67%. **v.** Et_3SiH, $B(C_6F_5)_3$ (cat.), HF, **vi.** NaSEt, DMF, reflux.

Biswas *el.al* [8] reported a very short and rapid synthesise of haliannuol C. In this strategy, direct Bargellini condensation [9] of appropriate coumarin (**23**) has been employed as a crucial step for the synthesis of the important precursor *o*-carboxyvinyl phenoxyisobutyric acid (**24a**). Treatment of diacid (**24a**) with diazomethane furnished the diester (**24b**). Reduction of diester with lithium aluminium hydride affords corresponding diol (**25**), which was then converted to vinyl carboxylate (**26**), utilising a Claisen rearrangement to install the key vinyl residue. Oxidation of ester–alcohol (**26**) with Jones' reagent at room temperature produced the corresponding carboxylic acid, which was esterified with diazomethane to furnish ethyl, methyl diester (**27**). A Dieckmann cyclisation of diester (**27**) and subsequent deethoxycarbonylation followed by reduction of the benzoxepanone (**29**) furnished *O*-methylheliannuol C along with its epimer. Finally demethylation of *O*-methylheliannuol C provided a short and facile synthesis of this allelochemical (**Scheme 3**).



Scheme-3.

Reagents and conditions: **ia**. NaOH, acetone, $CHCl_3$, reflux, 6 h, 75%; **b**. CH_2N_2, Et_2O, 0°C, 98%; **ii**. LAH, Et_2O, -20°C, 4 h, 90%; **iii**. $CH_3C(OEt)_3$, C_2H_5COOH, reflux, 5 h, 75%; **iva**. Jones' oxidation, 70%; **b**. CH_2N_2, Et_2O, 0°C, 1 h, 99%, **v**. LDA,THF, -10°c, rt, 72%, **vi**. LiCl, DMSO, 160°C, 5h, 85%. **viia**. $NaBH_4$, MeOH, 0°C, 98%. **b**. NaSEt, DMF, reflux.

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Genetic Algorithm (GA) based study of Structure and vibrational spectroscopy of alkali metal ion-water clusters

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Abstract

A hybrid method based on Genetic Algorithm and ab-initio calculations, was used to calculate IR spectra of alkali cation micro hydration clusters (e.g $M^+(H_2O)_n$ with M=Na) in the vibrational O-H stretch region. The cluster structures were globally optimized within the common TIPS2 potential using a floating point genetic algorithm. From the global minima structures at hand, it was possible to provide spectral data for the elucidation of trends in peak patterns with respect to these cluster structures. The method could predict many of the prominent features of the Infrared spectra of these species that are sensitive to the cluster size.

Key-words: Genetic Algorithm (GA), Global optimization, ab-initio method, ion-water clusters, TIP4P/OPLS potential, IR-spectra.

1. Introduction:

In recent years there has been a spurt in researches on structure and vibrational spectroscopy of small to medium size clusters [1-11] of the type $M^+(H_2O)_n$ [M = H, Li, Na, K, Cs, Ca]. The advent of sophisticated experimental techniques, which can produce and analyze clusters of definite stoichiometry, as well as high level quantum chemical calculations which can predict with confidence structural features and energetic have contributed to the rapid growth of the field. In the presence of a metal ion (e.g Na^+), especially for the cluster of size ($n \leq 4$) the $Na^+..O^{\delta-}$ electrostatic interaction dominates and causes the breaking of the hydrogen bond network of the ion-free clusters. As a consequence a blue shift in the O-H stretching mode compared to the O-H....O stretch in the ion-free clusters is observed. With increase in the size ($n \leq 4$) of the cluster a multitude of factors come into play, due to the presence of a wide variety of water molecules that differs in their hydrogen bonding pattern. The IR spectra in the O-H stretch region are particularly sensitive to hydrogen bonding patterns. As the experimental IR spectra are yet to be available for such clusters ($Na^+(H_2O)_n$) theoreticians could make an effort to calculate the IR spectra in the O-H stretch region to investigate the possibility of obtaining structural information from experimental IR spectra,

in conjunction with theoretical data. One may note the effort by Hartke et al [10] that uses a combination of specialized version of GA [8] and local mode approach [13] and by our group that uses a combination of a floating point GA approach [5] and semiempirical and ab-initio method to characterize the structure and spectra of the hydrated metal ion clusters. The simulated spectra show a red shift of hydrogen bonded *O-H* stretch compared to the free *O-H* group. One could extract several structural features (e.g a structural transition from one isomer to other) from a detailed analysis of the simulated spectra.

Most of the theoretical work available in literature uses high-level ab-initio calculations to characterize these clusters. Our objective would be to use suitable empirical potentials generated from chemical intuition which account for most of the forces operating between the atoms in such clusters. Once the potential is set up, we explore the potential energy surface for the lowest energy structure which the potential can support. Since the topography of the surface can be quite complex with multiple minima, saddle points, maxima, etc., the exploration of the lowest energy structure is beset with difficulties. We propose to use the stochastic optimization technique of genetic algorithm (GA), a potent and true global optimizer for a global search of the energy surface. Having generated the structures we would use it to perform a quantum chemical calculation at the ab-initio level (B3LYP) to simulate the IR-spectra of aquated alkali matal ion clusters. While semi-empirical (AM1) level calculations were performed to simulate the IR-spectra of the globally optimized structures (obtained by our GA-based recipe) of halide ion-water clusters in our previous study [5]. We adopted the ab-initio method in the former case due to the non-availability of the suitable parameters for Na^+ ion at the semi-empirical level.

2. Methodology

The strategy adopted by us has been to use an empirical potential energy surface (PES) and invoke a stochastic search for identifying the structure corresponding to the global minimum energy point on the PES. It is therefore necessary first to define the PES.

2.1 The Potential energy surface (PES)

Many empirical potentials for structure elucidation in pure $(H_2O)_n$ clusters, solvated $Cl^-(H_2O)_n$ and $Na^+(H_2O)_n$ have been used [1-11], over the years in various simulation studies. Jorgensen et. al. developed a set of transferable inter-molecular potential functions (TIPS) for the simulation of organic liquids, pure water and aqueous solutions of different ions (e.g Cl^-, Na^+, Li^+etc) namely TIPS2, TIP3P and TIP4P [14-17]. Very similar models of water-water and ion-water interactions have been employed by several authors in later studies. The state of the empirical potentials for pure water clusters has advanced far beyond the simple TIP4P-type potentials and e.g. the recently proposed potential is by Burnham and Xantheas [18], claim good agreement of the cluster structures and energetics with ab-initio results. To characterize the structures of $Na^+(H_2O)_n$ system, we have used the TIPS2 potential [16] proposed by Jorgenson et al for modeling water-water and ion-water interaction. Although this potential can be regarded to be "standard" which is used with success in many other areas, a model involving polarization (e.g.TIP4P-FQ [19]) might also be used. However, polarizability appears to be more important for the solvation of anions [1,19,20] than for the solvation of cations. In fact, the performance of the simple TIPS2 or TIP4P/OPLS (with 4-interaction sites in water molecule) model in arriving at quantitatively correct cluster

structures is much better than one might have guessed, as pointed out by Jorgenson et al [17], there is rather good agreement with ab-initio calculations. Energetics are modeled less well, but the discrepancies with ab-initio and experimental data is not very large [21]. Since there are no indications of intracluster reaction $Na^+ + H_2O \rightarrow NaOH + H^+$ in experimental study [8] it is of no use to going beyond the simple *4*-point model.

3. The Optimization Step

Once the potential energy surface is set up, we invoke the stochastic optimization technique of genetic-algorithm. Genetic Algorithms have emerged as a popular technique for solving a host of difficult optimization problems. Genetic Algorithms are search techniques whose mode of operation and architecture are inspired by the laws of genetic evolution and the Darwinian concept of survival of the fittest [22,23]. The application of GA requires us to represent the potential solutions chromosomally. For a cluster of n H_2O molecules, such a representation boils down to representation in terms of strings containing, let us say, the Cartesian coordinates of the different atoms in the cluster. This means, we need strings containing $9n$ variable. For the present problem, the water molecules are treated as rigid bodies and intra molecular motions like bond stretching and bending are considered to be absent. That means, the *O-H* distance, and *HOH* angles are fixed for all the water molecules leaving the $(3n)$ Cartesian coordinates of the (n) oxygen atoms of the water molecules and the three Eulerian rotation angles for each water molecule as the basic geometric variables making up the potential solution strings [24]. The Eulerian angles fixes the Cartesian coordinates of the two *H*-atoms of each water molecule first with respect to the body fixed coordinate system with its local origin on the oxygen atom; then on applying Eulerian rotations ($\acute{a}$, $\hat{a}$, $\tilde{a}$) the coordinates of the hydrogen atoms in the rotated body fixed frame of the water molecules are produced and finally they are translated to the space fixed frame. Effectively therefore, for the i^{th} water molecule we need to have a string containing the three Cartesian coordinates of the oxygen atom $\left(R^x_{O_i}, R^y_{O_i}, R^z_{O_i}\right)$ and the three Eulerian angles α_i, β_i, γ_i. The collection of six such variables defines a sub-string and a collection of n such substrings makes up a string defining a possible cluster geometry. This makes the chromosomal representation convenient to handle and crossover operation easy to implement during the genetic evolution. The crossover operation was designed so as to mix the variables in the k^{th} string representing i^{th} H_2O molecule with variables in the l^{th} string representing the water molecule. The pair of strings meant to undergo crossover with a probability $p_c = 0.25$ are given by

$$S^i_k = (R^x_{o_k}, R^y_{o_k}, R^z_{o_k}, \beta_k, \gamma_k) \qquad (1)$$

$$S^i_l = (R^x_{o_l}, R^y_{o_l}, R^z_{o_l} \alpha_l, \beta_l, \gamma_l) \qquad (2)$$

and then producing the crossed-over pair (crossed at one or more sites) as mentioned in the following.

$$(S^i_k)' = (R^{x'}_{o_k}, R^{y'}_{o_k}, R^{z'}_{o_k} \alpha'_k, \beta'_k, \gamma'_k) \qquad (3)$$

$$(S^i_l)' = (R^{x'}_{o_l}, R^{y'}_{o_l}, R^{z'}_{o_l} \alpha'_l, \beta'_l, \gamma'_l) \qquad (4)$$

where α_k' designed as

$$\alpha_k' = (1-f)\alpha_k + f\alpha_1 \qquad (5)$$

with f chosen randomly from the range *0 d" f d" 1*. The same is the form of β_k' etc. The form of α_i' chosen to be

$$\alpha_i' = f_{ax} + (1-f)\alpha_i \qquad (6)$$

The same rule followed in designing β_i', etc.

Mutation of any string is a low intensity change that takes place with low probability p_m ($\approx$0.01 - 0.05). The mutated variable (say $\hat{a}_i$) in the k^{th} string is given by

$$\left(\beta_i^k\right) = \beta_i^k + (-1)^M r_i^{\Delta} \qquad (7)$$

where M is a random integer, r_i is a random number between 0 and 1, Ä is an intensity factor chosen randomly from a given range.

The diversification operator is introduced to maintain the diversity in the population in presence of highly dominating structure. This operator is being operated on all the strings except the fittest one. Diversification of any string is a low intensity change that takes place with a low probability $p_{div} \approx$ 0.001.

When a halide ion is present in addition to the *n*- water molecules, three Cartesian coordinates of the halide with respect to the space fixed coordinate system is to be included in the strings. The selection operator requires us to define a fitness value for each string. Let V_i be the potential energy for the specific configuration of the halide ion + n water molecules in space as coded by the i^{th} string and V_L an estimated lower bound to the energy. Then, we can define a fitness value F for the string as,

$$F_i = e^{-\sigma_i} \qquad (8)$$

where $\sigma_i = |V_i - V_L|$, clearly, $0 \leq F_i \leq 1$. The fitness proportional selection of individuals is carried out at the beginning by a Roulette-wheel procedure [15].

We note here the work by Hartke et al that uses a different GA based global optimization algorithm [8] to find the minimum structures of Na^+-water clusters. The additional features incorporated in their algorithm are: phenotypic operations introduced by Deaven and Ho [25] is followed; the directed mutation approach is used to speed up the final convergence; and the "niche" procedure is adopted to preserve diversity in the population in the presence of strongly dominating structure [6,7]. In the "phenotypic" operation approach the crossover operator has to be designed according to the probable geometry of the cluster structures being studied. For example in the case of Na^+-water cluster it turns out that preserving the cavity of water molecules around the Na^+ ion which is fixed at the origin is important in their calculations. So the clusters are cut here by planes containing the origin during the phenotypic crossover operation. Our method appears to be more

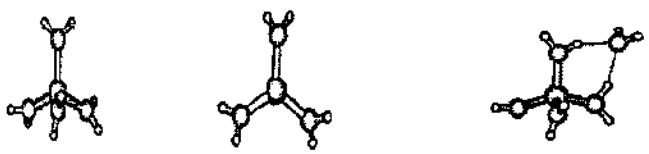
general where a simple genotypic (discussed in early part of this section) crossover is carried out to effect the structural changes in the clusters, and the same crossover algorithm can be used for optimization of any type of water clusters containing cations or anions. The introduction of niche criterion by Hartke et al [8] based on the average distance of Na^+ ion and the center of mass of the water molecules proves to be an important structural parameter to capture the main isomers. One may incorporate this method of "identification of different isomers" in our GA-based strategy to test the performance of these niche criteria in general for optimization of alkali ion or halide ion-water clusters.

4. Results and Discussion

4.1 Structure of Na^+ ion-water cluster

There are several theoretical studies on the characterization of the structure of $Na^+(H_2O)_n$ clusters that involves high level ab-initio calculations [26] and also calculations based on model water-water and ion-water potentials (e.g. TIP4P/OPLS) [8]. In this section we shall discuss on the globally optimized structures of $Na^+(H\_2O)_n$ clusters with n=3-8 obtained by applying our GA-based formalism on a model potentials (TIPS2 [17]) that simulates water-water and ion-water interactions. We shall have a comparative study of the structures obtained in our calculations with those obtained by ab-initio methods [26] and by Hartke et al [8].

Figure 1 shows our global minimum structures for $Na^+(H_2O)_n$, n= 3, 4 and 5. In the structures for n= 3 and 4 the Na^+ ion is placed at the center and the water molecules encapsulate the central ion in highly symmetric fashion. The hydrogen bonded network for ion-free water clusters are absent here and all the water molecules behaves as free H_2O in these tri and tetra-coordinated sodium ion clusters.



(a) (b) (c)

Figure 1. The global minimum structure of $Na^+(H_2O)_n$, where n=3, 4, 5

The result here is in good agreement with the ab-initio method and with the method adopted by Hartke et al. For the $Na^+(H_2O)_5$ system, we have obtained the *4+1*-isomer as the global minimum structure, where the four water molecules are in first solvation shell and the fifth one is in the second solvation shell making a hydrogen bonded network with two water molecules. Kim's ab-inito study [26] shows that the *4+1* isomer is lowest in energy and it is lower than the *5+0* structure at T= 0 K (without zero-point energy (ZPE) correction) by 8 *KJ/mol*, these two isomers have virtually the same free energy at ambient conditions. Our GA-based study is a zero temperature one and it searches the PES to find the global minimum energy structures at T= 0K. Our algorithm

appears to search PES based TIPS2 potential appropriately to find the global minimum structure at $T = 0K$ for the pentamer. However, the GA-based strategy adopted by Hartke et al that uses TIP4P/OPLS potential (which is based on the same four site model of water molecules but differs slightly from the TIPS2 model in terms of parameters sets used) produces a different isomer, the *5+0* one as the global minimum.

Figure 2 shows our global minimum structures for $Na^+(H\_2O)_n$, n= 6, 7 and 8 There are three isomers possible for the Na^+-water hexamer, namely *4+2*, *5+1*, *6+0*. The molecular dynamics (MD) simulations [27,28] and high level ab-initio study that includes many body effects to some extent and uses polarization functions in the basis set [26] show that there is small energy difference between these three isomers and their preference is governed by zero temperature energy and finite temperature effects. The ab-initio study shows *4+2* isomer to be the lowest in energy and it is lower in energy by 9 *KJ/mol* from the *5+1* isomer (without ZPE correction). We obtained the *4+2* isomer as the global minimum in our study based on GA (a zero temperature study) and the model TIPS2 potential. However, our results contradict the results obtained by Hartke et al [8] that uses a different GA-based approach and the same type of model potential (with slightly different parameter sets) that does not include polarization. One may note that the *5+1* isomer found to be the lowest in energy in some polarizable water models [27,28]. It is surprising that the *6+0* isomer

(a) (b) (c)

Figure 2. The global minimum structure of $Na^+(H_2O)_n$, where n=6, 7, 8.

is not the lowest in energy, in the light of facts that several studies [16,29-32] (using TIP4P potentials) find a coordination number of *6* in bulk solvation. Our study shows when we increase

the size of the $Na^+(H_2O)_n$ cluster up to *8* a distorted octahedral core with *6* water molecules in first solvation shell (see figure 2(c)) is formed and the remaining two water molecules starts to form the second solvation shell around the central ion. The transition to bulk solvation pattern of the kind described begins to take place as one goes beyond cluster size of $n > 6$ (see figure 2(b)). The study by Hartke et al shows the same pattern of Na^+ ion solvation with cluster size of $n = 8$. Their study shows that with the increase of number of water molecules beyond n=8, a second shell of water molecules starts to form the second solvation shell around the central ion and for $n \geq 8$ the first shell adopts a *6+0* distorted octahedral configuration. A detailed energy component analysis for the reason behind this observed phenomenon is discussed in the report by Hartke et al [8].

4.2 *Vibrational Spectra of Na^+ ion-water clusters*

The global minimum clusters structures of the systems $Na^+(H_2O)_n$ where n=3-8, obtained by our GA based study, are used to simulate the ab-initio an-harmonic IR-spectra. We use standard B3LYP basis in the calculations of the present study. However for quantitative analysis of the IR spectra that may be used in conjunction with the experimental one to characterize the structure of the $Na^+(H_2O)_n$ clusters would require correlated ab-initio calculations.

One may note that even harmonic IR spectra for large alkali cation water clusters by full ab-initio method are difficult and computationally challenging, though it can be realized to compute spectra of small pure neutral water clusters [32,33]. Our combined GA and ab-initio method based approach turns out to be computationally cost effective in this context. The GA plus an empirical local mode approach [13] by Hartke et al [10] to simulate the IR spectra of same type of systems also found to be computationally effective in case of even larger alkali cation and larger cluster size. In this section we shall analyze our simulated IR spectra in the *O-H* stretch region and compare them with the results obtained by Hartke et al [10]. On addition of the Na^+ ion to the ion-free water clusters we observe a blue shift in both the bands (arises due to two *O-H* stretching mode symmetric and asymmetric) in the simulated IR spectra for the $Na^+(H_2O)_n$ clusters of size $nd \leq 4$ (figure 3). The splitting in the *O-H* stretch bands in the ion-free water clusters are contributed to the hydrogen bonded network. This H-bonded network is ruptured on addition of a cation that causes the appearance of two simple bands in the simulated IR-spectra of the solvated clusters of smaller size ($nd \leq 4$). One naturally anticipates the observed blue shift in *O-H* stretch band due to the strengthening of the *O-H* bond in the absence of hydrogen bonded network in the aquated clusters.

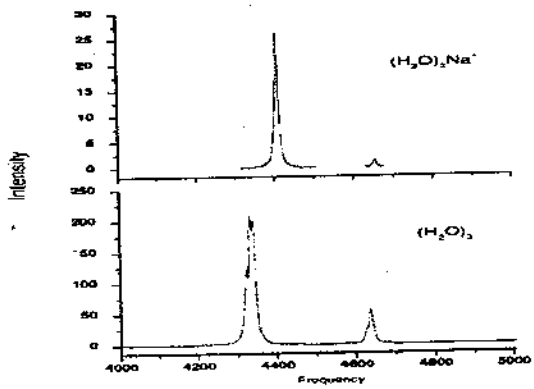


Figure 3. The Spectra of $(H_2O)_3$ and $(H_2O)_3Na^+$

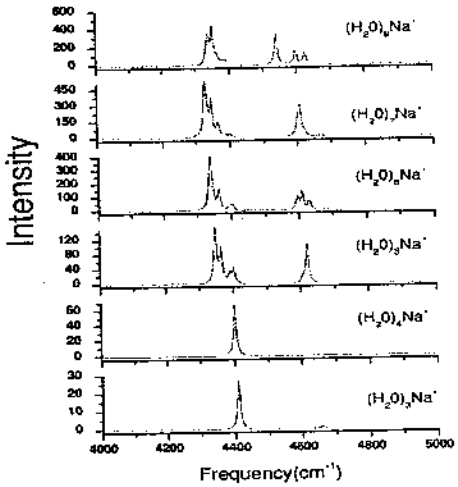


Figure 4. The Spectra of $(H_2O)_nNa^+$, where n=3 to 8

Figure 4 depicts the simulated IR spectra for $Na^+(H_2O)_n$ systems for n= 3-8. The spectra show a marked red shift in both the sets of *O-H* stretch (symmetric and asymmetric) band with increase in size of the clusters. The increase in complexity of the nature of the two *O-H* stretch bands with increase in size of the clusters (for n e□ 4) is contributed to the presence of a wide variety of water molecules e.g. single donor single acceptor of H-bonds (DA), double donor of H-bonds (DD), double acceptor of H-bonds (AA) etc for larger clusters. One anticipates the red shift as we move from free water molecules in smaller clusters (for n d□ 4) to water molecules in a hydrogen bonded network in the larger clusters (for $n >$ 4).

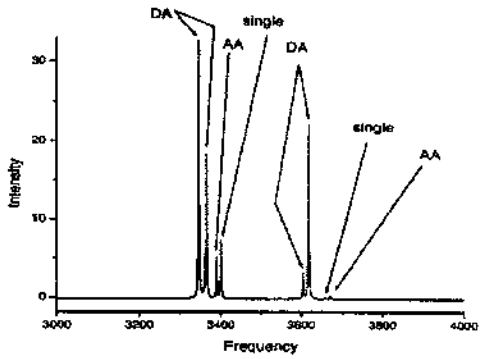


Figure 5. simulated high resolution Spectra of $(H_2O)_5Na^+$

A close analysis of the spectra (figure 4) shows a sharp change in the nature of the bands as we move from n=4 to n=5, where the splitting of two simple bands into a number of peaks along with a red shift is distinct. This observation is entirely consistent with the global minimum structures obtained for the two isomers (tetramer and pentamer, figure 1) where we see a transition from a structure containing free water molecules to a structure with a network of hydrogen bonded water molecules. To assign particular normal mode stretch corresponding to each peak, which can be used to analyze the various types of water molecules present in system, we take up the case of the pentamer. To get highly resolved spectra where assignment of peaks will be easier (see figure 5) we have simulated the IR spectra for the pentamer using a smaller bandwidth in the Lorenzian fitting of the frequency vs intensity data, obtained from ab-initio force calculations of GA-optimized Na^+-water clusters. The *O-H* stretch (symmetric and asymmetric) bands for water molecules of the type DA (single donor and single acceptor of hydrogen bond) appear in the region (3350 cm^{-1}) and (3600 cm^{-1}). The double splitting of these particular type of band arises due to the coupling of two *O-H* stretch modes of similar energy where the coupling is mediated by the hydrogen bonded network through a double acceptor (AA) type water molecule (see figure 1.c). The band for the *O-H* stretch of the double acceptor (AA) type water molecules arises in the higher energy side and falls in the region of the band that appears due to single free water molecule. This aspect of appearance of two bands (responsible for *O-H* stretches for different type of water molecules, AA and free water) in the same energy region is also observed in the simulation of IR-spectra of alkali ion-water clusters by Hartke et al [10]. The bands of these types appear in the region ($3400

cm^{-1}) and (3680 cm^{-1}) in our simulated spectra, for the symmetric and asymmetric *O-H* stretch respectively. There is a possibility for the appearance of the overtones in the experimental spectra due to these two similar types of oscillator frequency.

Further a close analysis of the spectra in figure 4 shows a sharp change in the nature of the bands when we move from n=6 to n=8. The splitting of bands on high energy side and a marked red shift are distinct as one moves from n=6 to n=8. This observation is consistent with the geometry of the global minimum structure obtained for the octamer. As expected from general trend a distorted octahedral core of the first salvation shell is formed for the cluster of size ne" 8 and the remaining water molecules are placed in the second solvation shell keeping Na^+ ion at the center. The local symmetry of the water molecules are lost in this case, causing a larger splitting in the IR- spectral bands.

The other feature that our simulated spectra reproduce is the gradual increase in intensity of the *(O-H...O)* stretches with increasing cluster size.

5. Conclusions:

We have shown that GA based calculations for locating minimum on model PES of $Na^+(H_2O)_n$

clusters coupled with ab-initio calculations using B3LYP basis, is a viable strategy for the characterization of the structure and vibrational spectra of $Na^+(H_2O)_n$. Many of the features can be understood semi quantitatively on the basis of these analyses which are cost - effective. This study

can be extended by using a correlated ab-initio calculation on the GA-optimized structures for quantitative reproduction of the experimental spectra. It would be interesting to study the change of the IR-spectral pattern as we move from ion free water clusters to a micro hydrated cluster that contains both Na^+ and Cl^- ions. One may extend this study to simulate the bulk salvation pattern of *NaCl* solution and the corresponding IR-spectra.

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Public theatre in Bengal: History behind its foundation

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Abstract

The aim of the article is to trace the evolution of the public theatre in Bengal. Drama that once flourished in India gradually fell out of practice after the Islamic conquests. It was revived during the Chaitanya age. The first public theatre of Bengal was founded by Lebedev in 1795. However it ceased to exist after only two performances. Around the eighteenth century *yatra*, *kabi* and *half-akhrai* gained popularity. These were part of the popular culture of Bengal and obscenity was inherent in these. The newly educated youths abhorred such vulgar forms of entertainment and longed for something as refined as the English drama. This made them to come up with quite a few amateur theatres. Though these theatres were short-lived, they created a taste for drama among the public. This gradually culminated in the foundation of the public theatre. Needless to say, the newly established theatre did not have female artistes. When they were recruited, there were protests from various quarters. However they were retained and this proved beneficial to the theatre as well as the society at large.

Though India can boast of dramatists like Kalidasa, Bhasa, Bhavabhuti, Bhattanarayana, Sriharsha and many others, drama gradually fell out of practice in the country. This can be traced to the Islamic conquests that began in the twelfth century. The Muslim rule discouraged or forbade theatre entirely. It was then that *yatras* dealing with religious and mythological themes came into existence. Bengal had to wait till the Chaitanya age (1486 - 1533), when plays again came to be staged. Around the eighteenth century, gradually, *kabi*, *half-akhrai*, *panchali*, *kathakata* and *kirtan* gained popularity. These were part of the popular culture of Bengal. When the English came to India, they brought with them the drama that was their national amusement. Later when western education was imparted to Indians, they began to dislike the existing forms of entertainment of Bengal. They longed for something as refined as the English drama. This longing saw its manifestation ultimately in the opening of the public theatre. This article aims to show the various phases that led to the foundation of the public board in Bengal. It mentions contribution of Lebedev and the various amateurs to the founding of the public theatre. The article also discusses the introduction of actresses on the public stage.

The history of the modern Bengali stage begins with Gerasim Stepanovich Lebedev who opened the Bengally Theatre in November 1795. He was born in 1749 in the town of Yaroslavl. From the scanty information that is available, Hayat Mamood draws the conclusion that his father was the clergyman in the private chapel of some rich landlord and that Count Andrei Kirillvich Razumovski might have been the first master. Lebedev's talent in music drew the attention of the Count. In 1777 the former left Russia as a part of the envoy to Naples with Razumo never circumstances forced them to halt for a year at Vienna where Lebedev learnt violoncello. After

visiting London, Paris and Madras, he came to Calcutta in 1787 and used musical performances to earn his livelihood.

Lebedev himself wrote to one of his friends that he wanted to learn Bengali, Hindustani and Sanskrit because without the knowledge of these languages it would be difficult for a wanderer like him to acquire accurate knowledge about the places. However finding a teacher was not easy. Mikhail Medvedev writes: 'It took many years to find an Indian who would care to decipher to a white man the letters of ancient Sanskrit – the "golden key" that unlocked the door to the priceless treasures of oriental science and knowledge' [1]. When he was on the verge of dropping the plan altogether, his *sarkar* introduced him to Goloknath Das, who was a school teacher. It was an interesting coincidence that he was no less eager to learn music from Lebedev, than the latter was to learn the Indian languages.

Lebedev began learning the Indian languages under Goloknath Das. The former did not stop here. He later translated Joddrell's *The Disguise* and *Love is the Best Doctor*, an adaptation of Moliere's *L'Amour Medecin* into Bengali. He was clever enough to observe that '[...] the Indians preferred mimicry and drollery to plain grave solid sense, however purely expressed – I therefore fixed on those plays and which were most pleasing, filled up with a group of watchmen chokey-dars; Savoyards, *Canera*; thieves ghoonia; lawyers, Gomasta and amongst the rest a corps of petty plunderers' [2]. Having noticed that the Bengalis loved songs, he set to music the words of the famous Bengali poet Bharatchandra Ray. After finishing the translation Lebedev invited several scholars to read it closely. After receiving their approbation, it was his linguist Goloknath Das who suggested staging the plays. Lebedev agreed and solicited the Governor General Sir John Shore for a regular license. Once it was granted, he built his own theatre at Domtollah. The performers belonged to both the sexes.

While these preparations were going on, Lebedev gave out an advertisement in the *Calcutta Gazette* on November 5, 1795 announcing the opening of the Bengally Theatre shortly that would be "decorated in the Bengalee [sic] style" [3]. *The Disguise* was presented on November 27, but only in one act. This was so because only a few Europeans could follow Bengali and a lengthy act might be boring for them. The success of the first show inspired Lebedev to come up with another presentation, which was partly in original and partly in translation. He says that on both the performances the house was full.

In spite of having an overflowing house, the performances returned only half of what Lebedev had actually spent. His hope of making profit made him seek the permission of the Governor General to allow him give performances in both English and Bengali and it was granted. Mrs. Antonova refers to an advertisement that was written but may or may not have been ultimately published where Lebedev intended 'to invite the Asiatic Inhabitants only, at and in the Vicinity of Calcutta' [4]. However he could never again stage another show. Apparently this may seem to be due to the jealousy of the Calcutta Theatre. But the latter actually did not have much to fear because both the theatres were staging plays in two different languages and more so when Lebedev managed to stage only two shows in a span of four months. Besides the Calcutta Theatre did not have any problem when the Wheeler Theatre was founded in 1797. Hayat Mamood says that though there is an absence of sufficient proof, it seems that the British did not trust Lebedev in the least. They feared him to be a Russian spy. They did not have any proof for espionage and therefore could not take any legal action against him. But they were desperate to get him out of Calcutta.

There seemed to be a cold war between England and Russia even in the eighteenth century. Still the Anglo-Russian relation was running smoothly. Friction came during the rule of Catherine the Great (1762 – 1796) when England felt threatened by the policy of southward expansion and feared it would disturb the balance of power that existed in Europe. It is true that all the countries realized that the strongest blow could be struck at England in India. Thus the over-curiosity of the Russian embassy in London towards the Indian affairs made the British feel unsafe. The Russian embassy could get better information once Lebedev arrives in Calcutta. Again, in a letter to Simbarski, Lebedev also mentions his hope of reward from the successors of the Queen Mother Ekterina Alexievna for his mission in foreign lands under hostile conditions. Though there is no direct evidence against Lebedev, yet the British fear seems justified. At last they were compelled to hatch a conspiracy against him so that the Russian would leave in his own will. Lebedev was so badly persecuted that he was compelled to leave Calcutta on December 3, 1797, almost a pauper.

The next stage of theatre in Bengal comes with the emergence of various English playhouses in the city. The Bengalis were naturally attracted towards this new form of entertainment. But, the tickets of entry to these theatres the tickets being too expensive, only the rich could occasionally attend. People therefore turned to other forms of entertainment. The Indians were '[...] commonly seen on the race-course, booking their bets [...]' [5]. Fights of bulbuls were popular as well. The Bengalis loved to attend *yatra*, *kabi* and *half-akhrai*. Obscenity was inherent in these forms of recreation. The educated youths of the Hindu College found these traditional forms of entertainment abhorring. They attended the shows at English playhouses and longed for something refined like the English drama. The English theatres of Calcutta were indeed the forerunners of the Bengali stage. 'Apart from being historically and chronologically the predecessors of the native theatres they were also the ultimate inspiration and model before the latter' [6].

After Lebedev left India, Calcutta had to wait for thirty five years till Prasannakumar Tagore's Hindu Theatre came up in 1831. Many private theatres mushroomed in the next forty years. However these theatres were mainly meant for the aristocrats. Prasannakumar founded the Hindu Theatre that opened with the last act of *Julius Caesar* and a portion of *Uttararamacharita* of Bhavabhuti, translated by Dr. Wilson who also supervised the acting. The *Samachar Darpan* commended the performance in an article on January 7, 1832. They wrote: 'People in various roles always look the same under the native proprietors and the costume designer [...] There is no doubt that the English ones are a thousand times better than their Indian counterparts. It can be believed that the actors dressed by them shall exactly resemble the person they are impersonating' [7]. It is interesting to note that the writer of this article looks upon the Hindu Theatre as a *yatra*. Indeed many nineteenth-century journals referred to the English theatre as foreign *yatra* [8].

There were some very rude comments as well. Someone signed the 'East Indian' wrote in the *Asiatic Journal*: '[...] what can be worse than to have the best dramatic compositions / in English language murdered outright, night after night, foreign manners misrepresented and instead of holding the mirror upto nature caricaturing everything human? [...] A theatre among the Hindus with the degree of knowledge they at present possess will be like building a palace in the waste' [9]. Another article rudely suggested the native aspirants to confine themselves to the representation of parts to which their complexion would be appropriate. The writer did not deny the histrionic skill of the Indians, but felt they should not venture beyond the part of Othello. This theatre ceased to exist after staging *Nothing Superfluous*.

The students of the Hindu College and the Sanskrit College performed different scenes from Shakespeare and other plays on various occasions. The acting was under the direct supervision of Dr. Wilson. He and Captain Richardson were the more noted ones amongst the teachers who directed the staging of plays at educational institutions. Herman Jeffroy, the Head Master of the Oriental Seminary, also helped his students in developing a love for drama. The David Hare Academy, founded in 1851, staged *The Merchant of Venice* under the training of Mr. Clinger, the Head Master of the Calcutta Madrasa. Some other ex-students of the Oriental Seminary established the Oriental Theatre in 1853 and staged *Othello*, *The Merchant of Venice*, *Henry IV* Part I and Meredith Parker's *Amateur*.

The Jorasanko Theatre founded at the house of Pearymohan Basu opened with *Julius Caesar* in 1854. All the plays performed by Bengalis in the first half of the nineteenth century were in English. The only exception was Nabinchandra Basu's theatre that opened on October 6, 1835 with *Bidyasundar*. Different scenes were acted in the different parts of his garden-house. The audience had to move to watch the play. The theatre was also popular for introducing women on the stage.

In spite of the presence of quite a few theatres, a permanent playhouse could not be seen. This was so because the performances being in English, they did not appeal to the general public. Even the educated youths had to exert themselves to grasp the meaning of the play in a foreign tongue. The actors too had to be careful about their accent and pronunciation. They were afraid of offending the refined taste of their English audiences. This could very likely divert their focus from proper acting. Thus the absence of Bengali plays turned out to be the main reason behind this lack of a sustained interest. It explains why forty odd years rolled by before the first public theatre opened its doors.

Leaving apart Lebedev's translated plays and *Bidyasundar*, *Abhijnana Sakuntala* by Nandakumar Ray is the first Bengali play to be staged on January 30, 1857. Since then the dramatic tradition in Bengal has remained uninterrupted. In the same year, members of the Jnanapradayini Sabha staged *Abhijnana Sakuntala*. They also performed *Mahasweta*. *Kulin Kulasarbaswa*, by Ramnarayan Tarkaratna dwelled on the evils of polygamy practiced by the *kulins*. This play was performed at the house of Ramjay Basak in 1857. About the performance of this play Mahendranath Mukhopadhyay writes: 'Rajendrababu and Jagaddurlabhbabu used to play the role of Brahmin pandits with a huge tummy and a tuft of never-cut hair at the back of their head. Rajendrababu would carry a snuff-box made of shell. When the both of them engaged in debate, the audiences would roll with laughter' [10]. The play was so popular that it was staged many times.

Kaliprasanna Sinha's Bidyotsahini Rangamancha opened with Ramnarayan Tarkaratna's translation of Bhattanarayana's *Veni-Samhara* in 1856. The founder also translated and staged Kalidasa's *Vikramorbasi* in 1857. He then composed *Sabitri Satyaban Natak*. It is the first of the few dramatic pieces that were actually composed in Bengali and was staged on June 5, 1858. The Paikpara Raja Pratapchandra Sinha and his brother Iswarchandra founded the Belgachia Natyasala. There was not a more popular theatre in its day. It was famous for acting, stage décor and music. They staged Ramnarayan Tarkaratna's translation of Sriharsha's *Ratnavali*, where the performance of Kesabchandra Gangopadhyay was superb. *Sarmishtha*, composed by Michael Madhusudan Dutt, was staged on September 3, 1859. This theatre was closed after the untimely death of Iswarchandra Sinha.

When the question of widow remarriage was being debated in Bengal, many plays were composed on this theme. The most prominent among those was Umeschandra Mitra's *Bidhaba Bibaha Natak*, staged by Kesabchandra Sen and the youths of his group on April 23, 1859. Since it was staged at the mansion of Ramgopal Mallik, where the Metropolitan College was founded, it was named the Metropolitan Theatre. Pratapchandra Majumdar, the biographer of Kesabchandra Sen wrote that Vidyasagar was present on more than one occasion and was so moved by the performance that he failed to check his tears.

Before Jatindramohan Tagore founded the Pathuriaghata Banganatyalay, his younger brother Saurindranath presented Ramnarayan Tarkaratna's translation of Kalidasa's *Malavikagnimitra* on the stage of their ancestral house in 1859. Jatindramohan opened his theatre with his dramatic version of *Bidyasundar*, which he did after omitting the obscenities. It was staged with *Jeman Karma Temni Phal* in December 1865. *Bujhle Ki Na*, *Malatimadhava*, *Chakshudan* and *Ubhaysamkat* were later staged. The theatre was suspended for a year, before reopened with *Rukmini Haran* and *Ubhaysamkat* in 1872.

The Sobhabazar Private Theatrical Society was founded by the princes of the Sobhabazar royal family. Their aim was to flush out the superstitions and evil practices of the society. They opened with Michael Madhusudan Dutt's *Ekei Ki Bale Sabhytata* on July 18, 1865. The *Sambad Prabhakar* wrote that if anyone amongst the audience is of the nature of the dramatis personae, he must have simultaneously felt ashamed and amused at seeing the dramatic representation of his secret games. They next staged Michael Madhusudan Dutt's *Krishnakumari*.

After seeing Gopal Urey's *yatra*, Saradaprasanna Gangopadhyay, Gunendranath Tagore and Jyotirindranath Tagore felt inspired to open a theatre. They first staged *Krishnakumari* and then *Ekei Ki Bale Sabhytata*. They too wanted to stage plays that would deal with social problems. They advertised for plays on the issues of polygamy, condition of the Hindu women and the atrocities of the zamindars. The best writers were to be awarded. The topic of polygamy was later withdrawn because Ramnarayan Tarkaratna promised to compose one. He came up with *Naba Natak* which was staged on January 5, 1867.

Baladeb Dhar and Chunilal Basu, actors of Pathuriaghata Natyasala, founded a theatre that opened with Manomohan Basu's *Ramabhishek Natak* in early 1868. Plays for this theatre were written by this dramatist. After five years a few distinguished Bengalis built a theatre house for this group and it was christened the Bowbazar Banganatyalay. It opened on January 17, 1874 with *Sati Natak*. *Harischandra* was the next play to be staged.

About this craze for theatre, Jatindramohan Tagore wrote to Michael Madhusudan Dutt: 'Nowadays theatre is mushrooming in the country. It is sad that these are not long lived. Still this must be seen as a good sign, because this indicates that people are developing a taste for drama' [11]. These theatres were founded either by the nouveaux riche or the reformers. These were due to their passing fancy. Once they lost interest or found some new diversion, the hapless theatre ceased to exist. Sometimes the theatre closed with their death. Then people had no other choice than waiting for the arrival of another theatre aficionado. Newspapers and journals kept on complaining about the absence of a permanent playhouse in the city.

Private theatres were meant for the aristocrats. The guards often turned out ordinary people wishing an entry into any of the houses. This touched the self-respect of Girish Chandra Ghosh and soon together with Nagendranath Bandyopadhyay, Radha Madhab Kar and others, he formed the Baghbazar Amateur Theatre in 1868. They selected Dinabandhu Mitra's social sketch *Sadhabar*

Ekadasi, in which Girish Chandra appeared in the role of Nimchand. The latter feels grateful towards this dramatist because his simple social play that did not require expensive costumes enabled the youths to dare performing it. Still the project would have been quite unsuccessful without the excellent portrayal of Nimchand. Hemendranath Das Gupta therefore says: 'Dinabandhu and Girischandra were therefore called the real founders of the National Theatre and the Public stage of Bengal' [12]. After this they staged the same author's *Biye Pagla Buro* and *Lilabati*. Amritalal Basu has called *Sadhabar Ekadasi* 'the unconscious germ of the public stage' [13] and the success of *Lilabati* was 'the immediate stimulus leading to its formation' [14].

In the meantime, Nabagopal Mitra, editor of the *National Paper* suggested the name 'Calcutta National Theatrical Society' which was ultimately shortened to 'National Theatre' [15]. The need for a public theatre was voiced in newspapers. The idea behind starting the theatre was not commercial. The young actors wanted to sell tickets only to defray their expenses for the theatre. Girish Chandra Ghosh did not agree to this and therefore parted company with his friends. The house of Madhusudan Sanyal of Jorasanko was taken on a monthly rent and the first public theatre was to be housed here. The play chosen for the first performance on December 7, 1872 was Dinabandhu Mitra's *Nildarpan*, a ruthless exposure of the oppression of the British indigo planters of the poor Bengali ryots. The youths desperately needed a permanent place for holding rehearsals. They found an opportunity when Bhuban Mohan Neogi offered them a place for holding rehearsals in his house on the bank of Ganga. He left the big hall on the first floor of his house and another room at the disposal of the young thespians. He also gave them an organ. Rehearsals went on with enthusiasm.

There was an advertisement of *Nildarpan* in *Sulabh Samachar* on November 19, 1872. The next day the *Englishman* wrote: 'A few native gentlemen of Baghbazar have established a Theatrical Society, named The Calcutta National Theatrical, their object being to improve the stage, as also encourage native youths in the composition of new Bengali dramas from the proceeds of the sales of tickets' [16]. The stage was soon built at Madhusudan Sanyal's house. Though practically there was no pavilion and the audience had to sit under the canopy of canvas, yet the play was a great success. Amritalal Basu says that tickets were priced at Rs.2/- for the first class to be seated on chairs taken on rental basis from Janbazar, Re.1/- for the second class, seated on makeshift benches and eight annas for the third class, seated on the stairs and the raised terrace in front of the building [17]. The cast was distributed among the youths. They were encouraged by Sisirkumar Ghosh, editor of the *Amrita Bazar Patrika*, Manomohan Basu, editor of the *Madhyastha* and Nabagopal Mitra. The first show of the first public theatre of Bengal was held in gaslight. The performance commenced at 8:00 p.m., the doors being opened at 7:00 p.m. It was over by midnight. All the parts were played very well. But the author keenly missed Girish Chandra Ghosh who was extraordinary in serious parts.

The performance was highly commended by the *Amrita Bazar Patrika* and the *Sulabh Samachar*. The *Education Gazette* published an article on December 13 requesting people to encourage the public theatre. They wanted everyone to unite so that the cheap forms of entertainment could not once again flourish. The *Englishman* however felt the play was damaging to the prestige of the British and should be stopped [19]. Before the second performance, they wrote on December 20: 'Considering that the Rev. Mr. Long was sentenced to one month's imprisonment for translating the play, which was pronounced by the High Court a libel on Europeans, it seems strange that Govt. should allow its representation in Calcutta, unless it has gone through

the hands of some competent censor, and the libelous parts been excised' [20]. In reply, the Secretary of the National Theatre wrote to the *Englishman* (and the letter was published on December 23) that they merely wanted to depict the picture of the rural Bengal and had no intention of vilifying the British. They went so far as to add that they held the Englishmen in high esteem. The *Madhysatha* wrote on Paush 15, 1279 that the same was declared from the stage at the end of the second performance of *Nildarpan*. Another curious complaint was made by this same journal on Magh 6, 1279: 'When Bengali plays are staged in the Bengali community of Bengal, in a playhouse that is being referred to as national, it cannot be understood why the managers are using English in the nomenclature and printing tickets, etc. Is not writing "National Theatre" in the Bengali script ludicrous? Would not writing "Jatiya Natyasala" and printing tickets in Bengali be better?' [21].

Girischandra satirized the players for taking a rash step by making the theatre public without a better house and a better stage by composing a song and putting it in the mouth of Radha Madhab Kar while playing a farce in a *yatra* performance. According to the *Viswakosh*, the song did not create any bad feeling. In fact Amritalal Basu says that they relished the song and sang it in chorus. 'Ardhendu Sekhar also said, "all our names were so cleverly put into the song that it reflected much credit on the poetic imagination of Girish"' [22].

Though the National Theatre happens to be the first public theatre of the country, the responsibility of making certain reforms was borne by another theatre that was to come up soon. This was the Bengal Theatre that was opened on August 16, 1873 by Saratchandra Ghosh, the grandson of Asutosh Deb. According to Hemendranath Das Gupta, Saratchandra got the inspiration of opening a public theatre after seeing the performance of the National Theatre at Jorasanko. He gave a concrete shape to his plans by the able co-operation of Biharilal Chattopadhyay. The Bengal Theatre was the first to have a building of its own.

The Bengal Theatre is credited with making certain reforms on the stage. Hemendranath Das Gupta writes: 'To turn the theatre into a school of art, it is necessary to introduce female artist on the stage, as male actors cannot do it for any length of time – boys from respectable / classes cannot be available and the standard is not reached even by the best boy-artists' [23]. Before the recruitment of actresses, the female roles had been invariably played by youths and less frequently by boys. It is never possible to have the same effect with men in female roles as can be achieved with actresses. Change in forms of representation required same-sex impersonation. Michael Madhusudan Dutt told Saratchandra Ghosh: 'It is very unnatural that men with signs of moustache do appear for females. If you mean theatre, you must take actresses. Introduce women and I shall write dramas for you' [24]. Therefore at the suggestion of this famous poet as well as Pandit Satyabrata Samasrami and Mr. O.C. Dutt, Saratchandra introduced actresses in his theatre. The four actresses to be recruited were Jagattarini, Syamasundari, Elokesi and Golapsunadari.

Since women from respectable families would not appear on the public stage, the actresses had to be recruited from the prostitute quarters. Though the idea appears very natural to the people of the twenty-first century, many educated people opposed the idea vehemently at the period of its introduction. Intellectuals either dissociated themselves from the theatre or held an ambivalent attitude towards it. The Bengali stage lost the association of Iswarchandra Vidyasagar after the introduction of the actresses, though he used to take much interest in it earlier. Bankimchandra Chattopadhyay was actively involved in the Chuchura presentation of Lilabati and his novels were adapted for the stage. But he had reservations about the public theatre. According

to Rimli Bhattacharya, he was not pleased with the stage version of his novels and believed that the Bengali language was not yet ready for drama. 'Some scholars have ascribed his distance from the public theatre to the death of his daughter. She is believed to have been murdered by her husband because he was having an affair with a stage actress' [25]. Sibnath Sastri also believed that educated men from respectable families ought to feel ashamed in appearing on the stage with the prostitutes or even financially supporting the theatre by buying tickets [26].

Newspapers were eloquent in their protests. While recording the success of the opening play of the Bengal Theatre, the *Hindu Patriot* of August 18, 1873 wished 'this dramatic corps had done without actresses'; the *Bharat Samskar* of August regretted that 'sons of respectable families should act with the women of the town' and the '*Amrita Bazar Patrika* while admitting that female roles are better done by actresses uttered a warning against the novel experiment' [27]. The *Madhyastha* of Bhadra 14, 1280 B.S viewed the move with strong disfavour. Even the *Englishman* could not help commenting on August 18 that they too were against the introduction of actresses'. Seeing the outcry raised when women appeared on the stage, the group of Amritalal Basu 'stuck to their original determination to employ only males' [28].

Favourable reactions also existed. Kshetranath Bhattacharyya was of the opinion that the absence of female artistes will soon be considered a defect. He writes: 'Some of the prostitutes are writing to receive education. If a few such educated women are secured, happy consequences will outweigh any mischief done' [29].

Bipinchandra Pal writes in 'The Bengalee Stage' that the objection of the general public regarding the presence of the women on the stage [...] is based upon the fact that the Bengalee [sic] actresses are recruited from a community which stands outside the pale of respectable society [...] one must respect an opposition that bases itself upon considerations with the public morality. Even the most ardent advocates of our national stage must admit that it would have been the best for all concerned if our actresses could be recruited from the respectable classes of the community [30].

However he feels that the evil influence of the boy-actresses is more than that of their female counterparts. He therefore concludes that the stage had a choice of evils and the lesser of the two evils was wisely chosen.

The women who performed on the public theatre were social outcasts. They lived under the shadow of an accursed birth, which they carried till their death. 'Lacking the identity of the patriarch that society recognized as the only identity, residential locality and single status were reason enough for the women concerned to be identified as a prostitute' [31]. Even those who did not hail from the prostitute quarters were looked upon as public women because they consented to appear on the stage. This show of disrespect was not the lot of only the actresses. Even the actors were looked down upon because they worked with the actresses, who were but the 'necessary evil' [32] of the stage.

Michael Madhusudan Dutt suggested using the talents of the prostitutes for their rehabilitation. Thus they were recruited in theatre. They found in drama a means of earning a respectable livelihood and come out of their earlier stifling environment. At least a few of the miserable creatures whose pitiful accidents of birth condemned them to a life of untold misery and degradation found a respectable means of livelihood. Besides, the requirement of their profession imposes considerable discipline upon the performers. Theatre thus facilitates 'the alleviation of human misery and elevation of human character' [33]. The introduction of actresses in the public theatre indeed proved beneficial to the society at large.

The public theatre of Bengal thus saw the light of the day after much struggle. Every stage contributed towards its development. Lebedev built a very short-lived public theatre. But he gave a clear indication of the sort of plays that would remain popular. The amateur theatres too helped in developing a taste for drama among the Bengalis. They came out in competition with their contemporaries. This ultimately led to the foundation of public theatre that has continued staging plays till date.

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The Story of Lithium ion Battery

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Abstract

High energy demand with technological development initiated large scale research in lithium ion battery in recent years. Among the three components of an electrochemical cell, cathode is the most important component. The disadvantages of present day commercial lithium ion battery have triggered the search for new cathode material. Considering its low cost and environment friendly character, the spinel cathode material has proved to be a potential candidate for the future commercial lithium ion electrochemical cell.

Introduction

The first use of battery was found to have taken place between 250BC to 640AD, when a clay jar with iron rod, surrounded by a copper cylinder, was discovered. When filled with vinegar it produces 1.1 volts DC. It is believed that the Parthians who ruled Baghdad (c. 250 BC) used batteries to electroplate silver. The Egyptians are said to have electroplated antimony onto copper over 4300 years ago.

In 1791, Galvani noticed that a circuit created with two different metals, when completed by including the leg of a dead frog, caused the leg to twitch. Alessandro Volta invented the Voltaic Pile and discovered the first practical method of generating electricity. He demonstrated (1793) that an electrical current is generated when metals and chemicals come into contact. Constructed of alternating discs of zinc and copper with pieces of cardboard soaked in brine between the metals, the Voltaic Pile produced electrical current. Alessandro Volta's voltaic pile was the first "wet cell battery" that produced a reliable, steady current of electricity.

In 1859, the French physicist Gaston Planté invented the first rechargeable battery based on the lead-acid cell. The advantage of a secondary (rechargeable) battery is the possibility of regeneration of the chemicals by charging.

Present day Scenario

The recent energy demand with the development of technology is the driving force behind the investigation to get high energy density batteries. A reliable secondary battery of high energy density is needed for a variety of new technologies. Li-ion battery was proved to be a potential candidate in this regard. Among them, transition-metal-oxide

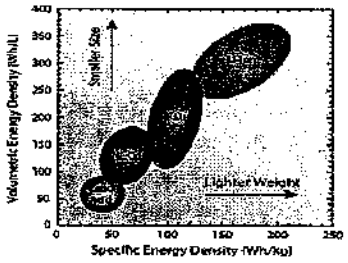

Fig.1

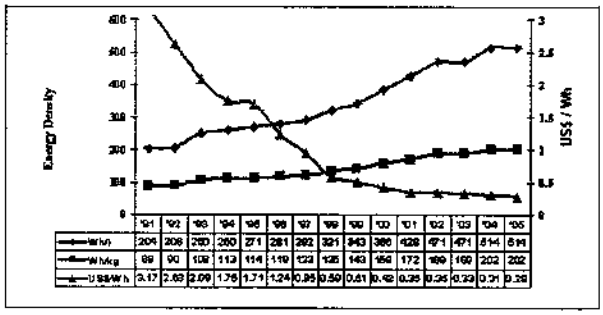

Fig.2

Lithium-ion batteries have shown good performance. The advantages of Li-ion battery are remarkable: It is rechargeable and it has higher energy density compared to others (Fig.1). In the last two decades continuous research on lithium ion battery has improved its energy-density and decreased its cost (Fig.2). It is small in size and environment-friendly. So it can be used in robotics, mobile phone, video camera etc. It holds its charge. A lithium-ion battery pack loses only about 5 percent of its charge per month, compared to 20 percent loss per month for other rechargeable batteries such as Ni-MH batteries.

A battery consists of a group of interconnected electrochemical cells. How they are connected and packaged depends upon the specific application for which they are designed. We will consider in this article only the elemental building block of a battery, viz., the electrochemical cell.

An electrochemical cell interacts with the external world through two metallic posts: one makes contact with a negative electrode (the anode) and the other a positive electrode (cathode). An electrolyte, which is essentially a salt containing lithium, separates the cathode and anode. During cell discharge electrons pass from anode to cathode through the external load and ions flow inside the cell to convert chemical energy into electrical energy. The electronic current flowing through the external circuit matches the ionic current flowing inside the electrochemical cell. Present day lithium-ion batteries use a solid reductant as anode and a solid oxidant as the cathode. On discharge, the anode supplies Li^+ ions to the electrolyte and electrons to the external circuit. The cathode is the host material into which Li^+ ions are inserted from electrolyte as guest species and charge is compensated by the flow of electrons from the external circuit into the cathode. For a secondary battery this reaction should be reversible. On charging, removal of electrons by external field releases Li^+ ions back into the electrolyte and the addition of electrons to the anode attract Li^+ ions from the electrolyte to the anode to maintain the charge neutrality and original composition. Since both the anode and cathode are the hosts for the Li^+ ion the electrochemical cell is called a "rocking chair" cell. The open circuit voltage V_{oc} of the electrochemical cell is determined by the electrochemical potential (i.e., the Fermi level) of the anode and cathode.

Present day commercial lithium-ion battery uses a lithium salt dissolved in an organic solvent as electrolyte. The higher mobility of lithium ion in the liquid electrolyte maintains a low value of internal resistance to draw power from it. Choice of cathode, on the other hand, is the most

important factor of an electrochemical cell. Since oxide materials have relatively wide band gap, so use of an oxide material results in a higher V_{oc}. Moreover, the cathode should be such that insertion/de-insertion of Li^+ ion into the material should not change its structural integrity in order to ensure its cyclability. This is the reason why $LiCoO_2$ was used as cathode material in the first commercialized lithium-ion battery. $LiCoO_2$ has a layered structure (Fig.3) [1]. In a lithium-ion battery the lithium ions are transported to and from the cathode or anode, with the transition metal, cobalt (Co), in Li_xCoO_2 getting oxidized from Co^{3+} to Co^{4+} during charging, and reduced from Co^{4+} to Co^{3+} during discharge at V_{oc}=4V.

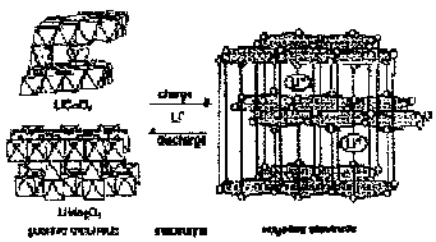

Fig.3

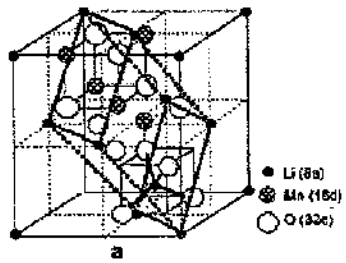

Fig.4

Although $LiCoO_2$ battery has many advantage still its cost and toxicity has led to further investigations into the rechargeable battery. Because of its easy synthesizability, high redox potential (~4V), low cost and environment-friendly-character the spinel $LiMn_2O_4$ has come out as a potential candidate to replace the layered $LiCoO_2$ as cathode material. $LiMn_2O_4$ has a spinel structure (Fig.4) which has a closed packed array of 32 oxygen atoms which form 64 tetrahedral and 32 octahedral sites in a unit cell that contains 8 units of $LiMn_2O_4$ molecule. 1/8th (=8) of the tetrahedral sites are filled by Li and ½ of the octahedral sites (=16) are filled by Mn - rest are vacant. $Li_{1-x}Mn_2O_4$ with $0 \leq x \leq 1$ gives a $V_{oc} \approx 4V$ when coupled with Li anode. During charging Mn^{3+} oxidizes to Mn^{4+} to maintain the charge neutrality of the cathode. Insertion of Li into $LiMn_2O_4$ displaces the Li from the tetrahedral 8a to the octahedral 16c sites in $Li_{1+x}[Mn_2]O_4$. The open circuit voltage drops to 3V and a Jahn-Teller deformation reduces the capacity and cyclability [2]. The loss of capacity over repeated cycling and low electronic conductivity of the cathode are the major disadvantages undermining its commercial use. The conductivity of the cathode is improved by mixing the cathode material with a conducting material, such as carbon, thus making it a composite. The problem of capacity fading (Fig.5) is dealt with by doping of Co into cathode ($Li_{1-x}Co_yMn_{2-y}O_4$); thus slightly compromising the capacity for improved cyclability (Fig.6) [3]. The improvement is attributed to the stronger bond strength of the cathode material while the decreased capacity is due the reduction of active Mn^{3+} which is replaced by Co^{3+}. Search is on to get a better cathode material for a better lithium- ion battery meeting the increasing power demand.

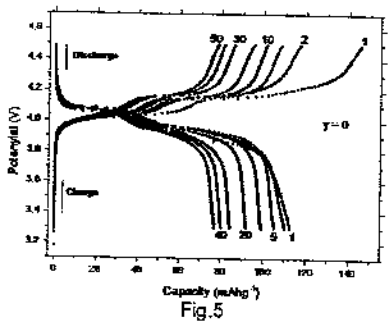

Fig.5

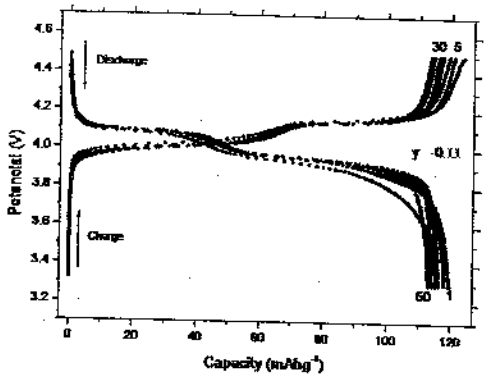

Fig.6

Conclusion: The Spinel structured manganese based cathode material has proved to be a promising candidate for lithium ion battery. The cyclabilty of the material is improved by doping with trivalent cobalt which improves the structural integrity of the material over repeated cycling. The low cost and environment-friendly material is a potential candidate to replace laminar cobalt based material presently used in commercial lithium- ion battery.

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Evolution of Map From its Origin to the Recent Period

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Abstract

A map is a drawing or a graphical representation of the whole or a part of the earth surface. Map can be regarded as a means for communication, irrespective of language or culture. The map is an indispensable part of Geography. There are evidences that map was evolved separately in different parts of world. Earlier maps were very crude in form and were very much local in nature due to the dearth of knowledge about the whole world. In some cases maps were influenced by religious dogmas and spiritual interpretations. Instead of these constraints and conservativeness, map was evolved through time. Hence in this paper an attempt has been made to show journey of map from its recorded origin to the modern time.

Key words: Map, Chart.

Introduction

A map is a drawing or a graphical representation of the whole or a part of the earth surface. It represents the location and distribution of various natural and cultural phenomena of the surface of the earth. Maps of heavenly bodies can also be made. It is the medium for conveying geographic information and can be regarded as means of communication, irrespective of language or culture. The Geographers as well as the planners, historians, economists, agriculturists, geologists and others working in the basic sciences and engineering, have found the map to be an indispensable aid.

Evolution of Map

Evidences of mapmaking suggest that the map was evolved independently in many separate parts of world. Primitive people like Eskimos, Beddouins, Polynesians, Banjaras had remarkable abilities to draw sketches of their known areas. Those maps were drawn on a piece of skin, wood, bone or terracotta. Egyptians used geometrical methods for land measurements and for establishing land ownership lines after each flood in the Nile.

Earliest direct evidence of mapping comes from the Middle East. Centuries before the Christian era, Babylonians drew maps on clay tablets. Among these the oldest sample found so far, have been dated about 2300 BC. This is the earliest proof of graphic depictions of parts of the earth. Few land drawings found in Egypt are also as old as the clay tablets. A tablet unearthed in Iraq shows the earth as the disk surrounded by water with Babylon as its centre. It is also found that

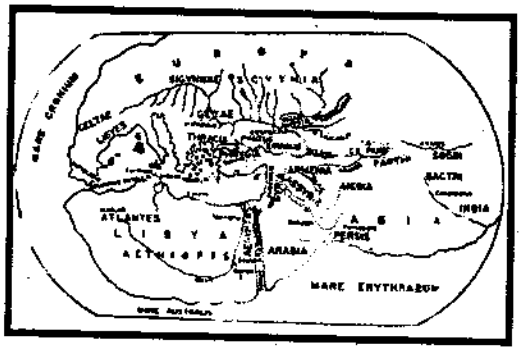

Fig -1

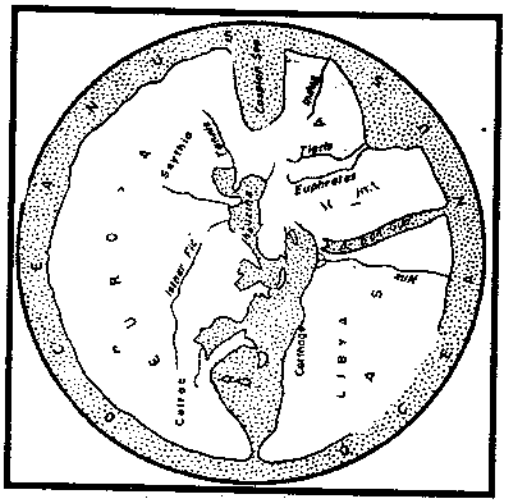

Fig -2

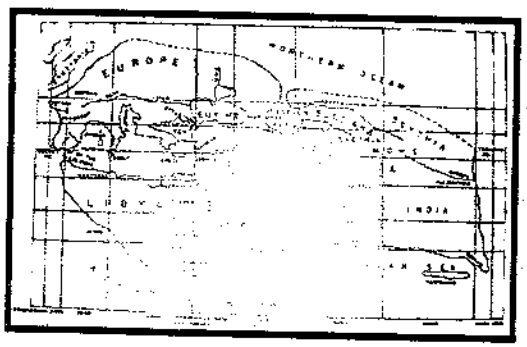

Fig -3

Babylonians and Egyptians had tried to show the form and extent of the earth as a whole. Their map making was preoccupied with more practical needs, such as the establishment of boundaries.[1]

Ancient Indians made remarkable progress in the field of map making. It appears that the whole of Eurasia and parts of Africa were frequently visited by the ancient Indians. They considered the whole world was divided into seven regions. The world as a whole was considered as round and surrounded by water. The southern waters were considered as salty and northern ones to be milky[2].

Greek maps: The ancient Greeks were famous for their quest of Geographic knowledge and skill of map making. They had an advantage of being close to Babylonians who were pioneers in the fields. Anaximander prepared the first map of the world (as known at that time) (Fig-1). Hecataeus produced the first book on geography about 500 BC. He believed that the earth as a flat disk surrounded by ocean (Fig-2). After that Herodotus improved the shape and extent of the then known regions of the world and stated that the Caspian Sea as an inland sea. But he did not represent the earth to be the circular. By the mid of 4th Century B C the theory of spherical earth was well accepted among Greek scholars, and about 350 BC Aristotle prepared six points of views to establish the spheroid shape of the earth. After that By 200 BC Eratothenes measured the circumference of the earth being 24662 miles or about 39459 km. He tackled the problem of representing the spherical earth on a plane surface by extending two parallels eastwards, one passing through Gibralter and the Caspean Sea and the other through Egypt and south India. He established the zero meridian approximately following the Nile (Fig-3). By 150 AD Ptolemy's monumental work "Geographike Hyphegeis" revealed Ptolemy's map of the world (Fig-4). He proposed co ordinate systems and a system of projections and that are still used today.

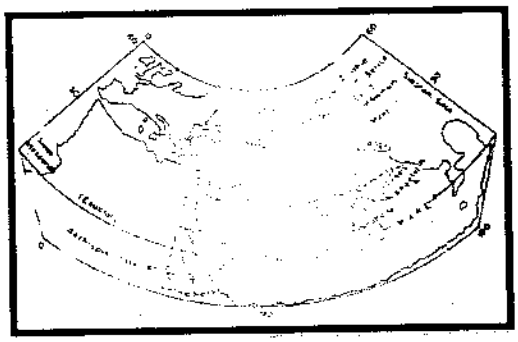

Fig -4

Roman maps: The Roman general Marcus Vispsanius Agrippa prepared world map (Fig-5) before Ptolemy's era by about 12 BC based on the survey of the then extensive system of Roman military road. This map was displayed in Rome for the information of citizens. The shape of the map was almost circular. References of many other Roman maps have been found but very few actual specimens survived in the dark ages.

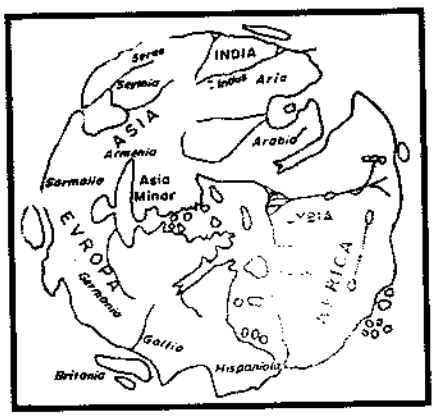

Fig- 5

Fig -6

Medieval maps: During the Medieval period, European map making were dominated by sacred doctrines and interpretation of spiritual scriptures. After 6th century AD, T-O map format was common (Fig-6). These maps were oriented with the east at the top and Jerusalem was depicted at the centre. O represented the circular world whereas the horizontal line represented Don – Nile meridian and vertical lines represented the axis of the Mediterranean.

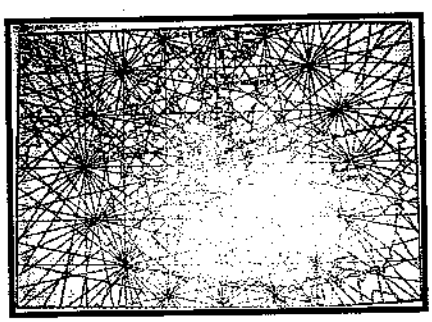

Fig -7

Towards the end of the medieval period i.e. by the end of 13th century, the Portolan sea charts were developed mainly by the Italian cartographers. Charts (Fig-7) were decorative. The coastline and the names except the names of

important harbours were usually given in black. Islands, rivers, deltas, rocks and shoals were shown in red[3]. There were very few land details.

Arabians also contributed important work in map making. One such example is Al- Adrisi's world map. He prepared a world map in AD 1154. The map provides better information on Asian areas than had been available before. The map was drawn for the Norman King Roger II of Sicily. In this map the entire portion of Eurasian continent, northern part of the African continent were shown.

Chinese map making was developed independently. The oldest known Chinese map was dated about 1137. The most of the areas had been mapped crude form before the arrival of European. The Jesuit missionaries of the 16th century found enough information to prepare an atlas and Chinese maps thereafter were influenced by the west[4].

Maps of the modern period: Geographic knowledge was slowly increased during the 15th & 16th centuries. The discoveries of unknown places gradually changed the world maps of those days. New places were added in the old ones. In 1513 Martin Wald seemuller prepared an edition with more than twenty modern maps.

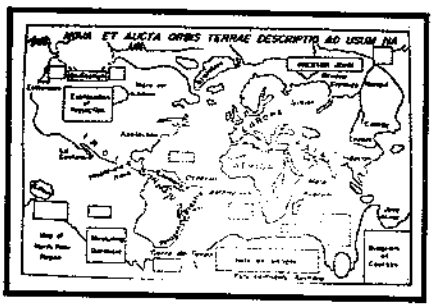

Fig -8

The most important aspect of early modern period's maps was their increasing accuracy, which was possible due to continuous explorations. Another significant characteristic was a trend toward artistic & colourful rendition. The first map printing was made from woodcuts. Latter they were engraved on copper, a process that made it possible to reproduce the much finer line. The process remained the basis of fine map reproduction until the comparatively the recent advent of photolithography[5].

In 1554, G. Mercator published the map of Europe (Fig-8) and developed the projection that bears his name. The Mercator projection paved the way to plot bearings as straight line. Mercator's world map is conspicuous not only in respect of using projection which gave correct shape & bearings but also in the sense that it gave a new conception of the world which was different from conception of Ptolemy.

Maps of the recent periods: From the 18th century onward many countries of Europe began to undertake the systematic topographic mapping of their own territory by national surveys. Topographic maps (Fig-9) were prepared by the military to assist in planning for battle and for defence purposes but gradually it became civilian in character. These were large scale maps and provided detail ground information. The accuracy of mapping was considerably increased with the improvement of surveying techniques.

Fig -9

Development of camera in the year 1920 made it possible to use aircraft for surveying. It helped the detail mapping of the visible face of the earth in short time which was never before obtained. Uses of artificial Satellites for data collection made a revolutionary impact on mapping. The very first images from space were taken from sub-orbital flights. United State launched V-2 flight in 24th October 1946, which took images in every 1.5 seconds. The first orbital satellite 'Explorer 6' was launched by the United State took photographs of Earth on 14th August 1959. Satellite data help to prepare detail maps of a larger area within a very short time. As satellite repetitively revolves around the earth in a fixed orbit, gradual changes of physical and cultural features of the earth surface can be recorded. Mapping practises get tremendous impetus after the introduction of Geographic Information System which is designed to capture, store, manipulate, analyze, manage, and present all types of geographical data. Now detail mapping of the earth in various aspects e.g. land use and land cover maps, Agriculture crop mapping, Structural mapping of the earth, Flood mapping, Drought mapping, Soil moisture mapping, Topographic and base line thematic mapping, Ocean resource mapping, Coastal area mapping With the development of technology map will be more sophisticated means of communication. etc can be done very easily with the help of Satellite data and the integrated system of GIS.

CONCLUSION:

Lastly it can be stated that the simple sketches and drawings of the ancient peoples has been converted into modern sophisticated maps. Mans thrust for knowledge, courage for explorations, power of imaginations, continuous hard working for new inventions and intense desire to upgrade the existing technology helped gradual development of map.

Map Source : Fig - 1, 2, 3, 4, 5, 7, 8 - Misra, Ramesh, Fundamentals of Cartography

Fig - 6 - http://en.wikipedia.org/wiki/t-ando.map.

Fig - 9 - http : // en.wikipedia.org/wiki/topographic-map

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Practical implementation of dance therapy in modern India

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Abstract

Dance provides an active, non-competitive form of exercise that has potential positive effects for physical and mental health. Dance therapy is based on the idea that body and mind are co-relational. The use of dance as therapy is a popular issue in the Western world, but such attempts are not so pronounced in India. In recent days some organizations and persons like Tripura Kashyap, Syed Sallauddin Pasha, Vyjayanthi Kashi, Priti Patel, Alakananda Roy, Sohini Chakraborty, Acharya Pratishtha Sharma and few others are working with physically and mentally challenged children and in special cases using dance as a mode of therapy. During this study practical implementation of dance therapy was conducted by the author among the poor, street children and slum girls and boys, special children having problem of autism and Down syndrome, aged person and for the management of drug addiction among the in-house boys.

Key-words: Dance therapy, physical health, mental health.

I. Introduction

Dance increases total body movement, which helps to improve circulatory, respiratory, skeletal, and muscular systems [1]. It provides an active, non-competitive form of exercise that has potential positive effects for physical health as well as mental and emotional wellbeing [2]. It has the potential to motivate and excite people and it can be a way of engaging people in physical activity [3]. Scientific research proved that in males and females dance practice and physical activity can have a positive effect on physical fitness and it also increases lung function, lung capacity, flexibility and aerobic capacity [4]. As a physical activity, it is believed that dance can make a significant contribution to the healthy-living agenda. Dance therapy as exercise increases the neurotransmitters called endorphins which increase a state of well-being [5]. Now-a-days in many hospitals and medical settings it has been used as a form of therapy not only for mental health, but also for physical health as well.

A great deal of research is in progress in different parts of the world regarding this new approach, but such types of theoretical background and practical implementation are limited in our country

and thus necessitate in-depth research on the topic. Moreover health related discussion, and therapeutic implementation of dance forms particularly in Indian context has never been discussed. Thus in this study it is a unique approach to contribution. In the present study different interviews are conducted with professionals and therapists of various fields, and also with the patients and clients of dance therapy to know the actual scenario in Indian perspective. Besides during this study therapeutic workshops are also conducted with children with captive life, special children, poor, street, slum children, and aged person.

II. Significance of Dance Therapy

Dance therapy is defined as the specialized, professional and psychotherapeutic use of movement and dance for emotional, cognitive, social, behavioral and physical conditions. Dance therapy is based on the idea that body and mind are co-relational, that the physical state of the body can affect the emotional and mental wellbeing both positively and negatively [6]. That means if dance therapy is used correctly, it will help to improve a person's emotional and mental state [7].

During the past few decades, the practice of dance therapy either singly or in combination along with some physical parameters has been advocated to give good results not only in healthy volunteer but also in the management of various symptoms of many diseases with special reference to psychosomatic components. The therapeutic approach of dance can be applicable in different diseases like irritable bowel syndrome, coronary atherosclerosis, hypertension, asthma, cardiac disease, anaemia, arthritis, gynaecological problems, muscular cramps, diabetes, stress etc [6]. In western world dance has been used for long time as therapy for patients, whether alternate or complementary. The use of dance as therapy is a popular issue in the Western world. But such attempts are not so pronounced in India.

III. Indian Perspective

The relationship of health with various types of dances is of great interest now-a-days. Indeed, the approach of therapy is maintained in India knowingly or unknowingly from a very long time. In Ayurveda, over five thousand years old scientific technique, held dance as a power of healing (therapy) and inner awareness (psychology). Indian philosophy also supports the facts of Sangeet (song, dance and music) for benefit of human health physically as well as mentally [8]. In modern India such types of works are in a very nascent stage. Now-a-days some organizations and persons are being involved to do some work for betterment of life by dance therapy. The examples of such hearty wishes were done by Shristi Institute of Dance Therapy, Bangalore; Asian pacific therapeutic theatre; Bhoomika, Delhi; Nrityanjali Dance Therapy Centre, Indore; Sanved, West Bengal; Indian Spinal Injuries Centre, Delhi etc.

The first of its kind in India, Rabindra Bharati University, West Bengal has introduced dance therapy as a Post-Graduate Course in University level (UGC approaved). The students and experts of their field are involved to use dance therapy, not only in healthy volunteers, but also in the management of various symptoms of many diseases with special reference to psychosomatic components.

In recent days Tripura Kashyap, Syed Sallauddin Pasha, Vyjayanthi Kashi, Priti Patel, Alakananda Roy, Sohini Chakraborty, Acharya Pratishtha Sharma and few others are working with physically and mentally challenged children and in special cases using dance as a mode of therapy. During the past three to four decades, the practice of dance therapy using different dance forms, either singly or in combination along with some physical parameters, has been advocated to give good results.

During this study practical implementation of dance therapy was conducted by the author among the members of Pratyush, Barasat and Hridaypur Nabasopan, in therapeutic mode to develop self esteem and self identity, among the poor, street children and slum girls and boys. The workshop covered the theoretical and practical aspects of dance therapy, relation of dance and public health, dance related injuries, dance science and education, therapeutic dance movements. Similar therapeutic sessions were also conducted with the inmates of Kishalaya Home, Barasat for the management of drug addiction among the in-house boys and also to develop education and togetherness through dance. Dance therapy workshops were also conducted with the special children of Bikasayan, Kolkata having problem of autism and Down syndrome, and with the aged person of Dinantey, Madhyamgram. In all the cases the therapeutic sessions resulted into development of self-efficacy, self-confidence, psychological busting and improved physical and mental fitness.

IV. Practical Use of Dance Therapy in India

In India today the dancers and dance therapists are conscious about this matter and in therapeutic sessions they actually improvise different dance movements according to the need. Indian Spinal Injuries Centre (ISIC), a medical care centre, in Delhi, has introduced dance therapy as a healing tool for its patients.

Among one of India's first trained dance therapists Tripura Kashyap has many important works in this field. She is trained in dance movement therapy at Hancock Center in Wisconsin, U.S.A. She worked as a dance therapist at many rehabilitation and treatment centers, half-way home, hospitals, special schools and de-addiction centers in Bangalore and other cities. She has conducted several training programs on dance therapy for special educators, teachers and therapists all over India.

Sohini Chakraborty and her team at Kolkata Sanved have been trying to bring new meaning to the lives of victims of rape, violence, slavery and trafficking, as well as people facing mental challenges or living with diseases such as AIDS. They use dance and movement to build positive attitudes and body image among participants, to discover, recognize and develop one's individual potential, to achieve psycho-social rehabilitation, counselling, empowerment, healing and a mode of expression for victims. Kolkata Sanved also helps adolescent and child victims of sexual exploitation to assert their rights as individuals and to find a place in the mainstream.

Asian pacific therapeutic theatre works with physically and mentally challenged, helps children to transcend their limitations and celebrate life meant for creating awareness about the potential and ability of the special children. Children with cerebral palsy, mental retardation, speech and learning

disabilities performed "Ramayana on wheels" by moving around in wheel chairs, tricycles and with the help of crutches, walkers and sticks with confidence and total involvement. Syed Sallauddin Pasha, a pioneer Indian dance therapist, is the founder and Director of Asian pacific therapeutic theatre. He is working as a consultant dance therapist in many special need institutions, universities, pedagogic schools in India and abroad.

At the Indian Institute of Cerebral Palsy from 1990, Priti Patel works with cerebral palsied children who are born without the natural rhythm mainstream people. The children in spite of their severe physical disability have responded wonderfully to dance and movement. The change occurs when the patient allows himself to experience and feel the action in his body. She had begun with the aim of treating them towards health and well being.

Vyjayanthi Kashi is another well-known dance therapist. She has worked with physically and mentally challenged children in India and abroad and is constantly thriving to make them come out of their seclusion and isolation. She has made a difference in the lives of many HIV positive patients, depressed women, the challenged using the strength and sanctity of Indian dance as a healing therapy and extends solace to the world.

For the last few years Alakananda Ray are engaged in training programme of martial and folk dance forms with the inmates of Alipur Central Correctional Home. It results into psychological change among them and freedom of inner mind from the life of a prisoner. For the first time in the history of India, the inmates have been regularly allowed to come out of their confinement to perform a dance drama "Balmiki Pratibha". Today, the ones who had stopped speaking are talking, because dance has motivated them to lead normal lives within the prison bars.

V. Conclusion

As dance is mainly dealing with physical movements, it has an immense role in health science as well as from the therapeutic point of view. These types of exercise need much confidence, body control, regular practice and proper movements. The breathing time and muscular control are very important in these regards. Dance therapy can prevent a person from some diseases and may also help to avoid unwanted problems. But for a wide application this topic should be highlighted by Government Organizations, NGOs and public sectors in forms of seminars, symposium, workshops, news, etc. to attract general people's interest. But no concrete reference is established till date and no hand-to-hand work is going on in this direction. The proper willing and interest on the said topic are required to establish this very important science to nourish from its now nascent stage to mature one. The present study is an attempt to this direction.

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The anthropogenic impact on avian migration and floral resources: a case study of Santragachi wetland, Howrah, West Bengal

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Abstract

This paper mainly deals with the environmental degradation of Santragachi wetland in Howrah district of West Bengal. This wet land is dependent on constant, recurrent or shallow inundation at or near the surface. It is rich in terms of physical, chemical and biological features and plays an inevitable part in controlling and maintaining the strength of ecosystem and the avian migration. In Howrah as well as in West Bengal or in India the Santragachi wetland is considered as the key stone for maintaining the avian migration and thereby controlling the ecosystem. Presently, due to anthropogenic activities the numbers of migratory birds are reducing every year. Therefore, an attempt has been made in this paper to show the various anthropological activities, their impacts and effects on this wetland.

Keywords: Wetland, migratory bird, anthropogenic activity, urbanization.

Introduction

Wetlands form the transitional zone between land and water, where saturation with water is the dominant factor determining the nature of soil development and the types of plant and animal communities living in and on it [1]. They are usually formed in the depressions by flooding or by groundwater seeps. Type of wetland is determined primarily by local hydrology and the unique pattern of water flow through an area. In general, there are two broad categories of wetlands - coastal and inland. Enhanced appreciation of wetlands in the recent past has led to the signing of many international agreements for protecting them, among which the Ramsar convention is the most important.

On the eve of notification of Indian Wetland Rule 2010, Prime Minister Dr. Manmahon Singh addressed to the nation on 2nd December, 2010, "Wetland conservation has been accorded a high priority in India. Since 1987, the National Wetlands Conservation Programme of India has been financially supporting wetland conservation activities all over India. Under the Programme 115 wetlands have been identified for conservation and management till date. India is also a party to the Ramsar Convention under which 25 wetlands from India are included in the list of wetlands of international importance."

Santragachi is one of the most important wetlands of India which is being under the above-mentioned programme from the very beginning. In this area wet lands has made the surrealistic peace, but unfortunately that has been under continuous threat of damage due to human activities.

The study area:

Santragachi wetland (jheel) is located in Howrah district, West Bengal, India. It extends from 22°052 N to 22°342 N latitude and 88°002 E to 88°262 E longitude and is located in ward no-45 under Howrah Municipal Corporation. The wetland covers a large area of 13,75000 sq. feet or 32 acres and it is in close proximity to south eastern railway and Kona expressway which connects Vidyasagar Setu (2nd Hooghly bridge). The surrounding areas are Ramrajatala, Jagachcha, Buxarah which are dominated by slum dwellers.

It is interesting to note that the selected study area is a large wetland which attracts large number of migratory birds in winter every year and this is one of the famous wetland in Howrah district and also in West Bengal.

Objectives of the present study:

The present investigation has been conducted with the following objectives:

i. To identify the environmental set up of the study area.

ii. To identify the causes of environmental degradation of wetland area.

iii. To analyze the pattern of seasonal variation of birds migration.

iv. To study the anthropogenic activities responsible for less movement of migratory birds.

Methodology:

The study has been conducted during the year 2009-11 and the primary data has been obtained through household survey with scheduled questionnaire. Data has also been collected from secondary sources like Census of India 2001, District Statistical Handbook 2006, District Gazetteer, Howrah etc. The data collected from both the primary and secondary sources were analyzed by cartographic techniques.

Physical aspect of the study area:

The study area falls in the 'Lower Gangetic Plains' bio-geographic area and is part of the alluvial plains of West Bengal with an elevation of 5 meters from sea level. The study area has a fairly stable, warm and humid climate throughout the year. The temperature in winter goes down to about 8°C and it rises to about 35°C in summer. The mean annual rainfall varies from 150 to 200 cm.

Floral and faunal assemblages of the study area:

The study area, Santragachi wetland is famous for marsh. It is home of several kinds of migratory birds, few reptiles, amphibians, butterflies, insects and even some rare mammals like fishing Cats and large Indian Civets which are less commonly available in other parts of Bengal. Several birds

often visit this area every year especially during winter from the northern and south eastern parts of the world. Notable among them are Northern Pintail, Northern Shoveler, Gadwall, Saras crane from North America and Australia and other local migratory birds such as, Comb Duck, Pygmy Goose, are spotted here during winter [2]. However, it might be noted that Lesser Whistling Duck is the most dominant species available. The wetland is the home of several fish's viz. *Clarius batrachus*, *Heteropneustus fossilis*, *Anabas testudineus*, *Rohu* sp., *Catla catla* etc. Several types of herbs shrubs and trees surround the Santragachi wetland, predominantly *Demodium trifolium, Boerhavia diffusa, Oxalis corniculata, Polygonum plebium* , *Colocasia esculenta, Amaranthus spinosus, Calotropis giganta, Cleodendrum gigantean, Opuntia dieallenii, Delonix regia* and *Ficus religiosa.* Aquatic vegetation includes *Eichhornia crassipes, Pistia* sp., water lily, water hyacinth etc [3]. The floral and faunal diversity of the Santragachi wetland are shown in fig. 1.

Quality of water of the study area:

The wetland is situated within a locality. Industrial wastes and water from household are discharged into the wetland which degrades the physiochemical properties of water. Around 75% people residing in the area agreed with the view that waste water passes through underground drains and flows into the wetland.

General Assessment:

The wetland area is adversely affected by several anthropogenic activities. Dumping of solid wastes and plastics in and around the bank of the wetland by the residents within the vicinity of the wetland area also adversely affects the ecological communities. The anthropogenic activities in the wetland are shown in fig. 2.

Anthropogenic Impact on Santragachi Wetland

Impact of roads and railways:

The area is situated near to Kona Expressway which connects South Bengal to Howrah district. Both heavy vehicles and light weight vehicles ply everyday through this way. The inflows and outflows of vehicles are shown in fig. 3. From the figure it is evident that this area is affected by the dangerous impact of air pollution as the number of vehicles increases. Toxic gases emitted from vehicles also pollute the local environment. Santragachi railway station is one of the busiest and important railway junctions of South-Eastern Railway of India. This wetland is in proximity to railway junction and station. From the field verification data it is evident that a large number of trains pass everyday which also has a profound impact of noise pollution.

Impact of settlement and industries:

The study area possesses a large settlement having around 1, 000, 00 population. In recent past there was a spurt in population growth rate thereby increasing urbanization of this area within a short time span. Deforestation is also another reason for the increasing environmental degradation of the area. About 66% of the population agreed to the view that construction of buildings is another reason for the degradation of the biotic resources.

Figure 4 depicts the rapid industrial growth of this area. Since this area is well connected by road and railways, rapid industrialization has occurred and the industrial growth is shown in the graph.

The area has several small and large scale industries and few manufacturing units. Emission of several toxic gases from these industries during night and also improper disposal of industrial effluents in the surrounding water bodies are the major source of air and water pollution respectively.

Increase of population:

The rapid growth rate of population shown in figure 5 is another reason of degradation of biotic resources in Santragachi area.

Figure 6 is explaining the perception of local people how different pollutions are affecting on the environmental condition of the jheel.

Impact on migratory birds:

The area at present plays host of about 85 migrant bird species during winter. Of these some are extremely rare and may not be observed every year. About 50 species of birds are regularly recorded by one or more observers.

The figure 7 is showing the path of migratory birds and the number of birds coming to this area in the year 2009. From the figure it can be stated that in the month of December, the number of the birds of various species are highest whereas in the month of November the number is lowest. The numbers of migratory birds over the year 2005 to 2011 are shown in a spiral diagram in figure 8. On routine investigation of the study, in the adjoining areas death of some birds has also been recorded, so it can be concluded that these birds might be under continuous biotic hazard. On the contrary it can be said that as the water quality degrades it poses a health hazard to most of these migrating birds thereby increase in death rates. From the field survey 79% people said that about 2-6 birds die every month due to emission of toxic gases, bird flu, and also due to poor water quality of the wetland.

Impact on water quality:

From the field investigation and questionnaire survey it is clear that people of the area are aware of the harmful effect of wetland pollution still many of them dumps solid wastes, plastic bottles, packets and other non biodegradable substances along the bank and roadside areas of the wetland. Since this area is home to migratory birds, during winter season tourists often visit this place and their careless activities such as open dumping, litter, and disposal of plastics in the area also degrades the quality of the wetland.

Present condition:

Nowadays local people are much aware and they have been trying to spread the message to protect the jheel from the harmful effect of pollution to others including tourists who visit the place from different parts of India. Different forums are also being organized to save the wetland by propaganda through newspaper and media.

Assessment of the present condition:

However the wetland is now under jurisdiction of South-Eastern Railway and Forest Department of West Bengal and the area has been declared as 'Plastic free Zone' of Howrah city. Several activities like bathing of human and animals, washing clothes, cleaning of automobiles etc. are strictly prohibited for the sake of protection of the large wetland. Another Non Government organization 'Prakiti Samsad' and 'Birds lovers association of Bengal' have taken several steps to

conserve this wetland. Further, according to the Department of forest of West Bengal there has been increase in number of migratory birds during last 5 years (nearly 12,000 birds visited here recently). The wetland also faces severe water crisis during summer season which affects the aquatic biota to a considerable extent.

Main findings of the study:

The area is rich in terms of migratory birds is the pollution and plastic free zone of Howrah city. But presently Santragachi Jheel area is characterized by encroachment of land for constructional works, like multistoried building and also urbanization which requires cutting and felling of trees. There is no doubt that these kinds of activities give rise to ecological disturbances in the jheel. Thus, for the society, social awareness should be enhanced both from the part of government as well as from common people. Pollution, which has been a direct effect, resulting from the problems like deforestation, emission of toxic gases from vehicles and insignificant amount from industries is the main reason behind the degradation of environment of the area.

Policy intervention for development:

Wetlands are not delineated under any specific administrative jurisdiction. The primary responsibility for the management of these ecosystems is in the hands of the Ministry of Environment and Forests. Although some wetlands are protected after the formulation of the Wildlife Protection Act, the others are in grave danger of extinction. Effective coordination between the different ministries viz. energy, industry, fisheries, agriculture, transport and water resources, is essential for the protection of these ecosystems.

India is a signatory to the Ramsar Convention on Wetlands and the Convention of Biological Diversity [4]. Apart from government regulation, development of better monitoring methods is needed to increase the knowledge of the physical and biological characteristics of each wetland resources, wetland dynamics and their controlling processes. India being one of the mega diverse Nations of the world should strive to conserve the ecological character of these ecosystems along with the biodiversity of the flora and fauna associated with these wetland ecosystems. Further, national wetland strategy should encompass (i) Conservation and collaborative management, (ii) Prevention of loss and restoration (iii) Sustainable management. These include: Protection, planning, managing and monitoring comprehensive inventory, legislation, coordinated and integrated approach and research.

Concluding remarks:

Through this case study on present environmental status of Santragachi wetland (jheel), one major issue has come out that the local environment cannot be ignored. Another important issue of this is that lack of authentic data appears to be a real challenge for the researchers. In many cases, the researchers have to depend on the information level domain instead of data level domain. It should be noted that the good accuracy not only depends on technical perfection but also on the number of data sources. Although the present result is satisfactory, some further edition of data from different sources for geo-processing is needed for a future version of advancement.

Nevertheless, there is a stern hope after the implementation of Indian Wetland Rule 2010 that Santragachi wetland will come out from the grave danger of population explosion and will continuously enrich our aesthetic pleasures.

Figures

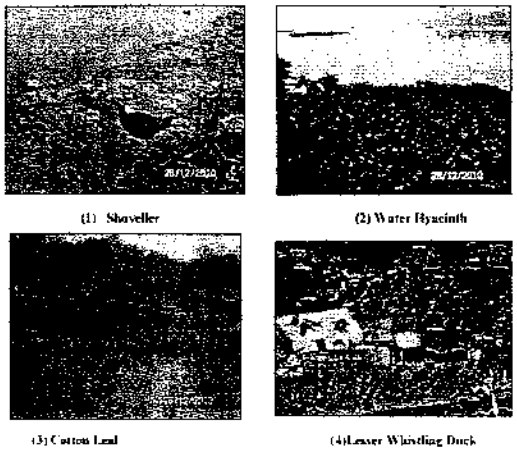


Fig. 1: The floral and faunal assemblages

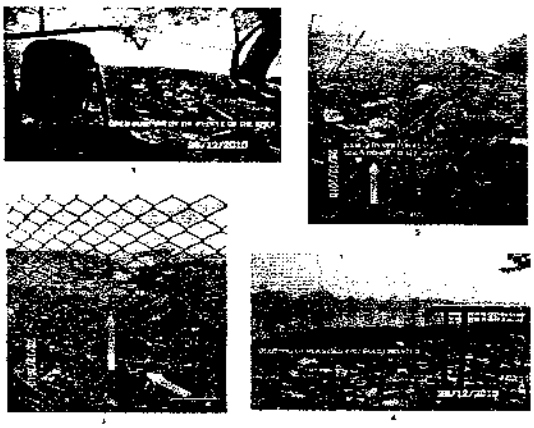


Fig. 2: Environmental Impact on Santragachi Jheel

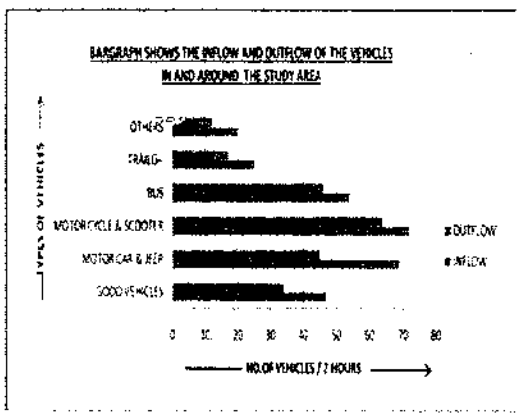


Fig .3: The inflow and outflow of vehicles through Kona Expressway

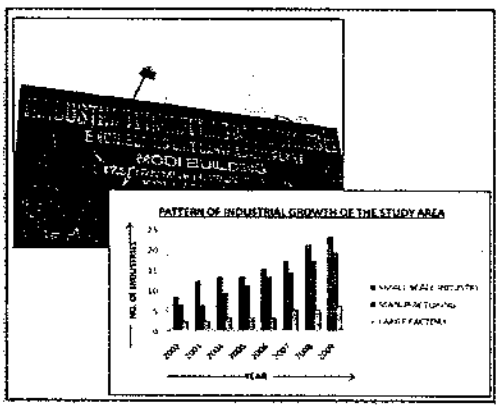
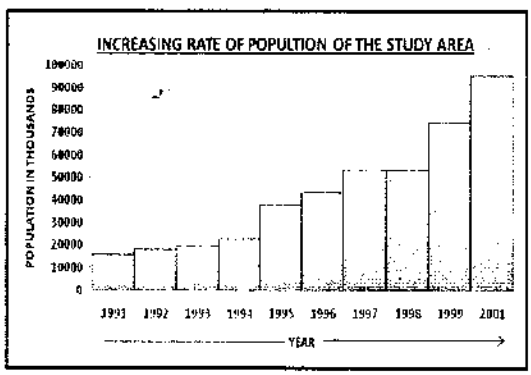


Fig.4: Industrial growth of the study area from 2002 to 2009



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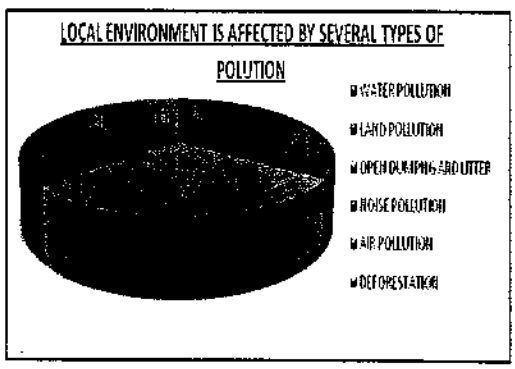

Fig. 6: People's Perception showing growth of urbanization can affect

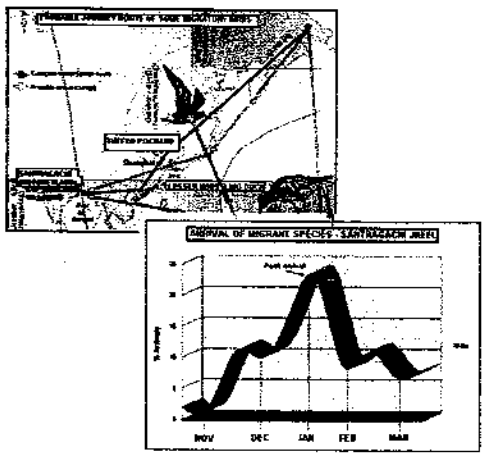

Fig. 7: Graphical representation showing the path of migration

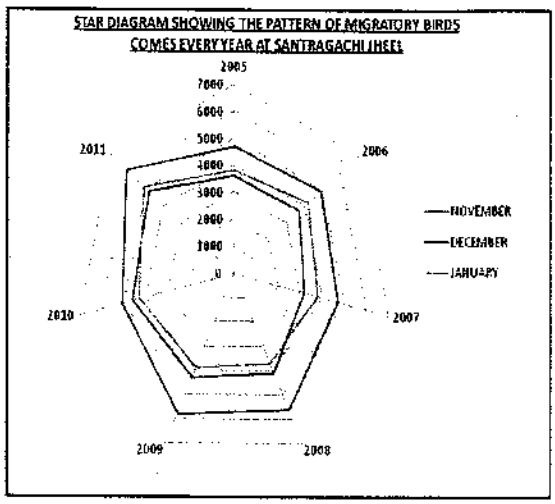

Fig.8: Star diagram showing visiting migratory birds over the years from 2005 to 2011.

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Chaos: Indeterminable Future Ahead of a Deterministic Past

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Abstract:

With the advent of computers and availability of powerful numerical techniques, much progress has been made in chaos theory, not only theoretically but also experimentally. The chaos theory, a theory of a deterministic system with inherent complexity and unpredictable future, has been explained concisely in this paper. The experimental aspect is discussed. The present status and activities in this field are also described.

Keywords: Chaos, nonlinearity, jerk oscillation, Chua's circuit, controlled chaos

Introduction:

Chaos, as the name suggests, stands for "apparently" intractable behaviour of a simple and well-behaved system. The activities of a chaotic system may seem to be erratic, random and "noise" like but after recent progress in this field in the last two decades it is now well understood that the chaotic behaviour arises in very simple systems which are almost free of noise. Moreover these systems are completely deterministic in nature; that means precise knowledge of the conditions of a system at one time is sufficient to predict exactly the future activities of the system. Nestled between the conflicting ideas of randomness and determinism, "chaos" emphasizes the fact that a deterministic system can exhibit aperiodic behaviour if initial conditions are varied a little, thereby rendering long term prediction impossible. In other words, the deterministic nature makes the system unpredictable! This sensitivity to initial conditions is popularly known as the "butterfly effect", as described in the title of a paper of Edward Lorenz in 1972 to the American Association for the Advancement of Science in Washington, D.C. entitled *Predictability: Does the Flap of a Butterfly's Wings in Brazil set off a Tornado in Texas?* The flapping of wings represents a small change in the initial condition of the system, which causes a chain of events leading to large-scale alteration. Had the butterfly not flapped its wings, the trajectory of the system might have been vastly different [1,2].

Chaos is exhibited only by nonlinear dynamical systems. The nonlinear system obeys nonlinear time evolution equation. One of the methods of showing the time evolution of a system is by differential equations. As an example, the differential equation of motion of a point mass subjected to the force exerted by an ideal spring is given by,

$$\frac{d^2x}{dt^2} = -\frac{k}{m}x \qquad \text{(i)}$$

This equation is linear and has second time-derivative of x. Another equation of motion, given by

$$\frac{d^2x}{dt^2} = bx^2 \qquad \text{(ii)}$$

is nonlinear. If the parameter in a linear equation (e.g. k, the spring constant in equation (i) is changed, the qualitative nature of the system remains the same. But in a nonlinear system, the change in a parameter may lead to sudden change in qualitative as well as quantitative nature of the system.

Based on the framework of nonlinear dynamical systems, this paper describes some simple chaotic equations of motion and also a few experimental studies of chaos.

Some examples of chaotic behaviour:

A very basic chaotic system is represented by the logistic equation that is used for a simple-minded biological population growth model:

$$x_{n+1} = Ax_n(1-x_n) = f(x_n) \qquad \text{(iii)}$$

where x_n is the population in the n^{th} generation. Its behaviour is very easy to study. Starting from some value $x=x_0$, one can get $x_1=f(x_0)$ then $x_2 = f(x_1)$ and so on. This is known as a sequence of iterations and the sequence of x values generated in this manner is known as a trajectory or orbit. At x=0 and $(1-1/A)$, $f(x_n)= x_n$ and those points are known as fixed points of the iterated map.

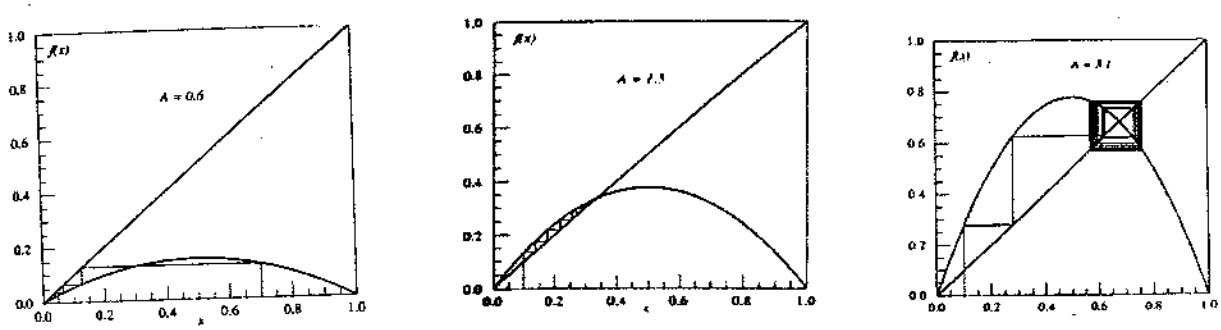

Fig.1 Left: Graphical representation of the iteration in equation (iii) starting from x_0=0.7 with A=0.6, Middle: Graph of the equation (iii) for x_0=0.1. The trajectory moves away from x=0 and is attracted towards x=1/3. So here x=1/3 is attractor and x=0 is repeller, Right:Period 2 bifurcation is shown for A=3.1 and x_0=0.1.

Since all trajectories in the domain $0<x<1$ (for $A<1$ and starting values between 0 and 1) approach $x=0$ point, that point is known as attractor. For $A>1$, the same point behaves as repeller, repelling the trajectories. For $A=3.1$ and $x_0=0.25$ one get $x=0.250$, 0.558, 0.755, 0.574, 0.758, 0.569 and so on for $n=0$, 1, 2, 3, 4, 5, 6, ... Thus the values are seen to be oscillating between 0.764 and 0.558 and similar values are repeated after every two iterations. This is called period-2 or period-doubling

bifurcation (Fig. 1 Right and Fig. 2) at $A=3.1$. At $A=3.45$, period-4 bifurcation occurs. Period-8, 16 etc bifurcations occur with increase in A. For $A>3.57$, the values never seem to repeat and it signals the onset

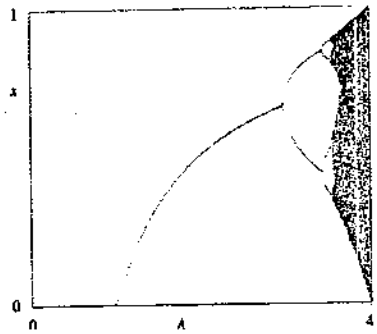

Fig. 2 The bifurcation diagram for logistic equation

of chaos. Two very nearby trajectories, after a few iterations, diverge from each other and it indicates the true chaotic behaviour of the system.

Lorenz model:

The Lorenz model [2] on chaos is based on a simplified system of equations describing the two dimensional flow of a fluid of uniform depth H. The equations are:

$$\begin{aligned} \dot{X} &= \sigma(Y - X) \\ \dot{Y} &= -XZ + rX - Y \\ \dot{Z} &= XY - bZ \end{aligned} \qquad \text{(iv)}$$

By setting all the time-derivatives in (iv) to zero and solving one can get the fixed points. The nature and stability of the fixed points can be studied by determining their eigenvalues and eigenvectors. For $\sigma=10$, $b=8/3$ and $r=28$, the trajectory's projection on the X-Z plane is shown in Fig. 3 (Right) that looks like a butterfly. The three dimensional trajectory in XYZ space is shown in Fig. 3 (Left) which shows chaotic features and attractors. The system behaves in a chaotic

manner and the chaotic nature can be proved by showing the divergence of nearby trajectories for some range of parameter values.

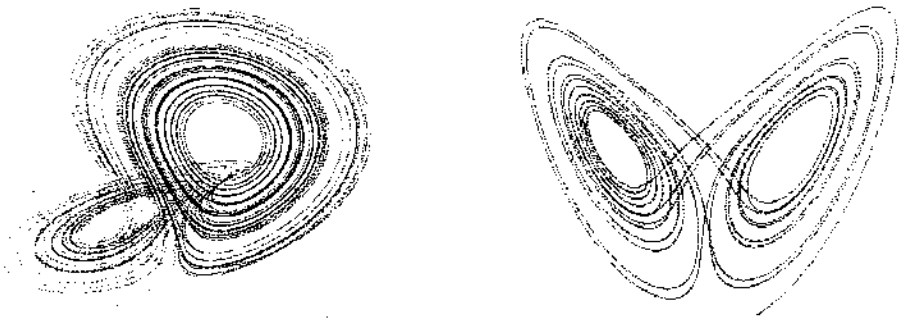

Fig. 3 Left: 3d trajectory of Lorenz attractor. Right: Projection on the X-Z plane.

The system is still deterministic, because by knowing the initial conditions of the system exactly, we can correctly predict the future behavior of the system using the time-evolution equations. If we make the Rossler model smallest change in those initial conditions, however, the trajectory quickly follows a completely different path. Since there is always some imprecision in specifying the initial conditions in any real experiment or real numerical calculation, we see that the actual future behavior is in fact unpredictable for a chaotic system.

Another simple chaotic system, known as the **Rossler model,** is defined in terms of three non-linear equations:

$$\begin{aligned} \dot{x} &= -y - z \\ \dot{y} &= x + ay \\ \dot{z} &= b + z(x - c) \end{aligned} \qquad \text{(v)}$$

For a=0.2, b=0.2 and c=5.7, Rossler [3] obtained a chaotic attractor.

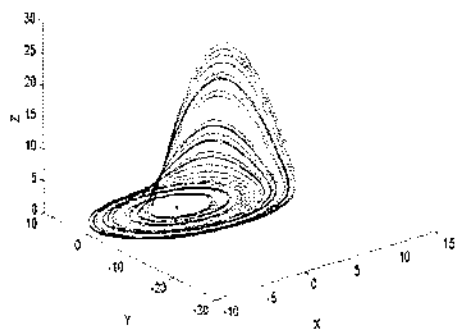

Fig.4 Rossler attractor with a=0.2, b=0.2 and c=5.7

Differential equations and chaos:

Chaos may arise in discrete time systems like in equation (iii) with only a single variable, whereas for continuous time systems it requires at least three variables. Can Newton's second law of motion in one dimension lead to chaotic behaviour? Since the two equations concerned with this are $dx/dt=v$ and $dv/dt=F/m$, which contain only two variables (x and v), chaos cannot follow.

Another simple equation viz. a sinusoidally driven mass connected to a linear as well as a nonlinear spring with restoring force ($-kx^3$), damping ($-b\dot{x}$) and obeying the equation:

$$\ddot{x} + b\dot{x} + \omega_0^2 x + kx^3 = A\sin\omega t \qquad \text{(vi)}$$

can show chaos for b=0.05 and A=7.5. This is called Duffing's equation. This equation can be converted into a set of autonomous equations in three variables (including t).

In a paper Sprott [4] demonstrated the chaotic properties of at least 18 autonomous differential equations (in variables x, y and z) which are simpler than the equations of Rossler and Lorenz. This has opened a vast field in chaotic dynamics: To analyze those equations numerically and experimentally, as well as to produce more new simpler equations leading to chaos.

Recently, Sprott has shown [5] that the simplest one dimensional equation in a single variable that leads to chaos is of the form $\dddot{x} = j(x, \dot{x}, \ddot{x})$, where j is known as the Jerk function (time derivative of acceleration). The starting equations for case A of Sprott are given by:

$$\dot{x} = y, \dot{y} = -x + yz, \dot{z} = 1 - y^2 \qquad \text{(vii)}$$

and can be modified into the form $\dddot{x} = -\dot{x}^3 + \ddot{x}\dfrac{(x+\ddot{x})}{\dot{x}}$ which is one of the simplest forms of chaotic equations. There are numerical search procedures to find simpler equations as explained in Ref. [5].

Experimental procedure:

It is possible to study chaos in a lab by constructing simple electronics circuits. The introduction of this idea has probably come after the realization of such an electronic circuit by Chua [6] in 1983. The basic Chua circuit consists of only one nonlinear element. The parallel combination of c_2 and L provides a lossless resonant circuit. The conductance G provides a coupling between this and the active nonlinear resistor R and c_1. R as an active resistor behaves as a power source. The dynamics is given by:

$$c_1 \frac{dv_{c1}}{dt} = G(v_{c2} - v_{c1}) - g(v_{c1})$$
$$c_2 \frac{dv_{c2}}{dt} = G(v_{c1} - v_{c2}) + i_L \qquad \text{(viii)}$$
$$L \frac{di_L}{dt} = -v_{c2}$$

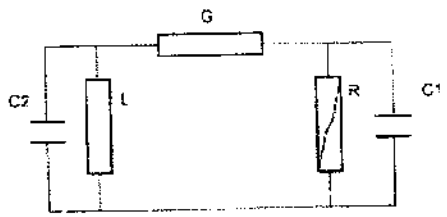

Fig. 5:Schematic representation of Chua's circuit

The only non-trivial circuit element to implement is the nonlinear resistor R (g = 1/R). There are several techniques that can be used in practical circuits, like creation of circuit using a single op-amp, two diodes and resistors or two bipolar transistors, two diodes and resistors or two op-amps and six resistors. One of the simpler circuits is given below (Fig. 6):

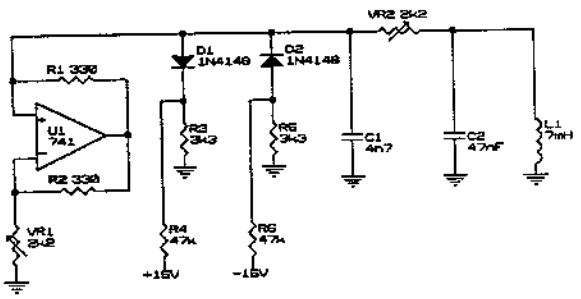

Fig. 6: Practical realization of Chua's circuit

The voltage v_{c1} and v_{c2} are fed to the X and Y channels of an oscilloscope operated in the X-Y mode. The different attractors can be seen in oscilloscope after circuit implementation, by varying L or the voltages across c_1 and c_2.

What's new in chaos:

Three decades have already gone by after the discovery of deterministic chaos. Researchers are trying to find more and more simple equations having chaotic dynamics. Experimentalists are engaged in realizing practical implementations of the 18 sets of equations shown by Sprott [4,5]. With the implementation of jerk circuits, people are trying to construct still simpler systems.

According to the Poincare-Bendixson theorem [5], an integer order of chaotic nonlinear system must have a minimum order of 3 for chaos to appear. To have a smaller order chaotic circuit,

fractional order nonlinear systems are being investigated with an aim to get simpler chaotic equations.

Construction of biological population models is important for the purpose of understanding animal population growth [7]. Chaos theory can effectively be used to find out under which conditions animal growth would be uncontrollable.

The possibility of self-synchronization of chaotic circuits has made researchers hopeful about using chaos in cryptography. Though growing interest could be seen in the recent past, not much progress has been made in this sector yet.

There have been some recent developments in the field of controlled chaos. Controlled chaos means to stabilize the chaotic trajectory by using external perturbations [8]. The dynamics of many deadly human diseases have been investigated and their dynamics have been mathematically expressed in terms of differential equations and chaoticity has been observed. If proper perturbations are set up, this chaos can be controlled thereby reducing the harmful effects of the diseases.

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Isolation and characterization of two multidrug resistant bacteria from drinking water samples at Barasat Govt. College

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Abstract: Drinking water has been regarded as the major source of water-borne diseases. To analyze the water quality of Barasat Govt. College, drinking water samples from six sources of the College were cultured in presence of one or more commonly used antibiotics. The MPN index per 100ml of one tap water sample was 210 though the MPN index of water from Water Purifier was nil. The present study revealed the presence of two different multidrug resistant bacteria in tap water samples. SP-1 was resistant against Norfloxacin and Cefoperazone whereas SP-2 was resistant against Norfloxacin, Cefoperazone and Cefotaxime at the concentration of 30µg/ml. Both the bacteria are Gram negative rods and can efficiently grown on Nutrient broth media. The optimum temperature for growth of both the bacteria was 37°C. SP-1 can be grown at 50°C whereas growth of SP-2 was arrested in this temperature. Both the bacteria were slightly acidophilic. The biochemical studies of these bacteria resemble to *Proteus sp.* (SP-1) and *Shigella sp.*(SP-2).

Key Words: Coli–form bacteria, Microbiological examination of water; Multidrug-resistant bacteria; Potable water; Water-borne diseases.

INTRODUCTION

Contamination of drinking water causes water-borne diseases and other associated infections which are the causes for crucial outbreaks of gastroenteritis and other diseases that affect the majority percentage of population in third world countries including India (Newspaper report : 'Drinking water contaminated with sewage', Times of India 12th July 2006 http://articles.timesofindia.indiatimes.com/2006-07-12/hyderabad /27813756_1_water-supply-ward-sewage). Eighty per cent of the total diseases in these countries are linked with contaminated water. The pathogens of these diseases multiply in the body of the infected. The inappropriate disposal of human excreta is the main cause of contamination of water of rivers, wells, lakes and shallow hand pumps and results in the propagation of these diseases. Thus the concentration of faecal coliform bacteria is a measureable marker of safety of potable water [1-2]. Majority of these infections are caused by enteric Gram negative bacteria such as *E. coli, Shigella, Salmonella,* etc. and ciprofloxacin is used as the first line of treatment [3]. The failure of ciprofloxacin or other related antibiotic for treatment of water-borne and related diseases has indicated one or more antibiotic resistance of these bacteria which may cause an outbreak. If

hospital sewage containing pathogenic bacteria (most of which are multi drug resistant) gets contaminated with drinking water then the outbreak becomes severe. A few years back a focal outbreak of gastroenteritis in Nagpur, India has been recognized as the heavy contamination of drinking water mainly by the hospital sewage caused by *Vibrio cholerae*. In public water supply systems in New Delhi, India, a bacteria has been found which is highly resistant to almost all antibiotics possibly caused by New Delhi metallo-beta-lactamase (NDM)-1 gene [4].

The astonishing discovery of penicillin in 1928 was followed by the discovery and commercial production of number of antibiotics. The antibiotic therapy is the most common and cheapest technology to prevent diseases [5]. Annually antibiotics are manufactured at an estimated scale of about 100,000 tons throughout the world. Though there are a large number of antibiotics but due to abuse of them a serious medical threat is rising day by day [6]. More strains of microbes are becoming resistant to antibiotic, and some have become resistant to many antibiotics and other chemotherapeutic agents, the phenomenon of multidrug resistance [7]. Increasing antimicrobial resistance against a large number of antibiotics restricted the treatment of invasive diseases caused by different pathogenic bacteria emphasize the need for alternative therapies [8-9].

The main objectives of this study are to perform the examination of water quality from different drinking sources of the College campus, identification of bacterial pool, drug resistance pattern of the water-borne microbes, their characterization and possible identification of the multidrug resistant bacteria.

MATERIALS AND METHODS

Sample collection and isolation of bacteria: Water samples (from both tap and Water Purifier) were collected from different sources of College in sterile test tube and brought to our laboratory and were analyzed within 6h of collection during the period of February, 2012 to June, 2012.

Water samples were cultured into solid Nutrient agar (NA) medium/ Nutrient broth with or without antibiotics and incubated at 37° C for 48h.

Growth of bacteria in selective media: Water samples were streaked on MacConkey agar medium. The medium prevents the growth of Gram positive bacteria. The organisms isolated were further sub-cultured on Nutrient agar medium (Pure culture). These pure cultures were subjected to Gram staining, biochemical tests and antibiotic resistant screening with antibiotic disc to identify possible drug resistant Gram negative organisms (preliminary screening).

Determination of coliform group of bateria (MPN): Water samples were prepared for MPN testing. Lactose broth of single and double strength were used as the presumptive medium. The medium containing Durham tube was inoculated with water sample and incubated at 37°C for 24h. Production of gas confirms the presence of coliform bacteria. To isolate coliform bacteria in pure culture, gas positive tubes of lactose broth were streaked on EMB agar plate and incubated at 30°C for 12h. Growth of colonies shows positive result. To complete the test, colonies from EMB agar were transferred to lactose broth with Durham tube and NA slants and incubated. Lactose broth producing acid and gas confirms the presence of coliform bacteria.

Antibiotic sensitivity testing: Antibiotic sensitivity testing was done by Well method according the to Das et al [8]. Bacterial strains were tested against six commonly used antibiotics i.e. Cefotaxime (CTX, 30µg/ml), Norfloxacin (NX, 30µg/ml), Oxytetracycline (OT, 30µg/ml), Penicillin (PC, 30µg/ml), Ciprofloxacin (CPX, 30µg/ml) and Cefoperazone (CPZ, 30µg/ml).

Characterization of bacteria: Gram staining, Acid fast staining, Endospore staining, Flagella staining were done according to standard methods. Indole production, MRVP test, Citric acid utilization, Nitrate reduction, Phenylalanine Production, Lysine Decarboylase Activity, H_2S production, Urease Production, Sugar Fermentation/Gas Production, Catalase activity/H_2O_2 Production, Starch hydrolysis,were done according to Dubey and Maheswari [10].

Effect of temperature and *pH* on bacterial growth: The effect of pH on bacterial growth was investigated by cultivating the organism at different temperature (20°-60°C) and different pH (4.5-9.5) in NA media. The organism was incubated for 24 hr. The bacterial growth was determined as OD value at 600 nm or by observing visual growth as mentioned.

RESULTS

Isolation of microbial colony by plating method: Though no microbial colony was found from water of any Water Purifier but all the tap water contain both bacterial and fungal colony (Table-1).

Table-1. Isolation of microbial colony[I] by plating method

Source of water	Bacterial colony	Fungal colony
Water purifier-1	Nil	Nil
Water purifier-2	Nil	Nil
Water purifier-3	Nil	Nil
Tap-1	15	3
Tap-2	13	4
Tap-3	17	6

[I]1 ml water was plated in each of the 3 plates.

MPN index of Water Purifier-3 and tap water-3 sample- Though Water purifier-3 has no bacteria but tap-3 water had MPN index of 1100/100ml (Table-2)

Table-2. MPN index of water

Water source	3 of 10ml each	3 of 1ml each	3 of 0.1 ml each	MPN index per 100ml
Water Purifier-3	Nil	Nil	Nil	Nil
Tap-3	3	2	2	210

Antibiotic Sensitivity test of Tap 3 water: When tap-3 water was plated against Norfloxacin antibiotic some resistant colonies were found as in Table-3.

Table-3. Antibiotic sensitivity test of Tap 3 water

Name of antibiotic	No. of bacterial colony	No. of fungal colony
Norfloxacin (30µg/ml)	2 (orange colony) 3 (yellow colony) 1 (white colony)	3

Isolation of antibiotic resistant strains: One each from two different coloured colonies were taken and named as SP-1 (orange), SP-2 (yellow).

Antibiotic sensitivity test : SP-1 and SP-2 were analyzed for their antibiotic sensitivity and the results were presented in Table-4.

Table-4. Antibiotic sensitivity test

Name of antibiotic	SP-1	SP-2
Penicillin	S	S
Norfloxacin	R	R
Oxytetracycline	S	S
Cefoperazone	R	R
Cefotaxime	S	R
Ciprofloxacin	S	S

R=Resistant. S=Susceptible

Characterization of SP-1, SP-2: Both the strains were characterized morphologically and biochemically. The results were tabulated in the following table (Table-5)

Table-5. Biochemical characterization of SP-1, and SP-2

Character	SP-1	SP-2
Cell form	Short rods	Slightly curved rods
Motility	+	+
Gram character	Gram negative	Gram negative
Acid-fast stain	-	Not clear
Endospore stain	-	Not clear
Flagella stain	+	+
Nitrate reduction to nitrite	+	+
Lactose fermentation	-	-/+
Glucose fermentation productivity	+	-/+
Catalase	+	+
Urease	+	+
Indole production	+/-	-
H_2S production	-	-
L-Lysine decarboxylase	–	+
Phenylalanine production	+	-
Starch hydrolysis	-	-
Methyl red test	+	-
VP test	-	-
Salt tolerance (upto 10% conc.)	+	+

Effect of temperature on bacterial growth: The SP-1 and SP-2 bacteria were incubated in NB media at different temperature (20-60°C). The optimum growth was found in 37°C in stationary condition in both the bacteria. The growth of both strains decreased over 40°C (Table-6). SP-1 was able to grow even in 50°C but SP-2 could not.

Table-6 : Effect of temperature on bacterial growth.

Temperature	SP-1	SP-2
20°C	+	+
25°C	++	++
30°C	++	++
37°C	+++	+++
40°C	++	+
50°C	+	-
60°C	-	-

Effect of pH on bacterial growth: The optimum pH of both the tested bacteria were 6.5 in the present experimental condition.

DISCUSSION

Resistance to commonly used antimicrobial drugs, particularly multiple drug resistances represents a critical public health problem because it complicates treatment of patient and the propensity results in longer hospital stays. Additionally as an outcome most of the population in developing countries cannot meet the expense of the newer and more expensive drugs [11] The problem of drug resistance in organisms causing water-borne diseases will continue to be ongoing in both developed and developing countries [6]. In the present investigation the tap water sources contained huge number of bacteria as evidenced from plate counting and MPN index (Table-1 and 2). Tarafdar and Bhattacharyya [12] showed the nonpotability of water sample from Gobordanga, 24 Pgs (N) having high MPN index. From the drinking water source of Barasat Govt. College campus, at least three morphologically different bacteria were found in Norfloxacin plate (Table-3). Two of these bacteria (SP-1 and SP-2) were further studied and found as multidrug resistant. SP-1 was resistant against Norfloxacin and Cefoperazone whereas SP-2 was resistant against Norfloxacin, Cefoperazone and Cefotaxime at the conc. of 30μg ml^{-1} (Table-4).

Both the bacteria can efficiently grown in Nutrient broth and are gram negative rods. A number of biochemical tests were performed to disclose their identity (Table-5). The optimum growth temperature of both the bacteria was found to be 37°C (Table-6). Both the bacteria were slightly acidophilic. They can grown in pH 4.5 with the optimum pH of 6.5. The morphological and biochemical tests preliminary indicated SP-1 as *Proteus sp.* and SP-2 as *Pseudomonas sp.* but to confirm the identity of isolated bacteria (SP-1 and SP-2) 16s rRNA and/nucleotide sequencing are necessary.

The present study shows the presence of multidrug resistant bacteria in drinking water of Barasat Govt. College. All the resistant bacteria may not be pathogenic but they may harbor resistance genes in their plasmids which can easily spread to pathogenic community by conjugation, which may be a serious threat to the community. It is observed that the analysis of

water from Water Purifier harbors no such bacteria but the number of such system was not sufficient in this College. The College authority should take the measures so that not a single user (mostly student) is forced to take the contaminated drinking water. The College authority and the water supply authority should shoulder responsibility to take the appropriate measures to control this grave problem for the service of other people who are innocently take this contaminated water.

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Isolation, Biochemical and Immunological Characterization of Yolk Protein of Freshwater Prawn *Macrobrachium rosenbergii.*

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Abstract

The isolation of vitellin from gravid ovaries was carried out using ultracentrifugation, seralose-6B and DEAE-cellulose columns. Native-PAGE analysis revealed the presence of single protein band in vitellin (yolk protein) fraction obtained after re-chromatography on DEAE-cellulose. Each vitellin shows two polypeptide subunits (82 kD and 75 kD) in SDS-PAGE. Purified proteins did not show any positive result with methyl green staining technique. Also neither it binds with hydroxyapatite matrix nor gives any positive reaction in alkali-labile phosphorus analysis (index of vitelleogenin (V_g) and yolk proteins (YP_S)). So the yolk protein is not phosphorylated like fish vitelleogenin. The purified vitellin has been suggested as a lipo-glyco-protein (since it stained positively with periodic acid-schiff and Sudan black) but not phosphoprotein.

Polyclonal antiserum against purified yolk protein (vitellin) was raised to conduct the immunological tests.Result of double immuno-diffusion test demonstrated the cross-reactivity of vitellin antiserum with fractions from different steps of purification, but not with peak-I fraction of Seralose-6B indicating immunologically dissimilar nature of the proteins.

Key Words: Yolk Protein, vitellin, prawn, Antibody, vitellogenin

Introduction

Vitellogenins (V_g) and yolk proteins (YP_S) are the precursor form and final product respectively in all oviparous animals. Crustacean YP_S are glyco-lipo-caroteno-protein in nature as reported by many earlier worker [1], whereas the same for insects and vertebrates are glyco-lipo-phospo-protein in nature [2].Time to time many scientists have isolated and characterized the major YP_S as lipovitellin /vitellin (V_t) in crustacea

and found its molecular weight ranges from $3-5\times10^5$ Dalton[3]. Also, the decapods V_t showed $2-11$ polypeptide subunits in SDS-PAGE [3-6]. Quinitio *et. al.* [7] have used the hydroxyapatite column to purify the V_t from the ovary of *Penaeus monodon* but they did not claim the proteins as phospo-protein where as many of the earlier worker have used the same type of column for the isolation and characterization of V_g and YP_S in vertebrates [8].

Since many decapods including freshwater prawns are commercially important, there is a need to study the biochemical nature of the proteins involved in gonadal recrudescence to get the clear picture of their involvement during reproduction [3]. Moreover the physiological function other than nutrition of V_g and V_t during embryonic development is also worthy to be find out [3]. An immuno-cyto-chemical demonstration of V_g accumulation during ovarian recrudescence in the fresh water prawn, *Macrobrachium rosenbergii* has been reported by PAP straining method using rabbit anti prawn V_g as the primary antibody [9].

We have undertaken the present study to find out the biochemical and immunological nature of purified major yolk protein (V_t) in fresh water prawn *Macrobrachium rosenbergii* to develop the enzyme linked immunosorbant assay (ELISA) for investigating the precursor (V_g) product (V_t) relationship, as well as for investigating effects of xenobiotics on vitellogenesis.

Materials and Methods:

Collection of samples

Freshwater Prawn *M. rosenbergii* (weighing 40-60 gm) used in this study were collected during late vitellogenic period (July) from their inhabitant at Simurali, West Bengal. The gravid ovaries were collected by opening the dorsal side of the prawn and were processed immediately for extraction of YP_S.

Extraction and purification of YP (vitellin)

Extraction of YP

The ovarian tissue (stage IV containing) were rinsed and homogenized in ice cold 0.6 M NaCl containing 0.05% PMSF and aprotinin (Zymofren: 4000 ki/ml). The homogenate was then passed through cheese cloth to remove the debris and then

centrifuged at 30000 rpm for 30 minutes by Ultracentrifuge (Beckman model-25). Three clear layers were observed: a floating orange fat layer, a carrot-yellow middle layer and a pellet. The clear middle protein layer was collected with the help of a 2 ml syringe fitted with 18 gauge long needle. It was re centrifuged to get more clear yolk preparation.

Gel filtration on seralore-6B

1 ml (75 mg of protein) of the clear yolk preparation was subjected to gel filtration on a column (93cm x0.9 cm) of seralose-6B (SRL, Bombay) and the elution was commenced using 50 mM Tris-HCl buffer (pH-8) containing PMSF (0.05%) and 2% NaCl as eluting buffer. Proteins were eluted in cold condition at the flow rate of 15 ml/hr and 3 ml fraction was collected with the help of a Radirac fraction collector (LKB). Following fractionation the absorbance of each fraction was read at 280 nm on a Beckman (Model-25) spectrophotometer. The presence of YP_S in protein peak was determined by electrophoretic analysis and immunological cross-reaction studies.

Ion exchange chromatography on DEAE-cellulose

The pooled fractions (No 23-31) of Peak-II of seralose-6B was dialysed against distilled water and then subjected to ion exchange chromatography on DEAE-cellulose (15 ml syringe was filled with gel matrix). Total 8ml, (48 mg of proteins) was loaded on to the gel matrix. Different concentration of NaCl viz 0.1M, 0.3M, 0.5 M and 0.7 M were used as step gradient to elute the protein and an amount of 3 bed volumes were eluted for each gradient. 1.5ml fractions were collected at each step and the absorbance of each fraction was read at 280 nm,

The peak eluted with 0.1 M NaCl was pooled (fraction no 24 to 32), dialysed, concentrated and again re-chromatographed on to the same DEAE- cellulose column. In this case lower concentration of NaCl like 0.05 M, 0.075 M and 0.1 M NaCl were used to resolve the protein.

Biochemical Characterization

Native-PAGE

Qualitative determination of YP_S from each semi purified and purified fractions were electrophoresed on 5% gel (Laemmli)[10]. Crude egg extract ($100\,\mu g$ protein), fraction obtained after gel filtration on Seralose-6B (peak II: $100\,\mu g$), fraction obtained after ion exchange chromatography on DEAE-cellulose (peak eluted with

0.1 M NaCl: 50 μg) and purified peak eluted with 0.1 M NaCl gradient of the second DEAE-cellulose column (30 μg) were used in native-PAGE.

After the run the gels were stained with coomassie blue for protein or with Sudan black for lipid and methyl green for phosphate.

SDS-PAGE

Purified yolk proteins (30 μg) along with molecular weight marker proteins (pharmacia) were used in 0.1 % SDS-PAGE according to the method of Laemmli[10]. After the run gels were stained with coomassie blue.

Estimation of alkali-Labile phosphorus

The presence of YP_S was determined by estimating the alkali-labile phosphorus in each protein peak as well as crude protein preparation according to the method of Nath and Sundararaj [11].

Adsorption chromatography on HA-Ultrogel

Purified yolk protein V_t from second DEAE-cellulose column was dialysed and then subjected to adsorption chromatography on HA-Ultrogel (15 ml syringe was filled with gel matrix). 10 mM phosphate buffer (pH-8) was used as equilibrating as well as washing buffer. The proteins loaded for adsorption were come out with the washing buffer. Protein did not bind with the gel matrix.

Immunological Characterization

Antiserum against purified V_t was obtained from rabbits by immunizing them by purified V_t (total 0.4 mg/ml) suspended in equal volume of complete Freund's adjuvent. Two booster of this suspension were injected interdermally on the thigh region at one week intervals. The rabbits were bled one week after the third injection and the serum was separated by conventional method. The serum was used as antivitellin antibodies.

Ouchterlony immune-diffusion test was performed to observe cross-reaction between the antiserum to V_t and protein peaks eluted in different steps of purification using 1% agar according to the method of Ouchterlony [12]. The well arrangements for this test are shown in Fig. 5.

Results

Purification

Fig. 1 shows the elution profile of egg extract on Seralose-6B. The elecrophorogram inserted above the figure revealed the presence of V_t in the second peak. Fig. 2 shows the illusion pattern of V_t fraction from seralose-6B column on DEAE-cellulose. The fraction eluted with 0.1 M NaCl contains mainly the V_t protein as revealed by native-PAGE (Electrophorogram inserted in Fig. 2). Two more protein bands along with the main protein band (arrow marked) also were observed in this case. The V_t protein was lipoprotein in nature since it stained positively with Sudan Black (Electrophorogram B of Fig. 2). Since the V_t protein was not purified totally the semi purified main fractions of DEAE-cellulose (Black bar indicated) were re-chromatographed on the same column. The purified fractions were eluted with 0.1 M NaCl (Fig.3).The electrophorogram of the peak revealed the presence of single protein band (Fig. 3A). This protein did not show any positive reaction for phosphate when it was stained with methyl green.

SDS-PAGE

SDS-PAGE of the purified V_t demonstrated the presence of 2 sub units of 82 kD and 75 kD respectively (Fig.4).

Biochemical characterization

The purified V_t fraction did not show any positive result when it was analysed for alkali-labile phosphorus (index for the presence of V_g as well as YP_S). Moreover the purified protein did not bind with the matrix of Hydroxyapatite-Ultrogel column when it was adsorbed for biochemical characterization. So the protein is non-phosphorylated

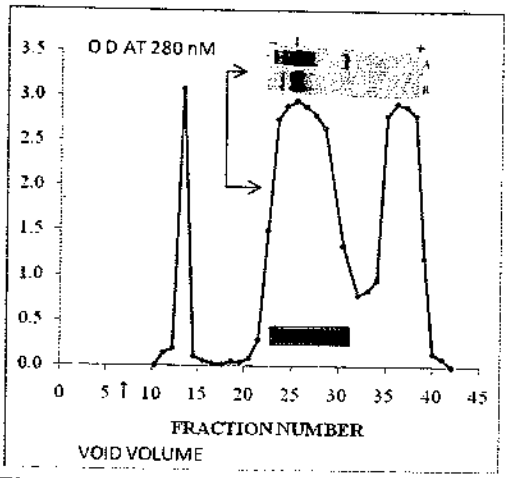

Fig.1. Gel filtration on Seralose-6B of crude yolk preperation after ultracentrifugation. The optical density of fractions are indicated by solid lines. Inset electrophorograms (5% polyacrylamide gel) of aliquots of the second peak obtained following gel filtration stained for protein(A) and lipid (B) are displayed at the upper middle of the figure.Black bar indicates the presence of V_t protein in this peak as well as the fractions used for further purification.

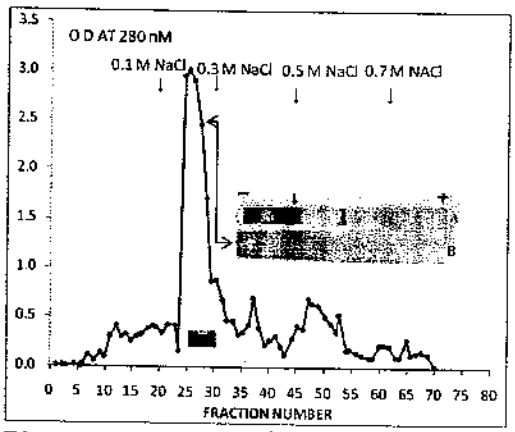

Fig.2. Elution pattern of DEAE-cellulose column of peak II of seralose 6B. The column was eluted by Tris-HCl buffer (pH-8) containing NaCl.The concentration of NaCl used as step gradient (arrow marked) were 0.1M,0.3M,0.5M and 0.7M NaCl respectively. Inset electro electrophorograms (5% polyacrylamide gel) of aliquots of the peak eluted with 0.1 M NaCl stained for protein (A) and lipid (B) are displayed at the lower right hand corner of the figure.

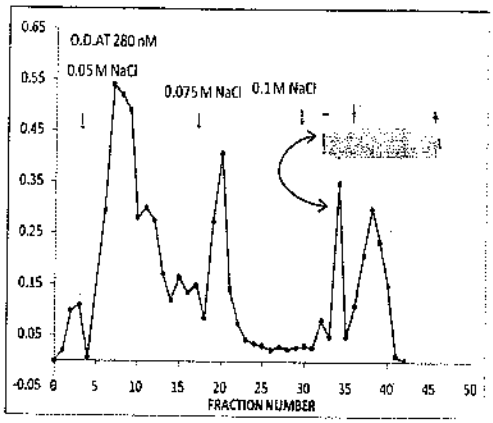

Fig.3. Elution profile of impure V_t fraction from DEAE-cellulose column on the same column. The NaCl concentration used as step gradient were very low 0.05M,0.075M and 0.1M for eluting the proteins. Inset electrophorograms (5% polyacrylamide gel) of aliquots of the peak eluted with 0.1 M NaCl stained for V_t protein is displayed at the upper right hand corner of the figure.

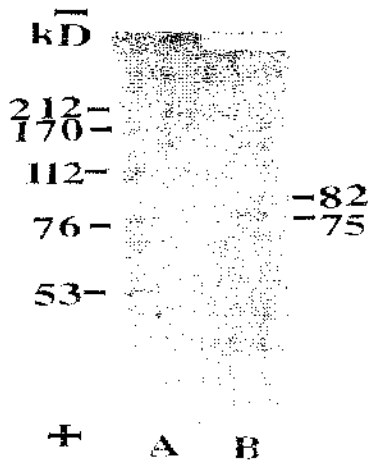

Fig.4. SDS-PAGE (0.1% gel) of the isolated V_t from *M. rosenbergii* ovaries (B). The molecular weights were based from mobilities relative to the standard proteins (Pharmacia) used (A).

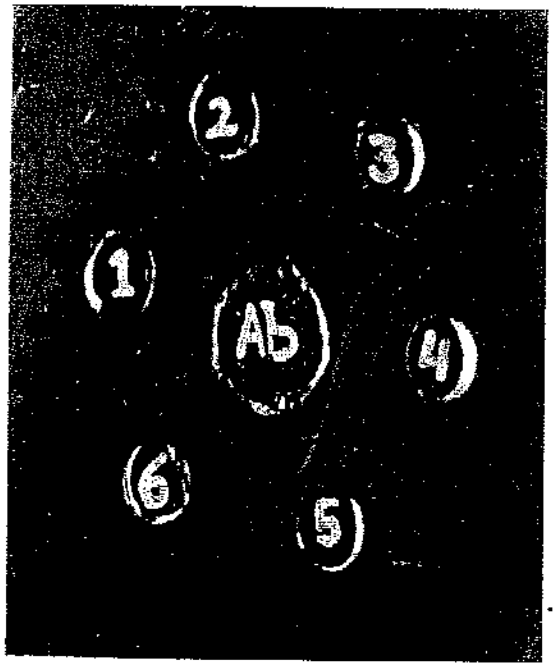

Fig.5. Immunodiffusion showing the crossrecativity of antiserum to V_t (Ab) with saline extract of gravid ovary(Well-1), egg extract after ultracentrifugation (Well-2), Peak-I & II of seralose-6B (Well-6,3),Peak eluted with 0.1 M NaCl in first & second DEAE cellulose (Well-4 & 5) respectively.

Immunological characterization

Antibodies against purified V_t was raised in white rabbits and the antiserum was used for Ouchterlony immuno-diffusion tests. Fractions used in different steps of purification (wells 1-5 of Fig. 5) showed precipitin lines against the antiserum to V_t. Fraction from first peak of Seralose-6B did not show any precipitin line against the antiserum to V_t (well 6 of Fig, 5).

Discussion

Sex proteins are considered now as good biomarkers for assessing environmental endocrine disruption. To develop an enzyme linked immunosorbant assay (ELISA) for investigating effects of xenobiotics on vitellogenesis attempt has been made for isolation, identification and characterization of the vitellin proteins (V_t) from the gravid ovaries of fresh water prawn *(Macrobrachium rosenbergii)*.

In the present study the YP_S from the matured oocytes of female prawn were extracted following the few steps of the method of Bhakta and Nath [8] which has been widely used to extract the egg yolk proteins in many non-mammalian vertebrates[13]. But Quinitio et.al.[7] have used only buffer extraction and centrifugation technique for the same. Previous investigators have used various approaches to purify V_t from ripe ovaries of prawn. First approach was precipitation, second was ultracentrifugation after $(NH_4)_2SO_4$ precipitation and the third approach used was a combination of gel filtration, ion exchange chromatography followed by affinity chromatography.

In the present work, V_t was purified following the method of gel filtration on Seralose-6B, ion exchange chromatography on DEAE-cellulose and re-chromatography on the same DEAE- cellulose column. The V_t peak was eluted by 0.1 M NaCl solution in both the anion

exchange column. Since the V_t protein was not purified totally after first DEAE-cellulose chromatography (Fig.2), the protein peak eluted by 0.1 M NaCl were pooled ,dialysed against distilled water and was chromatographed again on the same DEAE column. Here, low strength gradient like 0.05, 0.075 and 0.1 M NaCl were used to elute the impurities present in the earlier fractions (Electrophorogram Fig. 3). In this case the purified V_t was also eluded with 0.1 M NaCl. The use of similar kind of ion exchanger like DEAE-sepharose and DEAE-sephacel has already been established for separations of V_t in a number of crustaceans [1, 14].

The purification of V_t resulted in the purified proteins band (Fig.3) which is stained with Sudan Black showing that they are lipoproteins (Fig. 2). It also stained with PAS technique that reveals that V_t is lipo-glyco-protein [15]. Attempt has also made for phosphate staining by methyl green technique but it did not show any positive result. So the protein is non-phosphorylated. Many of the earlier workers [1, 7], demonstrated this proteins as lipo-glyco-caroteno-proteins in different crustacea. Quinitio et.al. [7] used the hydroxyapatite column (Phospho-protein affinity column) for the separation of V_t in *Penaeus monodon* but they did not coin the protein as phospho-protein whereas this particular column has been used by many earlier workers to purify V_g as well as YP_S in vertebrates [8]. Same hydroxyapatite-Ultrogel column was used to characterize the interested proteins but it did not bind with the gel matrix.

Many earlier workers had used the alkali-labile phosphorus assay method to detect the YP_S in fish [8, 11]. The use of alkali-labile phosphorus assay for detection of YP_S is justified because of the fact that, YP_S are the cleaved products of V_g and V_g is generally estimated as alkali-labile phosphorus. We have used the same alkali-labile phosphorus method to detect the phosphorus moiety in purified prawn V_t and it is found that it did not contain alkali-labile phosphorus. So from the above studies, we can conclude that the biochemical nature of the purified V_t is glyco-lipo-proteins but not phosphoproteins.

Most studies indicate that crustacean V_t has a molecular weight ranging from 300 to 700 kD [6]. SDS-PAGE of purified V_t of the present species of our interest showed two subunits of 82 kD and 75 kD. Some V_t are compound of two subunits like *M. rosenbergii* [11, 16]. Tuberly et. al. [17] isolated V_t from five different estuarine crustacean species and

depending on the species they detected 3-12 subunits [18] . Only 2 subunits of molecular weight 84-92 kD and 92-105 kD were estimated from SDS-PAGE of V_t in *M. rosenbergii* which supports our findings[19].

In the present study polyclonal antibody was raised against purified V_t. The antivitellin did not cross react with peak-I fraction of Seralose-6B (Fig.5 well- 6) but other similar samples from different steps of purification had cross reacted and gave precipitate line (cf. Fig.5 wells 1-5). The finding thus suggests that peak-I of Seralose-6B has different antigenic determinant. From these findings we are very sure that samples used for purification in each step contained V_t which are immunologically confirmed.

Polyclonal antibodies against V_t of prawns including other crustaceans have been produced for different immunological studies. For instance attempts have been made to localize V_t immunocytochemically in the ovary, hepatopancreas and sub-epidermal adipose tissue by the same group of workers [20].

This study is supported by the findings of Chen and Kuo in same type of species to establish the precursor product relationship but information on the transformation process of crustacean V_g into YP_S- V_t is still lacking.

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Ordering of life distribution in terms of reversed time

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Abstract: If a random variable X ($X \geq 0$, with probability one) denotes the lifetime of a unit, then the random variable $X_t = (t - X | X \leq t)$, for a fixed $t > 0$, is known as 'time since failure', which is similar to the residual lifetime of a random variable used in reliability and survival analysis. The reversed hazard rate function, which is related to the random variable X_t, has received the attention of many researchers in the recent past (cf. Shaked and Shanthikumar (1994)). In this paper we define a new ordering based on variance of the random variable X_t and establish its relationship with reversed hazard rate ordering.

Keywords: Reversed hazard rate function, Reversed hazard rate ordering, *RMR* ordering, *RVR* ordering.

1. Introduction:

Let a random variable X ($X \geq 0$, with probability one) denotes the lifetime of a unit having absolutely continuous distribution function $F(\cdot)$, survival function $\bar{F} = 1 - F(\cdot)$ and the density function $f(\cdot)$. Let the random variable $X_t = (t - X | X \leq t)$ denotes the time elapsed after failure till time t, given that the unit has already failed by the time t. The random variable X_t is analogous to the well known concept in reliability theory, known as residual lifetime $R_t = (X - t | X > t)$ of a unit surviving at time t. We call the random variable X_t, the reversed residual life (or time since failure). Since, here time is considered in backward direction, we sometimes call it reversed time.

In the literature, the function $\mu_F(x) = \frac{f(x)}{F(x)} \forall x$ such that $F(x) > 0$ is known as reversed hazard rate function (cf. Shaked and Shanthikumar (1994)). Nanda et. al (2003) have defined reversed mean residual function $\alpha_F = E(X_t)$ and reversed variance residual function $\beta_F = Var(X_t)$ of the random variable X (having distribution function F). In the analysis of left-censored data, the reversed hazard rate function plays the same role as the hazard rate function plays in the analysis of right-censored data (cf. Andersen, Borgan, Gill and Keiding (1993)). In

this paper, we define one ordering based on variance of random variable X_t. We establish a relationship between our proposed order and the reversed hazard rate ordering and also give a relationship between reversed mean residual life ordering (RMR ordering) and our proposed order.

2. New order of life distributions:

In this section we define a new ordering of random variables and study its relationship with the reversed hazard rate ordering.

Let X and Y two random nonnegative and absolutely continuous random variables, having distribution functions $F(\cdot)$ and $G(\cdot)$, reversed hazard rate functions $\mu_F(\cdot)$ and $\mu_G(\cdot)$ and the reversed mean residual life functions $\alpha_F(\cdot)$ and $\alpha_G(\cdot)$, respectively. The following definition of reversed hazard rate ordering is given in Shaked and Shanthikumar (1994).

Definition 2.1: *The random variable X is said to be larger than the random variable Y in reversed hazard rate ordering (written as $X \geq^{RH} Y$) if $\mu_F(t) \geq \mu_G(t)$, for all $t \geq 0$.*

The following definition of reversed mean residual life ordering is given in Nanda et al (2003).

Definition 2.2 : *The random variable X is said to be larger than the random variable Y in reversed mean residual life ordering (written as $X \geq^{RMR} Y$) if $\alpha_F(t) \leq \alpha_G(t)$, for all $t \geq 0$.*

Here we proposed a new order of life distribution based on variance of random variable X_t.

Definition 2.3 : *The random variable X is said to be larger than the random variable Y in reversed variance residual life ordering (written as $X \geq^{RVR} Y$) if for all $x \geq 0$,*

$$\frac{\int_0^x \int_0^t F(u)\,du\,dt}{\int_0^x F(u)\,du} \leq \frac{\int_0^x \int_0^t G(u)\,du\,dt}{\int_0^x G(u)\,du}$$

This can equivalently be written as $\frac{\int_0^x \int_0^t F(u)\,du\,dt}{\int_0^x \int_0^t G(u)\,du\,dt}$ is increasing in $x \geq 0$.

The following theorem is due to Nanda et al (2003).

Theorem 2.1 : *The reversed hazard rate ordering is stronger than reversed mean residual life ordering.*

The following theorem gives a relationship among reversed variance residual life ordering and reversed hazard rate ordering.

Theorem 2.2 : *The reversed hazard rate ordering is stronger than reversed variance residual life ordering.*

Proof : Given that $X \geq^{RH} Y$. This means that $\mu_F(t) \geq \mu_G(t)$, *for all* $t \geq 0$, which gives $\frac{F(t)}{G(t)}$ is increasing in $t \geq 0$. Then for fixed $\lambda > 0$, $F(t) - \lambda G(t)$ will have at most one change of sign and if one such change does occur, it occurs from – to +. This implies that, for each fixed real λ,

$\int_0^t [F(u) - \lambda G(u)]\, du$ will also have at most one change of sign and it one such change does occur, it occurs form – to +. This establishes that $\frac{\int_0^t F(u)\,du}{\int_0^t G(u)\,du}$ is increasing in $t \geq 0$. Again, since $\frac{\int_0^t F(u)\,du}{\int_0^t G(u)\,du}$ is increasing in $t \geq 0$ so for a fixed $\theta > 0$, $\int_0^t F(u)du - \theta \int_0^t G(u)du$ will have at most one change of sign and if one such chage does occur, it occurs from – to +. This implies that, for each fixed real θ, $\int_0^x \left| \int_0^t F(u)du - \theta \int_0^t G(u)du \right| dt$ will also have at most one change of sign and if one such change does occur, it occurs from – to +. This establishes that $\frac{\int_0^x \int_0^t F(u)\,du\,dt}{\int_0^x \int_0^t G(u)\,du\,dt}$ is increasing in $x \geq 0$.

Hence the result follows.

The following counterexample, which is also used in Nanda et. al (2003) for some other purpose, shows that the converse of the above theorem is not necessarily true.

Counterexample 2.1 : Let X and Y have the following probability distributions.

$$F(x) = P(X \leq x) = \begin{cases} \frac{x^2}{2}, & 0 \leq x \leq 1 \\ \frac{x^2+2}{6}, & 1 \leq x \leq 2 \\ 1, & x \geq 2 \end{cases}$$

and

$$G(x) = P(Y \leq x) = \begin{cases} \frac{x}{2}, & 0 \leq x \leq 2 \\ 1, & x \geq 2. \end{cases}$$

Then

$$\frac{F(x)}{G(x)} = \begin{cases} x, & 0 \leq x \leq 1 \\ \frac{x^2+2}{3x}, & 1 \leq x \leq 2 \\ 1, & x \geq 2, \end{cases}$$

which is not monotone as Figure 1 shows. Therefore $X \not\geq^{RH} Y$.

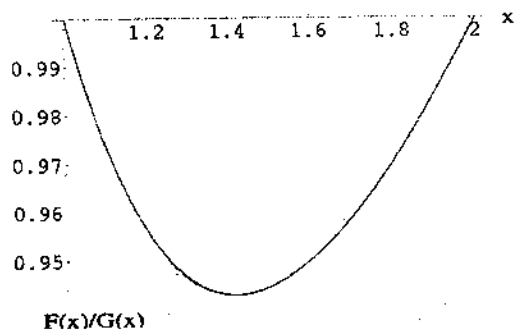

Figure 1: Plot of $F(x)/G(x)$, for $1 \leq x \leq 2$.

To show that there exists the RVR ordering between X and Y, we proceed as follows.

Case I: When $0 \le x \le 1$, $\int_0^x F(u)du = \frac{x^3}{6}$, $\int_0^x G(u)du = \frac{x^2}{4}$, $\int_0^x \int_0^t F(u)\,dudt = \frac{x^4}{24}$ and $\int_0^x \int_0^t G(u)\,dudt = \frac{x^3}{12}$. Thus, $\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x \int_0^t G(u)dudt} = \frac{x}{2}$.

Case II: When $1 \le x \le 2$, $\int_0^x F(u)du = \int_0^1 F(u)du + \int_1^x F(u)du = \frac{1}{6}\left(\frac{x^3}{3} + 2x - \frac{4}{3}\right)$,

$\int_0^x \int_0^t F(u)\,dudt = \int_0^x \frac{1}{6}\left(\frac{t^3}{3} + 2t - \frac{4}{3}\right)dt = \frac{1}{6}\left(\frac{x^4}{12} + x^2 - \frac{4}{3}x\right)$, $\int_0^x G(u)du = \int_0^x \frac{u}{2}du = \frac{x^2}{4}$ and

$\int_0^x \int_0^t G(u)\,dudt = \int_0^x \frac{t^2}{4}dt = \frac{x^3}{12}$. Therefore, $\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x \int_0^t G(u)dudt} = \frac{x^3+12x-16}{6x^2}$.

Case III: When $x \ge 2$, $\int_0^x F(u)du = \int_0^1 F(u)du + \int_1^2 F(u)du + \int_2^x F(u)du = x - \frac{10}{9}$,

$\int_0^x G(u)du = x - 1$, $\int_0^x \int_0^t F(u)\,dudt = \int_0^x \left(t - \frac{10}{9}\right)dt = \frac{x(9x-20)}{18}$ and

$\int_0^x \int_0^t G(u)\,dudt = \int_0^x (t-1)dt = \frac{x}{2}(x-2)$. Thus, $\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x \int_0^t G(u)dudt} = 9 - \frac{2}{x-2}$.

Combining all the cases, we get

$$\frac{\int_0^x \int_0^t F(u)\,dudt}{\int_0^x \int_0^t G(u)\,dudt} = B(x)(say) = \begin{cases} \frac{x}{2}, & 0 \le x \le 1 \\ \frac{x^3+12x-16}{6x^2}, & 1 \le x \le 2 \\ 9 - \frac{2}{x-2}, & x \ge 2 \end{cases}.$$

Clearly, for $x \in [0,1] \cup [2,\infty)$, $\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x \int_0^t G(u)dudt}$ is increasing in x. Again, for $1 \le x \le 2$, Figure 2 shows that $\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x \int_0^t G(u)dudt}$ i.e. $B(x)$ is increasing in x, giving that $X \geq^{RVR} Y$.

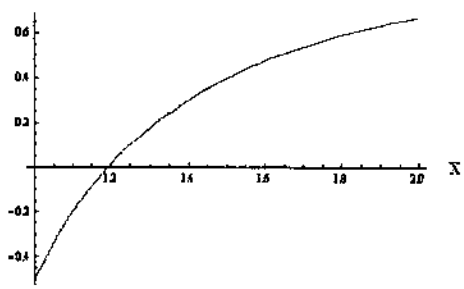

Figure 2: Plot of $B(x)$, for $1 \le x \le 2$.

Corollary 2.1 : $X \geq^{RMR} Y$ implies $X \geq^{RVR} Y$.

Below we redefine the different orderings (RMR and RVR) discussed in this chapter, in the spirit of the definitions given in Sing (1989).

It can be noted that $\mu_F(x) = \frac{d}{dx} \ln F(x)$.

Define $\alpha_F^*(x) = \frac{d}{dx}\left[\ln \int_0^x F(u)\,du\right]$ and $\beta_F^*(x) = \frac{d}{dx}\left[\ln \int_0^x \int_0^t F(u)\,du\,dt\right]$.

Now observe that

$$\alpha_F^*(x) = \frac{d}{dx}\left[\ln \int_0^x F(u)\,du\right] = \frac{F(x)}{\int_0^x F(u)du} = \frac{1}{\alpha_F(x)}$$

$$\text{and } \beta_F^*(x) = \frac{d}{dx}\left[\ln \int_0^x \int_0^t F(u)\,du\,dt\right] = \frac{\int_0^x F(u)du}{\int_0^x \int_0^t F(u)dudt}.$$

The following theorem characterizes the different reversed orderings in terms of $\alpha_F^*(x)$ and $\beta_F^*(x)$.

Theorem 2.2 : (i) $X \geq^{RMR} Y$ if and only if $\alpha_F^*(x) \geq \alpha_G^*(x)$ for all $x > 0$.
(ii) $X \geq^{RVR} Y$ if and only if $\beta_F^*(x) \geq \beta_G^*(x)$ for all $x > 0$.

Proof : (i) follows from the fact that $\alpha_F^*(x) = \frac{1}{\alpha_F(x)}$. To prove (ii), note that $\beta_F^*(x) \geq \beta_G^*(x)$ if and only if

$$\frac{\int_0^x \int_0^t F(u)dudt}{\int_0^x F(u)du} \leq \frac{\int_0^x \int_0^t G(u)dudt}{\int_0^x G(u)du}.$$

Hence the result follows.

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A Generalization of Binomial Theorem And Collatz Cycles

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ABSTRACT :

We define a new real valued function giving a generalization of Binomial Theorem and provide an expression of powers of two, which generates Collatz Cycles, by means of such a function.

1. INTRODUCTION

Very simple looking but a notorious as well as famous problem is **Collatz Conjecture**, introduced by Lothar Collatz in 1937 which is an unsolved problem in Mathematics till this date.

The Collatz function $f : \mathbb{N} \longrightarrow \mathbb{N}$ is defined by

$$f(n) = \begin{cases} \frac{n}{2}, & if\ n\ is\ even; \\ 3n+1, & if\ n\ is\ odd, \end{cases} \quad \forall\, n \in \mathbb{N}.$$

For any $k \in \mathbb{N}$, $n \in \mathbb{N}$, $f^k(n)$ is defined by $f^k(n) = (fofo \ldots\ldots of)(n)$, where $fofo \ldots\ldots of$ denotes the composition of f k-times. The Collatz Conjecture asserts that for every $n \in \mathbb{N}$ there exists a $k \in \mathbb{N}$ such that $f^k(n) = 1$.

Since for every $n \in \mathbb{N}$ there exists a $k \in \mathbb{N}$ such that $f^k(n) =$ an odd positive integer and not divisible by 3 (proved in Lemma (1)), the Collatz function may be modified as :

$C : \mathbb{N}' \longrightarrow \mathbb{N}'$ defined by

$$C(n) = \frac{3n+1}{2^\alpha}, \ \forall\, n \in \mathbb{N}', \text{ where}$$

$\mathbb{N}' = \mathbb{N}_{odd} - 3\mathbb{N}$, $\mathbb{N}_{odd} = \mathbb{N} - 2\mathbb{N}$ and α is the positive integer such that $\frac{3n+1}{2^\alpha}$ is an odd integer.

Then Collatz Conjecture becomes : for every $n \in \mathbb{N}'$ there exists a $k \in \mathbb{N}$ such that $C^k(n) = 1$.

Clearly $\mathbb{N}' = \{6n+1 : n \in \mathbb{N} \cup \{0\}\} \cup \{6n+5 : n \in \mathbb{N} \cup \{0\}\}$

For a given $n \in \mathbb{N}'$, if k be the smallest positive integer such that $C^k(n) = n$, then the sequence $n, C(n), C^2(n), \ldots\ldots\ldots, C^{k-1}(n)$ is called a Collatz cycle of length k starting with n. For example the length of the Collatz cycle starting with 1 is 1, as $C(1) = \frac{3.1-1}{2^2} = 1$, which is also called the trivial Collatz cycle.

For $n \in \mathbb{N}'$, $C(n) = n$ implies $n = 1$ (proved in Lemma (2)), i.e. a Collatz cycle of length 1 starts with 1 and ends with 1, that is, it is the trivial Collatz cycle only .

For some $n \in \mathbb{N}'$, $k \in \mathbb{N}$, $C^k(n) = 1$ implies that $C^{k+p}(n) = 1$, $\forall\, p \in \mathbb{N}$ and so the infinite Collatz sequence $n, C(n), C^2(n), \ldots\ldots\ldots, C^{k-1}(n), C^k(n) = 1, C^{k+1}(n) = 1, \ldots\ldots\ldots\ldots$ contains the trivial Collatz cycle only.

Also, for some $n \in \mathbb{N}'$, $k \in \mathbb{N}$, if $n, C(n), C^2(n), \ldots\ldots\ldots, C^{k-1}(n)$ be a Collatz cycle of length k, then $C^{k+p}(n) = C^p(n)$, $\forall\, p \in \mathbb{N} \cup \{0\}$, where $C^0(n) = n$.

Existence of a non-trivial Collatz cycle $n, C(n), C^2(n), \ldots\ldots\ldots, C^{k-1}(n)$ $(n > 1, k > 1, n \in \mathbb{N}', k \in \mathbb{N})$ implies that the Collatz conjecture is false. So another interesting problem comes to us, which is also an unsolved problem till this date, called **Collatz weak conjecture**, stated in terms of the function C as :

There is no non-trivial Collatz cycle, i.e. for every $n \in \mathbb{N}'$ with $n > 1$ there exists no $k \in \mathbb{N}$ such that $C^k(n) = n$.

Assume that there is a non-trivial Collatz cycle and let $C^k(n) = n$, for some $n \in \mathbb{N}'$ with $n > 1$ and $k \in \mathbb{N}$. Then $k > 2$ (By Lemma(2)). Now $C^i(n) = C\left(C^{i-1}(n)\right) = \frac{3C^{i-1}(n)+1}{2^{a_i}}$, where a_i is the positive integer such that $\frac{3C^{i-1}(n)+1}{2^{a_i}}$ is odd positive integer, $i = 1,2, \ldots\ldots, k$, and $C^0(n) = n$.

Then for $i = 1,2, \ldots\ldots, k$,

$$C^i(n) = \frac{3^i n + 3^{i-1} + 3^{i-2}.2^{a_1} + 3^{i-3}.2^{a_1+a_2} + \cdots + 3.2^{a_1+a_2+\cdots+a_{i-2}} + 2^{a_1+a_2+\cdots+a_{i-1}}}{2^{a_1+a_2+\cdots+a_i}} \quad \ldots\ldots\ldots(1.1)$$

Therefore

$$n = C^k(n) = \frac{3^k n + 3^{k-1} + 3^{k-2}.2^{a_1} + 3^{k-3}.2^{a_1+a_2} + \cdots + 3.2^{a_1+a_2+\cdots+a_{k-2}} + 2^{a_1+a_2+\cdots+a_{k-1}}}{2^{a_1+a_2+\cdots+a_k}} \quad \ldots\ldots (1.2).$$

Till this date it is known that the Collatz conjecture is verified for all positive integers $\leq 10^{360} - 1$ and up to June 2003 it has been known that length of a non-trivial Collatz cycle , in terms of the function C is at least $\frac{1027712276}{3} = 342570758.7 \approx 342570759$ (calculated by the help of [2]), if exists.

Our aim in this paper is to define a new real valued function giving a generalization of Binomial Theorem and express $2^{a_1+a_2+\cdots+a_k}$ (see (1.2)) in terms of such a function.

2. SOME RESULTS

Lemma 1. : For every $n \in \mathbb{N}$ there exists a $k \in \mathbb{N}$ such that $f^k(n) =$ an odd positive integer and not divisible by 3.

Proof : If n $(\in \mathbb{N})$ be even then $n = 2^a n_1$, for some $a \in \mathbb{N}$, $n_1 \in \mathbb{N}_{odd}$ and so $f^a(n) = n_1$. If n_1 be not divisible by 3, proof is completed. If n_1 be divisible by 3, then $f^{a+1}(n) = f(n_1) = 3n_1 + 1$ is even and not divisible by 3. Let $f^{a+1}(n) = 2^b n_2$, for some $b \in \mathbb{N}$, $n_2 \in \mathbb{N}_{odd}$. Then n_2 is not divisible by 3 and $f^{a+1+b}(n) = n_2$.

If n be odd, similarly the result can be established.

Lemma 2. : For $n \in \mathbb{N}'$, $(i)\ C(n) = n \Rightarrow n = 1$, $(ii)\ C^2(n) = n \Rightarrow n = 1$.

Proof : (i) $C(n) = n \Rightarrow \frac{3n+1}{2^a} = n$, where a is the positive integer such that $\frac{3n+1}{2^a}$ is odd positive integer. Therefore $(2^a - 3)n = 1$. This implies that $2^a - 3 \in \mathbb{N}$ and $n = 1$.

(ii) Let $C(n) = \frac{3n+1}{2^a}$ and $C^2(n) = \frac{3C(n)+1}{2^b}$, where a, b are the positive integer such that $\frac{3n+1}{2^a}$ and $\frac{3C(n)+1}{2^b}$ are odd positive integers. Then $C^2(n) = \frac{3^2 n+3+2^a}{2^{a+b}} = n$. This implies that $(2^{a+b} - 3^2)n = 3 + 2^a$ ..... (A).

From (A) it is clear that $2^{a+b} - 3^2 > 0$ and so $2^{a+b} - 3^2 \geq 7$ and $a + b \geq 4$ ..... (B)

Again

$2^{a+b} = 2^a \cdot 2^b = \frac{3n+1}{C(n)} \cdot \frac{3C(n)+1}{C^2(n)} = \frac{3n+1}{C(n)} \cdot \frac{3C(n)+1}{n} = \left(3 + \frac{1}{n}\right)\left(3 + \frac{1}{C(n)}\right) \leq 4^2 = 2^4 \quad (\because n, C(n) \geq 1)$

.This implies that $a + b \leq 4$ ..... (D) .

From (B) and (D), $a + b = 4$.

Case (a) : a=1, b = 3. Then (A) becomes $7n = 5$ which is not possible .

Case (b) : a=2, b = 2. Then (A) becomes $7n = 7$, $or, n = 1$.

Case (c) : a=3, b = 1. Then (A) becomes $7n = 11$ which is not possible.

Therefore $n = 1$.

Lemma 3. : Let $n, C(n), C^2(n), \ldots, C^{k-1}(n)$ be a non-trivial Collatz cycle of length $k > 1$ $(n \in \mathbb{N}', n > 1)$

and $C^i(n) = \frac{3C^{i-1}(n)+1}{2^{a_i}}$, where a_i is the positive integer such that $\frac{3C^{i-1}(n)+1}{2^{a_i}}$ is odd positive integer, for

$i = 1, 2, \ldots, k$. Then $2^{a_1+a_2+\cdots+a_k} = \left(3 + \frac{1}{n}\right)\left(3 + \frac{1}{C(n)}\right)\left(3 + \frac{1}{C^2(n)}\right) \ldots\ldots \left(3 + \frac{1}{C^{k-1}(n)}\right)$.

Proof : $2^{a_1+a_2+\cdots+a_k} = 2^{a_1} \cdot 2^{a_2} \cdot 2^{a_3} \ldots\ldots\ldots 2^{a_k}$

$$= \frac{3C^0(n)+1}{C(n)} \cdot \frac{3C(n)+1}{C^2(n)} \cdot \frac{3C^2(n)+1}{C^3(n)} \ldots\ldots\ldots \frac{3C^{k-1}(n)+1}{C^k(n)}$$

$$= \frac{3n+1}{C(n)} \cdot \frac{3C(n)+1}{C^2(n)} \cdot \frac{3C^2(n)+1}{C^3(n)} \ldots\ldots\ldots \frac{3C^{k-1}(n)+1}{n} \quad (\text{since}$$

$C^0(n) = n,\ C^k(n) = n$)

$$= \frac{3n+1}{n} \cdot \frac{3C(n)+1}{C(n)} \cdot \frac{3C^2(n)+1}{C^2(n)} \cdots\cdots\cdots \frac{3C^{k-1}(n)+1}{C^{k-1}(n)}$$

$$= \left(3+\frac{1}{n}\right)\left(3+\frac{1}{C(n)}\right)\left(3+\frac{1}{C^2(n)}\right)\cdots\cdots\left(3+\frac{1}{C^{k-1}(n)}\right)$$

3. INTRODUCTION OF A NEW REAL VALUED FUNCTION GIVING A GENERALIZATION OF BINOMIAL THEOREM.

Binomial Theorem states that, for any $a, b \in \mathbb{R}$, $k \in \mathbb{N}$,

$$(a+b)^k = a^k + ka^{k-1}b + \frac{k(k-1)}{1.2}a^{k-2}b^2 + \cdots\cdots + \frac{k(k-1)\cdots\cdots(k-i+1)}{1.2.3\cdots\cdots(i-1)i}a^{k-i}b^i + \cdots\cdots + \frac{k(k-1)\cdots 3.2.1}{1.2.3\cdots(k-1)k}b^k$$

$$= a^k + \theta_1 ka^{k-1}b + \theta_1\theta_2\, k(k-1)a^{k-2}b^2 + \cdots\cdots + \theta_1\theta_2\cdots\cdots\theta_i\, k(k-1)\cdots\cdots(k-i+1)a^{k-i}b^i + \cdots\cdots\cdots\cdots + \theta_1\theta_2\cdots\cdots\theta_k\,(k!)\,b^k,$$

where $\theta_i = \frac{1}{i} \in (0, 1]$, for $i = 1,2, \ldots, k$.

Getting motivation from this result, let us define a function $f_k : I^k \times \mathbb{R}^2 \longrightarrow \mathbb{R}$,

where $I = (0, 1]$ and $K \in \mathbb{N}$, by

$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ; a, b) = a^k + \theta_1 ka^{k-1}b + \theta_1\theta_2\, k(k-1)a^{k-2}b^2 + \cdots\cdots +$
$\theta_1\theta_2\cdots\cdots\theta_i\, k(k-1)\cdots\cdots(k-i+1)a^{k-i}b^i + \cdots\cdots + \theta_1\theta_2\cdots\cdots\theta_k\,(k!)\,b^k,$
$\forall\, \theta_i \in I\ \ (i = 1, 2, \ldots, k),\ \forall\, a, b \in \mathbb{R}$ ..... (3.1).

For a given $k \in \mathbb{N}$, the function f_k is called a General Binomial function of order k.

For $i = 1, 2, \ldots, k$, if θ_i be substituted by $\frac{1}{i}$ in (3.1), then

$f_k\left(1, \frac{1}{2}, \ldots, \frac{1}{k} ;\ a, b\right) = (a+b)^k,$

$\forall\, a, b \in \mathbb{R}$ and for a given $k \in \mathbb{N}$ ..... (3.2).

SOME PROPERTIES OF GENERAL BINOMIAL FUNCTION :

Property 1. : For a given $K \in \mathbb{N}$, $\forall\, \theta_i \in I\ \ (i = 1, 2, \ldots, k),\ \forall\, a \in \mathbb{R}$,

$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ; a, a) = a^k f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ; 1, 1)$.

Proof : Putting $a = b$ in (3.1), we get

for a given $k \in \mathbb{N}$, $\forall\, \theta_i \in I\ \ (i = 1, 2, \ldots, k),\ \forall\, a \in \mathbb{R}$,

$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ; a, a) = a^k + \theta_1 ka^{k-1}a + \theta_1\theta_2\, k(k-1)a^{k-2}a^2 + \cdots\cdots +$
$\theta_1\theta_2\cdots\cdots\theta_i\, k(k-1)\cdots\cdots(k-i+1)a^{k-i}a^i + \cdots\cdots + \theta_1\theta_2\cdots\cdots\theta_k\,(k!)\,a^k$
$= a^k\{1 + \theta_1 k + \theta_1\theta_2\, k(k-1) + \cdots\cdots + \theta_1\theta_2\cdots\cdots\theta_i\, k(k-1)\cdots\cdots(k-i+1) + \ldots\ldots +$
$\theta_1\theta_2\cdots\cdots\theta_k\,(k!)\}$
$= a^k f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ; 1, 1).$

Property 2. : For a given $k \in \mathbb{N}$, $\forall\, a, b \in \mathbb{R}$,

(i) $f_k\left(\frac{1}{k}, \frac{1}{k-1}, \ldots\ldots, \frac{1}{2}, 1; a, b\right) = \begin{cases} \frac{a^{k+1}-b^{k+1}}{a-b}, & \text{if } a \neq b \\ (k+1)a^k, & \text{if } a = b \end{cases}$

(ii) $f_k\left(\frac{1}{k}, \frac{1}{k-1}, \ldots\ldots, \frac{1}{2}, 1; a, -b\right) = \begin{cases} \frac{a^{k+1}+b^{k+1}}{a+b}, & \text{if } a+b=0 \text{ and } k \text{ is even} \\ \frac{a^{k+1}-b^{k+1}}{a-b}, & \text{if } a+b \neq 0 \text{ and } k \text{ is odd} \\ a^k, & \text{if } a+b=0 \text{ and } k \text{ is even} \\ 0, & \text{if } a+b=0 \text{ and } k \text{ is odd} \end{cases}$

Proof : (i) For $i = 1, 2, \ldots\ldots, k$, substituting $\theta_i = \frac{1}{k-i+1}$ in (3.1), we get

$$f_k\left(\frac{1}{k}, \frac{1}{k-1}, \ldots\ldots, \frac{1}{2}, 1; a, b\right) = a^k + a^{k-1}b + a^{k-2}b^2 + \cdots\ldots + a^{k-i}b^i + b^k$$

$$= \begin{cases} \frac{a^{k+1}-b^{k+1}}{a-b}, & \text{if } a \neq b \\ (k+1)a^k, & \text{if } a = b \end{cases}$$

(ii) Replacing b by $-b$ in the result of (i), if $a + b \neq 0$, then

$$f_k\left(\frac{1}{k}, \frac{1}{k-1}, \ldots\ldots, \frac{1}{2}, 1; a, -b\right) = \frac{a^{k+1}-(-b)^{k+1}}{a-(-b)} = \begin{cases} \frac{a^{k+1}+b^{k+1}}{a+b}, & \text{if } k \text{ is even} \\ \frac{a^{k+1}-b^{k+1}}{a+b}, & \text{if } k \text{ is odd} \end{cases}$$

If $a + b = 0$ then $b = -a$ and hence

$f_k\left(\frac{1}{k}, \frac{1}{k-1}, \ldots\ldots, \frac{1}{2}, 1; a, -a\right) = a^k + a^{k-1}(-a) + a^{k-2}(-a)^2 + \cdots\ldots +$
$a^{k-i}(-a)^i + \cdots\ldots + (-a)^k$

$$= \begin{cases} a^k, & \text{if } k \text{ is even} \\ 0, & \text{if } k \text{ is odd} \end{cases}$$

Hence the result.

Property 3. : (i) For a given $\in \mathbb{N}$, $\forall\, a, b \in \mathbb{R}$ and $\forall\, \theta, \theta_1, \theta_2, \ldots\ldots, \theta_k \in I$,

$f_k(\theta\theta_1, \theta\theta_2, \ldots\ldots, \theta\theta_k; a, b) = \theta^k f_k\left(\theta_1, \theta_2, \ldots\ldots, \theta_k; \frac{a}{\theta}, b\right) = f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k; a, \theta b)$

(ii) For a given K $\in \mathbb{N}$, $\forall\, a, b \in \mathbb{R}$, $\forall\ \theta_1, \theta_2, \ldots\ldots, \theta_k \in I$ and $\forall\, \theta \geq \max(\theta_1, \theta_2, \ldots\ldots, \theta_k)$

$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k; a\theta, b) = \theta^k f_k\left(\frac{\theta_1}{\theta}, \frac{\theta_2}{\theta}, \ldots\ldots, \frac{\theta_k}{\theta}; a, b\right) = \theta^k f_k\left(\theta_1, \theta_2, \ldots\ldots, \theta_k; a, \frac{b}{\theta}\right)$

Proof : (i) $f_k(\theta\theta_1, \theta\theta_2, \ldots\ldots, \theta\theta_k; a, b)$

$= a^k + \theta\theta_1 k a^{k-1}b + \theta\theta_1\theta\theta_2\, k(k-1)a^{k-2}b^2 + \cdots\ldots +$
$\theta\theta_1\theta\theta_2 \ldots\ldots \theta\theta_i\, k(k-1)\ldots\ldots(k-i+1)a^{k-i}b^i + \cdots\ldots + \theta\theta_1\theta\theta_2 \ldots\ldots \theta\theta_k\, (k!)\, b^k$

$= a^k + \theta_1 k a^{k-1}(\theta b) + \theta_1\theta_2\, k(k-1)a^{k-2}(\theta b)^2 + \cdots\ldots +$
$\theta_1\theta_2 \ldots\ldots \theta_i\, k(k-1)\ldots\ldots(k-i+1)a^{k-i}(\theta b)^i + \cdots\ldots + \theta_1\theta_2 \ldots\ldots \theta_k\, (k!)\, (\theta b)^k$

$= f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k; a, \theta b)$

Again,

$f_k(\theta\theta_1, \theta\theta_2, \ldots\ldots, \theta\theta_k; a, b) = a^k + \theta\theta_1 k a^{k-1}b + \theta\theta_1\theta\theta_2\, k(k-1)a^{k-2}b^2 + \cdots\ldots +$

$$\theta\theta_1\theta\theta_2 \dots\dots\theta\theta_i\, k(k-1)\dots\dots(k-i+1)a^{k-i}b^i$$

$$+\dots\dots+\theta\theta_1\theta\theta_2\dots\dots\theta\theta_k\,(k!)\,b^k$$

$$=\theta^k\left(\left(\frac{a}{\theta}\right)^k+\theta_1 k\left(\frac{a}{\theta}\right)^{k-1}b+\theta_1\theta_2 k(k-1)\left(\frac{a}{\theta}\right)^{k-2}b^2+\dots\dots+\theta_1\theta_2\dots\theta_k(k!)\,b^k\right)$$

$$=\theta^k f_k\left(\theta_1,\theta_2,\dots\dots,\theta_k;\ \frac{a}{\theta},\ b\right)$$

(ii) Clearly $\frac{\theta_1}{\theta},\frac{\theta_2}{\theta},\dots\dots,\frac{\theta_k}{\theta}\in I$.

Now,

$f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a\theta,b)=(a\theta)^k+\theta_1 k(a\theta)^{k-1}b+\theta_1\theta_2 k(k-1)(a\theta)^{k-2}b^2+\dots\dots\dots+\theta_1\theta_2\dots\theta_k(k!)b^k$

$$=\theta^k\left(a^k+\frac{\theta_1}{\theta}ka^{k-1}b+\frac{\theta_1}{\theta}.\frac{\theta_2}{\theta}k(k-1)a^{k-2}b^2+\dots\dots+\frac{\theta_1}{\theta}.\frac{\theta_2}{\theta}\dots\dots\frac{\theta_k}{\theta}(k!)b^k\right)$$

$$=\theta^k f_k\left(\frac{\theta_1}{\theta},\frac{\theta_2}{\theta},\dots\dots,\frac{\theta_k}{\theta};a,b\right)$$

Again,

$f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a\theta,b)=(a\theta)^k+\theta_1 k(a\theta)^{k-1}b+\theta_1\theta_2 k(k-1)(a\theta)^{k-2}b^2+\dots\dots+\theta_1\theta_2\dots\theta_k(k!)b^k$

$$=\theta^k\left(a^k+\theta_1 ka^{k-1}\frac{b}{\theta}+\theta_1\theta_2 k(k-1)a^{k-2}\left(\frac{b}{\theta}\right)^2+\dots\dots+\theta_1\theta_2\dots\theta_k(k!)\left(\frac{b}{\theta}\right)^k\right)$$

$$=\theta^k f_k\left(\theta_1,\theta_2,\dots\dots,\theta_k;\ a,\frac{b}{\theta}\right)$$

Property 4. : For a given $K\in\mathbb{N}$, $\forall\, a\in\mathbb{R}$ and $\forall\ \theta_1,\theta_2,\dots\dots,\theta_k\in I$,

(i) $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a,0)=a^k$

(ii) $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;0,a)=\theta_1\theta_2\dots\dots\theta_k(k!)a^k$

Proof : Immediately follows from definition.

MORE SCENARIO :

(α) We know that $(a+b)^k=(b+a)^k,\ \ \forall\, k\in\mathbb{N},\ \forall\, a,b\in\mathbb{R}$

i.e., $f_k\left(1,\frac{1}{2},\dots\dots,\frac{1}{k};\ a,b\right)=f_k\left(1,\frac{1}{2},\dots\dots,\frac{1}{k};\ b,a\right),\ \forall\, k\in\mathbb{N},\ \forall\, a,b\in\mathbb{R}$

But it is clear that $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a,b)\neq f_k(\theta_1,\theta_2,\dots\dots,\theta_k;\ b,\ a)$, in general.

Now equating $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a,b)$ and $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;\ b,\ a)$, and from definition of f_k we can easily determine the condition of equality of $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a,b)$ and $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;\ b,\ a)$.

Thus we see that f_k is not commutative with respect to a and b. Also it is clear that f_k is not commutative with respect to θ_i and θ_j, for $i,j\in\{1,2,\dots\dots,k\}$ with $i\neq j$.

(β) We have $(a+b+c)^k=((a+b)+c)^k=(a+(b+c))^k$, $\forall k\in\mathbb{N},\ \forall\, a,b,c\in\mathbb{R}$

i.e., $f_k\left(1,\frac{1}{2},\dots\dots,\frac{1}{k};\ a+b,c\right)=f_k\left(1,\frac{1}{2},\dots\dots,\frac{1}{k};\ a,b+c\right),\ \forall k\in\mathbb{N},\ \forall\, a,b,c\in\mathbb{R}$.

But $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a+b,c)=f_k(\theta_1,\theta_2,\dots\dots,\theta_k;\ a,\ b+c)$, in general.

Now following definition of f_k we can easily determine the condition of equality of $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;a+b,\ c)$ and $f_k(\theta_1,\theta_2,\dots\dots,\theta_k;\ a,\ b+c)$, stated as :

$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a+b\ ,\ c) = f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a,\ b+c)$ iff $\theta_i = \frac{1}{i}$, for $i = 1, 2, \ k$.

Thus this property holds for binomial function only, but not for general binomial function.

(γ) We have, $(a+b)^{p+q} = (a+b)^p (a+b)^q, \forall\, p, q \in \mathbb{N}, \forall\, a, b \in \mathbb{R}$

i.e.,

$f_{p+q}\left(1, \frac{1}{2}, \ldots\ldots, \frac{1}{p+q}\ ;\ a\ ,b\right) = f_p\left(1, \frac{1}{2}, \ldots\ldots, \frac{1}{p}\ ;\ a\ ,b\right).f_q\left(1, \frac{1}{2}, \ldots\ldots, \frac{1}{q}\ ;\ a\ ,b\right)\ ,\ \forall\, p, q \in \mathbb{N}\ \ ,$

$\forall\, a, b \in \mathbb{R}.$

We shall determine the condition under which

$$f_{p+q}(\theta_1, \theta_2, \ldots, \theta_{p+q}\ ;\ a\ ,b) =$$

$$f_p(\phi_1, \phi_2, \ldots\ldots, \phi_p\ ;\ a\ ,b) f_q(\psi_1, \psi_2, \ldots\ldots, \psi_q\ ;\ a, b) \quad \ldots\ldots\ldots (\gamma 1).$$

$\forall\, \theta_i \in I\ \ (i = 1,2, \ldots\ldots, p+q), \forall\, a, b \in \mathbb{R},\ \forall\, p, q \in \mathbb{N}$ and for suitable $\phi_i, \psi_j \in I\ \ (i = 1,2, \ldots\ldots, p;\ \ \ \ \ \ j = 1,2, \ldots, q).$

From the relation $(\gamma 1)$, we have

$$a^{p+q} + \theta_1 (p+q) a^{p+q-1} b + \theta_1 \theta_2 (p+q)(p+q-1) a^{p+q-2} b^2 + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_i\ (p+q)(p+q-1)\ldots\ldots(p+q-i+1) a^{p+q-i} b^i + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_{p+q} (p+q)!\, b^{p+q}$$

$$= (a^p + \phi_1\, p a^{p-1} b + \phi_1 \phi_2\, p(p-1) a^{p-2} b^2 + \cdots\ldots + \phi_1 \phi_2 \ldots \phi_j p(p-1)\ldots(p-j+1) a^{p-j} b^j +$$
$$\ldots\ldots + \phi_1 \phi_2 \ldots \phi_p (p!) b^p)(a^q + \psi_1\, q a^{q-1} b + \psi_1 \psi_2\, q(q-1) a^{q-2} b^2 + \cdots\ldots +$$
$$\psi_1 \psi_2 \ldots \psi_t\, q(q-1)\ldots(q-t-1) a^{q-t} b^t + \cdots\ldots + \psi_1 \psi_2 \ldots \psi_q (q!) b^q$$

which is an identity.

Therefore, we have

$\theta_1 (p+q) = \phi_1\, p + \psi_1\, q\ ,$

$\theta_1 \theta_2 (p+q)(p+q-1) = \phi_1 \phi_2\, p(p-1) + \phi_1 \psi_1 pq + \psi_1 \psi_2\, q(q-1),$

.....

.....

$\theta_1 \theta_2 \ldots\ldots \theta_i\ (p+q)(p+q-1)\ldots\ldots(p+q-i+1)$

$$= \begin{cases} \phi_1 \phi_2 \ldots \phi_i p(p-1)\ldots(p-i+1) + \psi_1 \phi_1 \phi_2 \ldots \phi_{i-1} qp(p-1)\ldots(p-i+2) + \cdots\ldots \\ \qquad + \psi_1 \psi_2 \ldots \psi_i q(q-1)\ldots(q-i+1), \quad \textit{if } p \geq i,\ q \geq i. \\ \phi_1 \phi_2 \ldots \phi_i p(p-1)\ldots(p-i+1) + \phi_1 \phi_2 \ldots \phi_{i-1} \psi_1 p(p-1)\ldots(p-i+2) q + \cdots\ldots \\ \qquad + \phi_1 \phi_2 \ldots \phi_{i-q} \psi_1 \psi_2 \ldots \psi_q p(p-1)\ldots(p-i+q+1)(q!), \quad \textit{if } p \geq i,\ q < i. \\ \phi_1 \phi_2 \ldots \phi_p \psi_1 \psi_2 \ldots \psi_{i-p} (p!) q(q-1)\ldots(q-i+p+1) \\ \qquad + \phi_1 \phi_2 \ldots \phi_{p-1} \psi_1 \psi_2 \ldots \psi_{i-p-1} p(p-1)\ldots 2.\, q(q-1)\ldots(q-i+p) + \cdots\ldots \\ \qquad + \psi_1 \psi_2 \ldots \psi_i q(q-1)\ldots(q-i+1), \quad \textit{if } p < i,\ q \geq i \\ \phi_1 \phi_2 \ldots \phi_p \psi_1 \psi_2 \ldots \psi_{i-p} (p!) q(q-1)\ldots(q-i+p+1) \\ \qquad + \phi_1 \phi_2 \ldots \phi_{p-1} \psi_1 \psi_2 \ldots \psi_{i-p-1} p(p-1)\ldots 2.\, q(q-1)\ldots(q-i+p) + \cdots\ldots \\ \qquad + \phi_1 \phi_2 \ldots \phi_{i-q} \psi_1 \psi_2 \ldots \psi_q p(p-1)\ldots(p-i+q+1)(q!), \textit{if } p < i, q < i,\ p+q \geq i. \end{cases}$$

.....

.....

$\theta_1\theta_2 \dots \dots \theta_{p+q}\,(p+q)! = \phi_1\phi_2 \dots \phi_p\psi_1\psi_2 \dots \psi_q(p!)(q!)$.

From these $(p+q)$ equations we can determine $(p+q)$ unknowns $\phi_1, \phi_2, \dots, \phi_p, \psi_1, \psi_2, \dots, \psi_q$ in terms of $\theta_1, \theta_2, \dots \dots, \theta_{p+q}, p, q$.

(δ) For a given $k \in \mathbb{N}, \forall \theta_1\theta_2 \dots \dots \theta_k \in I, \forall a, b, c(\neq 0) \in \mathbb{R}$,

$f_k(\theta_1, \theta_2, \dots \dots, \theta_k : a, b) = f_k(\theta_1, \theta_2, \dots \dots, \theta_k : a-c, b+c)$ iff $\theta_i = \frac{1}{i}$.

Proof : Let $f_k(\theta_1, \theta_2, \dots \dots, \theta_k : a, b) = f_k(\theta_1, \theta_2, \dots \dots, \theta_k : a-c, b+c)$

Then

$a^k + \theta_1 ka^{k-1}b + \theta_1\theta_2\, k(k-1)a^{k-2}b^2 - \dots \dots + \theta_1\theta_2 \dots \dots \theta_i\, k(k-1) \dots (k-i+1)a^{k-i}b^i + \dots \dots$
$+ \theta_1\theta_2 \dots \dots \theta_k\,(k!)b^k =$
$(a-c)^k + \theta_1 k(a-c)^{k-1}(b+c) + \theta_1\theta_2\, k(k-1)(a-c)^{k-2}(b+c)^2$
$+ \dots \dots + \theta_1\theta_2 \dots \dots \theta_i\, k(k-1) \dots \dots (k-i+1)(a-c)^{k-i}(b+c)^i + \dots \dots +$
$\theta_1\theta_2 \dots \dots \theta_k\,(k!)(b+c)^k$

which is an identity.

..... $(\delta 1)$.

Comparing coefficients of $a^{k-1}c$ from both sides of $(\delta 1)$, we get

$-k + \theta_1 k = 0$, or, $\theta_1 = 1 = \frac{1}{1}$.

Again comparing coefficients of $a^{k-2}c^2$ from both sides of $(\delta 1)$, we get

$\binom{k}{2} - \theta_1 k\binom{k-1}{1} + \theta_1\theta_2\, k(k-1) = 0$,

or, $\frac{k(k-1)}{2} - k(k-1) + \theta_2\, k(k-1) = 0 \quad (\because \theta_1 = 1)$

or, $\theta_2 = \frac{1}{2}$.

Thus the result holds for $i = 1, 2$.

Let $i > 2$ and the result holds for 1, 2, ..., i-1, *i.e.*, $\theta_r = \frac{1}{r}$, for $r = 1, 2, \dots \dots, i-1$

..... $(\delta 2)$

Comparing coefficients of $a^{k-i}c^i$ from both sides of $(\delta 1)$, we get

$(-1)^i\binom{k}{i} + \theta_1\, k(-1)^{i-1}\binom{k-1}{i-1} + \theta_1\theta_2\, k(k-1)(-1)^{i-2}\binom{k-2}{i-2} + \dots \dots \dots$
$+\theta_1\theta_2 \dots \dots \theta_{i-2}\, k(k-1) \dots \dots (k-i+3)\binom{k-i+2}{2}$
$-\theta_1\theta_2 \dots \dots \theta_{i-1}\, k(k-1) \dots \dots (k-i+2)\binom{k-i+1}{1} + \theta_1\theta_2 \dots \dots \theta_i\, k(k-1) \dots \dots (k-i+1) = 0$

Or, $\frac{\theta_i\, k(k-1)\dots(k-i+1)}{1.2\dots(i-1)} - \frac{k(k-1)\dots(k-i+1)}{1.2\dots(i-1)(i)} + \frac{k(k-1)\dots(k-i+1)}{1.2\dots(i-2)(2i)} - \dots \dots + (-1)^{i-2}\frac{k(k-1)\dots(k-i+1)}{1.2\ (i-2)i}$
$+(-1)^{i-1}\frac{k(k-1)\dots(k-i+1)}{1\ (i-1)i} + (-1)^i\frac{k(k-1)\dots(k-i+1)}{(i)} = 0$ (by $(\delta 2)$)

Or,
$\theta_i = 1 - \frac{i-1}{2!} + \frac{(i-1)(i-2)}{3!} - \dots \dots + (-1)^{i-1}\frac{(i-1)(i-2)\dots 3}{(i-2)!} + (-1)^i\frac{(i-1)(i-2)\dots 2}{(i-1)!} + (-1)^{i+1}\frac{(i-1)(i-2)\dots 2.1}{(i)!}$

$$= \frac{1}{i}\left\{1 - \frac{i(i-1)}{2!} + \frac{i(i-1)(i-2)}{3!} - \cdots\cdots + (-1)^{i-1}\frac{i(i-1)(i-2)\cdots 3}{(i-2)!} + (-1)^{i}\frac{i(i-1)(i-2)\cdots 2}{(i-1)!} + (-1)^{i+1}.1\right\}$$

$$= \frac{1}{i}\left\{-1 + \binom{i}{1} - \binom{i}{2} + \binom{i}{3} - \cdots\cdots + (-1)^{i+1} + 1\right\} = \frac{1}{i}\left\{1 - (1-1)^{i}\right\} = \frac{1}{i}.$$

Hence the result holds for i.

Hence the result.

Converse part is obvious.

(σ) We have the following recurrence relations on General Binomial Function.

(i) For a given $k\,(>1) \in \mathbb{N}, \forall \theta_1 \theta_2 \ldots\ldots \theta_k \in I,\ \forall a, b \in \mathbb{R}$,

$$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a,\ b) = a^k + \theta_1 kb f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k\ ;\ a,\ b).$$

(ii) For a given $k\,(>1) \in \mathbb{N}, \forall \theta_1 \theta_2 \ldots\ldots \theta_k \in I,\ \forall a, b \in \mathbb{R}$,

$$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a,\ b) = a f_{k-1}(\theta_1, \theta_2, \ldots\ldots, \theta_{k-1}\ ;\ a,\ b) + \theta_1 b f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k\ ;\ a,\ b)$$
$$+(k-1)\theta_1\theta_2 b^2 f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k\ ;\ a,\ b) + (k-1)(k-2)\theta_1\theta_2\theta_3 b^3 f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k\ ;\ a,\ b)$$
$$+\cdots\ldots + (k-1)(k-2)\ldots(k-i+1)\theta_1\theta_2\ldots\theta_i b^i f_{k-i}(\theta_{i+1}, \theta_{i+2}, \ldots\ldots, \theta_k\ ;\ a,\ b) + \cdots\ldots$$
$$+(k-1)(k-2)\ldots 2.\theta_1\theta_2\ldots\theta_{k-1} b^{k-1} f_1(\theta_k\ ;\ a,\ b) + (k-1)!\,\theta_1\theta_2\ldots\theta_k b^k$$

(iii) For a given $k\,(>1) \in \mathbb{N}, \forall \theta_1 \theta_2 \ldots\ldots \theta_k \in I,\ \forall a, b \in \mathbb{R}$,

$$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a,\ b) = a f_{k-1}(\theta_1, \theta_2, \ldots\ldots, \theta_{k-1}\ ;\ a,\ b) + \theta_1 ab f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1}\ ;\ a\ b)$$
$$+k\theta_1\theta_2 a b^2 f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1}\ ;\ a,\ b) - k(k-1)\theta_1\theta_2\theta_3 a b^3 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1}\ ;\ a,\ b)$$
$$+\cdots\ldots + k(k-1)\ldots(k-i+2)\theta_1\theta_2\ldots\theta_i a b^i f_{k-i-1}(\theta_{i+1}, \theta_{i+2}, \ldots\ldots, \theta_{k-1}\ ;\ a,\ b) + \cdots\ldots$$
$$+k(k-1)\ldots 4.\theta_1\theta_2\ldots\theta_{k-2} a b^{k-2} f_1(\theta_{k-1}\ ;\ a,\ b) + k(k-1)\ldots 3.\theta_1\theta_2\ldots\theta_{k-1} a b^{k-1}$$
$$+(k!)\theta_1\theta_2\ldots\theta_k b^k$$

Proof :

(i) $f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k\ ;\ a,\ b) = a^k + \theta_1 k a^{k-1} b + \theta_1\theta_2 k(k-1)a^{k-2}b^2 + \cdots\ldots + \theta_1\theta_2\ldots\ldots\theta_k (k!) b^k$

$$= a^k + \theta_1 kb\left(a^{k-1} + \theta_2(k-1)a^{k-2}b + \cdots\ldots + \theta_2\theta_3\ldots\ldots\theta_k(k-1)!\,b^{k-1}\right)$$

$$= a^k + \theta_1 kb f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k\ ;\ a,\ b).$$

(ii) At first we shall prove the following result by means of Lemma(4).

Lemma (4) : For a given $k\,(>1) \in \mathbb{N}, \forall \theta_2 \ldots\ldots \theta_k \in I,\ \forall a, b \in \mathbb{R}$,

$$a^{k-1} + 2(k-1)\theta_2 a^{k-2} b + 3(k-1)(k-2)\theta_2\theta_3 a^{k-3} b^2 + \cdots\ldots +$$
$$(k-1)(k-1)(k-2)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1} a b^{k-2} + (k!)\theta_2\theta_3\ldots\theta_k b^{k-1}$$
$$= f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k\ ;\ a,\ b) + (k-1)\theta_2 b f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k\ ;\ a,\ b) +$$
$$(k-1)(k-2)\theta_2\theta_3 b^2 f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k\ ;\ a,\ b) + \cdots\ldots +$$
$$(k-1)(k-2)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_i b^{i-1} f_{k-i}(\theta_{i+1}, \theta_{i+2}, \ldots\ldots, \theta_k\ ;\ a,\ b) + \cdots\ldots +$$
$$(k-1)(k-2)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1} b^{k-2} f_1(\theta_k\ ;\ a,\ b) + (k-1)!\,\theta_2\theta_3\ldots\theta_k b^{k-1}.$$

Proof of Lemma (4) : We shall prove it by mathematical induction on k.

Let $k = 2$.

Then the expression of left hand side =

$$a^{2-1} + (2!)\theta_2 b^{2-1} = a + (2!)\theta_2 b = a + \theta_2 b + \theta_2 b =$$

$= f_1(\theta_2;\ a,\ b) + \theta_2 b = f_{2-1}(\theta_2;\ a,\ b) + (2-1)!\,\theta_2 b^{2-1}$ = expression of right hand side.

Therefore the result holds for $k = 2$.

Let $k > 2$ and the result hold for $k = 2, 3, \ldots, k\text{-}1$.

Then, $a^{k-2} + 2(k-2)\theta_2 a^{k-3}b + 3(k-2)(k-3)\theta_2\theta_3 a^{k-4}b^2 + \cdots\ldots +$
$(k-2)(k-2)(k-3)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-2}ab^{k-3} + (k-1)!\,\theta_2\theta_3\ldots.\theta_{k-1}b^{k-2}$
$= f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b) + (k-2)\theta_2 b f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) +$
$(k-2)(k-3)\theta_2\theta_3 b^2 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$
$(k-2)(k-3)\ldots.(k-i)\theta_2\theta_3\ldots.\theta_i b^{i-1} f_{k-i-1}(\theta_{i+1}, \theta_{i+2}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$
$(k-2)(k-3)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-2}b^{k-3} f_1(\theta_{k-1};\ a,\ b) + (k-2)!\,\theta_2\theta_3\ldots.\theta_{k-1}b^{k-2}.$

Hence, $a^{k-2} + 2(k-2)\theta_3 a^{k-3}b + 3(k-2)(k-3)\theta_3\theta_4 a^{k-4}b^2 + \cdots\ldots +$
$(k-2)(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1}ab^{k-3} + (k-1)!\,\theta_3\theta_4\ldots.\theta_k b^{k-2}$
$= f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k;\ a,\ b) + (k-2)\theta_3 b f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-2)(k-3)\theta_3\theta_4 b^2 f_{k-4}(\theta_5, \theta_6, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$
$(k-2)(k-3)\ldots.(k-i)\theta_3\theta_4\ldots.\theta_{i+1}b^{i-1} f_{k-i-1}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$
$(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1}b^{k-3} f_1(\theta_k;\ a,\ b) + (k-2)!\,\theta_3\theta_4\ldots.\theta_k b^{k-2}.$
$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$ **(σ1).**

Now, $a^{k-1} + 2(k-1)\theta_2 a^{k-2}b + 3(k-1)(k-2)\theta_2\theta_3 a^{k-3}b^2 + \cdots\ldots +$
$(k-1)(k-1)(k-2)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1}ab^{k-2} + (k!)\theta_2\theta_3\ldots.\theta_k b^{k-1}$
$= \{a^{k-1} + (k-1)\theta_2 a^{k-2}b + (k-1)(k-2)\theta_2\theta_3 a^{k-3}b^2 + \cdots\ldots +$
$(k-1)(k-2)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1}ab^{k-2} + (k-1)!\,\theta_2\theta_3\ldots.\theta_k b^{k-1}\} + \{(k-1)\theta_2 a^{k-2}b +$
$2(k-1)(k-2)\theta_2\theta_3 a^{k-3}b^2 + \cdots\ldots + (k-2)(k-1)(k-2)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1}ab^{k-2} +$
$(k-1)((k-1)!)\theta_2\theta_3\ldots.\theta_k b^{k-1}\}$
$= f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k;\ a,\ b) + (k-1)\theta_2 b\{a^{k-2} + 2(k-2)\theta_3 a^{k-3}b + 3(k-2)(k-3)\theta_3\theta_4 a^{k-4}b^2$
$+ \cdots\ldots + (k-2)(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1}ab^{k-3} + (k-1)!\,\theta_3\theta_4\ldots.\theta_k b^{k-2}\}$
$= f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k;\ a,\ b) + (k-1)\theta_2 b\{f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-2)\theta_3 b f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k;\ a,\ b) + (k-2)(k-3)\theta_3\theta_4 b^2 f_{k-4}(\theta_5, \theta_6, \ldots\ldots, \theta_k;\ a,\ b)$
$+ \cdots\ldots + (k-2)(k-3)\ldots.(k-i)\theta_3\theta_4\ldots.\theta_{i+1}b^{i-1} f_{k-i-1}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$
$(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1}b^{k-3} f_1(\theta_k;\ a,\ b) + (k-2)!\,\theta_3\theta_4\ldots.\theta_k b^{k-2}\}$ **(by(σ1))**
$= f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k;\ a,\ b) + (k-1)\theta_2 b f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-1)(k-2)\theta_2\theta_3 b^2 f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-1)(k-2)(k-3)\theta_2\theta_3\theta_4 b^3 f_{k-4}(\theta_5, \theta_6, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$
$(k-1)(k-2)(k-3)\ldots.(k-i)\theta_2\theta_3\ldots.\theta_{i-1}b^i f_{k-i-1}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$
$(k-1)(k-2)(k-3)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1}b^{k-2} f_1(\theta_k;\ a,\ b) + (k-1)!\,\theta_2\theta_3\ldots.\theta_k b^{k-1}$
$= f_{k-1}(\theta_2, \theta_3, \ldots\ldots, \theta_k;\ a,\ b) + (k-1)\theta_2 b f_{k-2}(\theta_3, \theta_4, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-1)(k-2)\theta_2\theta_3 b^2 f_{k-3}(\theta_4, \theta_5, \ldots\ldots, \theta_k;\ a,\ b) +$
$(k-1)(k-2)(k-3)\theta_2\theta_3\theta_4 b^3 f_{k-4}(\theta_5, \theta_6, \ldots\ldots, \theta_k;\ a,\ b) + \cdots\ldots +$

$(k-1)(k-2)(k-3)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_i b^{k-i} f_{k-i}(\theta_{i+1},\theta_{i+2},\ldots\ldots,\theta_k;\ a,\ b)+\cdots\ldots+$
$(k-1)(k-2)(k-3)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}f_1(\theta_k;\ a,\ b)+(k-1)!\,\theta_2\theta_3\ldots\theta_k b^{k-1}$

Therefore the result holds for $k=k$.

Hence, by Mathematical induction, we have the Lemma.

Now we shall prove property (ii) by Mathematical induction on k.

Let $k=2$.

Then, the expression of left hand side $= f_2(\theta_1,\theta_2;\ a,\ b) = a^2+2\theta_1 ab+(2!)\theta_1\theta_2 b^2$

$= a(a+\theta_1 b)+\theta_1 ab+(2!)\theta_1\theta_2 b^2 = af_1(\theta_1;\ a,\ b)+\theta_1 b(a+\theta_2 b)+\theta_1\theta_2 b^2$

$= af_1(\theta_1;\ a,\ b)+\theta_1 bf_1(\theta_2;\ a,\ b)+\theta_1\theta_2 b^2$

$= af_{2-1}(\theta_1;\ a,\ b)+\theta_1 bf_{2-1}(\theta_2;\ a,\ b)+(2-1)!\,\theta_1\theta_2 b^2$ = expression of right hand side.

Therefore the result holds for $k=2$.

Let $k>2$ and the result hold for $k=2, 3, \ldots.$, k-1.

Now, $f_k(\theta_1,\theta_2,\ldots\ldots,\theta_k;\ a,\ b)$

$= a^k+\theta_1 ka^{k-1}b+\theta_1\theta_2 k(k-1)a^{k-2}b^2+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_i k(k-1)\ldots\ldots(k-i+1)a^{k-i}b^i$
$+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_k(k!)\,b^k$

$= a\{a^{k-1}+\theta_1 ka^{k-2}b+\theta_1\theta_2 k(k-1)a^{k-3}b^2+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_{k-1}k(k-1)\ldots\ldots 2.b^{k-1}\}+$
$\theta_1\theta_2\ldots\ldots\theta_k(k!)\,b^k$

$= a\{a^{k-1}+\theta_1(k-1)a^{k-2}b+\theta_1\theta_2(k-1)(k-2)a^{k-3}b^2+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_{k-1}(k-1)!\,b^{k-1}\}$
$+a\{\theta_1 a^{k-2}b+\theta_1\theta_2 2(k-1)a^{k-3}b^2+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_{k-1}(k-1)(k-1)(k-2)\ldots 2.b^{k-1}\}$
$+\theta_1\theta_2\ldots\ldots\theta_k(k!)\,b^k$

$= af_{k-1}(\theta_1,\theta_2,\ldots\ldots,\theta_{k-1};\ a,\ b)-\theta_1 b\{a^{k-2}+2(k-2)\theta_2 a^{k-3}b+3(k-1)(k-2)\theta_2\theta_3 a^{k-4}b^2$
$+\cdots\ldots+(k-1)(k-1)(k-2)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1}ab^{k-2}+(k!)\theta_2\theta_3\ldots\theta_k b^{k-1}\}$

$= af_{k-1}(\theta_1,\theta_2,\ldots\ldots,\theta_{k-1};\ a,\ b)+\theta_1 b\{f_{k-1}(\theta_2,\theta_3,\ldots\ldots,\theta_k;\ a,\ b)+$
$(k-1)\theta_2 bf_{k-2}(\theta_3,\theta_4,\ldots\ldots,\theta_k;\ a,\ b)+(k-1)(k-2)\theta_2\theta_3 b^2 f_{k-3}(\theta_4,\theta_5,\ldots\ldots,\theta_k;\ a,\ b)$
$+\cdots\ldots+(k-1)(k-2)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_i b^{i-1}f_{k-i}(\theta_{i+1},\theta_{i+2},\ldots\ldots,\theta_k;\ a,\ b)$
$+\cdots\ldots+(k-1)(k-2)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}f_1(\theta_k;\ a,\ b)+(k-1)!\,\theta_2\theta_3\ldots\theta_k b^{k-1}\}.$

(by Lemma (4)).

$= af_{k-1}(\theta_1,\theta_2,\ldots\ldots,\theta_{k-1};\ a,\ b)+\theta_1 bf_{k-1}(\theta_2,\theta_3,\ldots\ldots,\theta_k;\ a,\ b)+$
$(k-1)\theta_1\theta_2 b^2 f_{k-2}(\theta_3,\theta_4,\ldots\ldots,\theta_k;\ a,\ b)+(k-1)(k-2)\theta_1\theta_2\theta_3 b^3 f_{k-3}(\theta_4,\theta_5,\ldots\ldots,\theta_k;\ a,\ b)$
$+\cdots\ldots+(k-1)(k-2)\ldots(k-i+1)\theta_1\theta_2\ldots\theta_i b^i f_{k-i}(\theta_{i+1},\theta_{i+2},\ldots\ldots,\theta_k;\ a,\ b)$
$+\cdots\ldots+(k-1)(k-2)\ldots 2.\theta_1\theta_2\ldots\theta_{k-1}b^{k-1}f_1(\theta_k;\ a,\ b)+(k-1)!\,\theta_1\theta_2\ldots\theta_k b^k.$

Therefore the result holds for $k=k$.

Hence, by Mathematical induction, we have the property (ii).

(iii) At first we shall prove the following result by means of Lemma(5).

Lemma (5) : For a given $k\ (\geq 3)\in\mathbb{N}, \forall\theta_2\ldots\ldots\theta_{k-1}\in I,\ \forall a,b\in\mathbb{R}$,

$a^{k-2}+2(k-1)\theta_2 a^{k-3}b+3(k-1)(k-2)\theta_2\theta_3 a^{k-4}b^2+\cdots\ldots+$
$(k-1)(k-1)(k-2)\ldots 2.\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}$

$= f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b) + k\theta_2 b f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) +$

$k(k-1)\theta_2\theta_3 b^2 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$

$k(k-1)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_{i+1} b^i f_{k-i-2}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$

$k(k-1)\ldots.4.\theta_2\theta_3\ldots.\theta_{k-2} b^{k-3} f_1(\theta_{k-1};\ a,\ b) + k(k-1)\ldots.3.\theta_2\theta_3\ldots.\theta_{k-1} b^{k-2}.$

Proof of Lemma (5) : We shall prove it by mathematical induction on k.

Let $k = 3$.

Then, expression of left hand side =

$a + 2.2.\theta_2 b = (a + \theta_2 b) + 3\theta_2 b = f_1(\theta_2;\ a,\ b) + 3\theta_2 b$

$= f_{3-2}(\theta_2;\ a,\ b) + 3\theta_2 b^{3-2}$ = expression of right hand side.

Therefore the result holds for $k = 3$.

Let $k > 3$ and the result hold for $k = 3, 4, \ldots\ldots, k-1$.

Then, $a^{k-3} + 2(k-2)\theta_2 a^{k-4} b + 3(k-2)(k-3)\theta_2\theta_3 a^{k-5} b^2 + \cdots\ldots +$

$(k-2)(k-2)(k-3)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-2} b^{k-3}$

$= f_{k-3}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-2};\ a,\ b) + (k-1)\theta_2 b f_{k-4}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-2};\ a,\ b) +$

$(k-1)(k-2)\theta_2\theta_3 b^2 f_{k-5}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-2};\ a,\ b) + \cdots\ldots +$

$(k-1)(k-2)\ldots.(k-i)\theta_2\theta_3\ldots.\theta_{i+1} b^i f_{k-i-3}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_{k-2};\ a,\ b) + \cdots\ldots +$

$(k-1)(k-2)\ldots.4.\theta_2\theta_3\ldots.\theta_{k-3} b^{k-4} f_1(\theta_{k-2};\ a,\ b) +.$

$(k-1)(k-2)\ldots.3.\theta_2\theta_3\ldots.\theta_{k-2} b^{k-3}$

Hence, $a^{k-3} + 2(k-2)\theta_3 a^{k-4} b + 3(k-2)(k-3)\theta_3\theta_4 a^{k-5} b^2$

$+ \cdots\ldots + (k-2)(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1} b^{k-3}$

$= f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) + (k-1)\theta_3 b f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) +$

$(k-1)(k-2)\theta_3\theta_4 b^2 f_{k-5}(\theta_5, \theta_6, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$

$(k-1)(k-2)\ldots.(k-i)\theta_3\theta_4\ldots.\theta_{i+2} b^i f_{k-i-3}(\theta_{i+3}, \theta_{i+4}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$

$(k-1)(k-2)\ldots.4.\theta_3\theta_4\ldots.\theta_{k-2} b^{k-4} f_1(\theta_{k-1};\ a,\ b) +$

$(k-1)(k-2)\ldots.3.\theta_3\theta_4\ldots.\theta_{k-1} b^{k-3}$ ..... $(\sigma 2)$

Now, $a^{k-2} + 2(k-1)\theta_2 a^{k-3} b + 3(k-1)(k-2)\theta_2\theta_3 a^{k-4} b^2 + \cdots\ldots +$

$(k-1)(k-1)(k-2)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1} b^{k-2}$

$= \{a^{k-2} + (k-2)\theta_2 a^{k-3} b + (k-2)(k-3)\theta_2\theta_3 a^{k-4} b^2 + \cdots\ldots + (k-2)!\,\theta_2\theta_3\ldots.\theta_{k-1} b^{k-2}\} +$

$\{k\theta_2 a^{k-3} b + 2k(k-2)\theta_2\theta_3 a^{k-4} b^2 + \cdots\ldots + k(k-2)(k-2)(k-3)\ldots.2.\theta_2\theta_3\ldots.\theta_{k-1} b^{k-2}\}$

$= f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b) + k\theta_2 b\{a^{k-3} + 2(k-2)\theta_3 a^{k-4} b + 3(k-2)(k-3)\theta_3\theta_4 a^{k-5} b^2$

$+ \cdots\ldots + (k-2)(k-2)(k-3)\ldots.2.\theta_3\theta_4\ldots.\theta_{k-1} b^{k-3}\}$

$= f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b) + k\theta_2 b\{f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) +$

$(k-1)\theta_3 b f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) +($

$k-1)(k-2)\theta_3\theta_4 b^2 f_{k-5}(\theta_5, \theta_6, \ldots\ldots, \theta_{k-1};\ a,\ b)$

$+\cdots\ldots+(k-1)(k-2)\ldots(k-i)\theta_2\theta_3\ldots\theta_{i-2}b^i f_{k-i-3}(\theta_{i-3},\theta_{i-2},\ldots\ldots,\theta_{k-1};\ a,\ b)+\cdots\ldots+$
$(k-1)(k-2)\ldots4\,\theta_2\theta_3\ldots\theta_{k-2}b^{k-3}f_1(\theta_{k-1};\ a,\ b)+(k-1)(k-2)\ldots3\,\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}$
(by (σ2))

$= f_{k-2}(\theta_2,\theta_3,\ldots\ldots,\theta_{k-1};\ a,\ b)+k\theta_2 b f_{k-3}(\theta_3,\theta_4,\ldots\ldots,\theta_{k-1};\ a,\ b)+$
$k(k-1)\theta_2\theta_3 b^2 f_{k-4}(\theta_4,\theta_5,\ldots\ldots,\theta_{k-1};\ a,\ b)+$
$k(k-1)(k-2)\theta_2\theta_3\theta_4 b^3 f_{k-5}(\theta_5,\theta_6,\ldots\ldots,\theta_{k-1};\ a,\ b)+\cdots\ldots+$
$k(k-1)(k-2)\ldots(k-i)\theta_2\theta_3\ldots\theta_{i-2}b^{i+1}f_{k-i-3}(\theta_{i-3},\theta_{i-2},\ldots\ldots,\theta_{k-1};\ a,\ b)+\cdots\ldots+$
$k(k-1)(k-2)\ldots4.\,\theta_2\theta_3\ldots\theta_{k-2}b^{k-3}f_1(\theta_{k-1};\ a,\ b)+$
$k(k-1)(k-2)\ldots3.\,\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}$

$= f_{k-2}(\theta_2,\theta_3,\ldots\ldots,\theta_{k-1};\ a,\ b)+k\theta_2 b f_{k-3}(\theta_3,\theta_4,\ldots\ldots,\theta_{k-1};\ a,\ b)+$
$k(k-1)\theta_2\theta_3 b^2 f_{k-4}(\theta_4,\theta_5,\ldots\ldots,\theta_{k-1};\ a,\ b)+$
$k(k-1)(k-2)\theta_2\theta_3\theta_4 b^3 f_{k-5}(\theta_5,\theta_6,\ldots\ldots,\theta_{k-1};\ a,\ b)+\cdots\ldots+$
$k(k-1)(k-2)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_{i+1}b^i f_{k-i-2}(\theta_{i+2},\theta_{i-3},\ldots\ldots,\theta_{k-1};\ a,\ b)+\cdots\ldots+$
$k(k-1)(k-2)\ldots4.\,\theta_2\theta_3\ldots\theta_{k-2}b^{k-3}f_1(\theta_{k-1};\ a,\ b)+$
$k(k-1)(k-2)\ldots3.\,\theta_2\theta_3\ldots\theta_{k-1}b^{k-2}.$

Therefore the result holds for $k=k$.

Hence, by Mathematical induction, we have the Lemma.

Now we shall prove property (iii) by Mathematical induction on k.

Let $k=2$.

Then, the expression of left hand side $= f_2(\theta_1,\theta_2;\ a,\ b) = a^2+2\theta_1 ab+(2!)\theta_1\theta_2 b^2$
$= a(a+\theta_1 b)+\theta_1 ab+(2!)\theta_1\theta_2 b^2 = af_1(\theta_1;\ a,\ b)+\theta_1 ab+(2!)\theta_1\theta_2 b^2$
$= af_{2-1}(\theta_1;\ a,\ b)+\theta_1 ab+(2!)\theta_1\theta_2 b^2$ = expression of right hand side.

Therefore the result holds for $k=2$.

Let $k>2$, *i.e.*, $k\geq 3$ and the result hold for $k=2,3,\ldots\ldots,k-1$.

Now, $f_k(\theta_1,\theta_2,\ldots\ldots,\theta_k;\ a,\ b)$
$= a^k+\theta_1\ ka^{k-1}b+\theta_1\theta_2\ k(k-1)a^{k-2}b^2+\theta_1\theta_2\theta_3 k(k-1)(k-2)a^{k-3}b^3+\cdots\ldots+$
$\theta_1\theta_2\ldots\ldots\theta_i\ k(k-1)\ldots\ldots(k-i+1)a^{k-i}b^i+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_k\ (k!)\ b^k$
$= a\{a^{k-1}+\theta_1\ ka^{k-2}b+\theta_1\theta_2\ k(k-1)a^{k-3}b^2+\theta_1\theta_2\theta_3 k(k-1)(k-2)a^{k-4}b^3+\cdots\ldots+$
$\theta_1\theta_2\ldots\ldots\theta_{k-1}\ k(k-1)\ldots\ldots 2.\,b^{k-1}\}+\theta_1\theta_2\ldots\ldots\theta_k\ (k!)\ b^k$
$= a\{a^{k-1}+\theta_1\ (k-1)a^{k-2}b-\theta_1\theta_2\ (k-1)(k-2)a^{k-3}b^2+\theta_1\theta_2\theta_3(k-1)(k-2)(k-3)a^{k-4}b^3$
$+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_{k-1}\ (k-1)!\,b^{k-1}\}+a\{\theta_1\ a^{k-2}b+\theta_1\theta_2\ 2(k-1)a^{k-3}b^2+$
$\theta_1\theta_2\theta_3 3(k-1)(k-2)a^{k-4}b^3+\cdots\ldots+\theta_1\theta_2\ldots\ldots\theta_{k-1}\ (k-1)(k-1)(k-2)\ldots\ldots 2.\,b^{k-1}\}$
$+\theta_1\theta_2\ldots\ldots\theta_k\ (k!)\ b^k$
$= af_{k-1}(\theta_1,\theta_2,\ldots\ldots\theta_{k-1};\ a,\ b)+\theta_1 ab\{a^{k-2}+\theta_2 2(k-1)a^{k-3}b+\theta_2\theta_3 3(k-1)(k-2)a^{k-4}b^2$
$+\cdots\ldots+\theta_2\theta_3\ldots\ldots\theta_{k-1}(k-1)(k-1)(k-2)\ldots\ldots 2.\,b^{k-2}\}+\theta_1\theta_2\ldots\ldots\theta_k\ (k!)\ b^k$
$= af_{k-1}(\theta_1,\theta_2,\ldots\ldots,\theta_{k-1};\ a,\ b)+\theta_1 ab\{f_{k-2}(\theta_2,\theta_3,\ldots\ldots,\theta_{k-1};\ a,\ b)+$

$$k\theta_2 b f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) +$$
$$k(k-1)\theta_2\theta_3 b^2 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) +$$
$$k(k-1)(k-2)\theta_2\theta_3\theta_4 b^3 f_{k-5}(\theta_5, \theta_6, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$$
$$k(k-1)(k-2)\ldots(k-i+1)\theta_2\theta_3\ldots\theta_{i+1} b^i f_{k-i-2}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$$
$$k(k-1)(k-2)\ldots 4.\,\theta_2\theta_3\ldots\theta_{k-2} b^{k-3} f_1(\theta_{k-1};\ a,\ b) +$$
$$k(k-1)(k-2)\ldots 3.\,\theta_2\theta_3\ldots\theta_{k-1} b^{k-2}\} + \theta_1\theta_2\ldots\ldots\theta_k\,(k!)\,b^k \quad \textbf{(by Lemma (5), as, } k \geq 3\textbf{)}$$
$$= a f_{k-1}(\theta_1, \theta_2, \ldots\ldots, \theta_{k-1};\ a,\ b) + \theta_1 ab f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b) +$$
$$k\theta_1\theta_2 a b^2 f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) +$$
$$k(k-1)\theta_1\theta_2\theta_3 a b^3 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b) +$$
$$k(k-1)(k-2)\theta_1\theta_2\theta_3\theta_4 a b^4 f_{k-5}(\theta_5, \theta_6, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$$
$$k(k-1)(k-2)\ldots(k-i+1)\theta_1\theta_2\ldots\theta_{i+1} a b^{i+1} f_{k-i-2}(\theta_{i+2}, \theta_{i+3}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$$
$$k(k-1)(k-2)\ldots 4.\,\theta_1\theta_2\ldots\theta_{k-2} a b^{k-2} f_1(\theta_{k-1};\ a,\ b) +$$
$$k(k-1)(k-2)\ldots 3.\,\theta_1\theta_2\ldots\theta_{k-1} a b^{k-1} + \theta_1\theta_2\ldots\ldots\theta_k\,(k!)\,b^k$$
$$= a f_{k-1}(\theta_1, \theta_2, \ldots\ldots, \theta_{k-1};\ a,\ b) + \theta_1 ab f_{k-2}(\theta_2, \theta_3, \ldots\ldots, \theta_{k-1};\ a,\ b)$$
$$+k\theta_1\theta_2 a b^2 f_{k-3}(\theta_3, \theta_4, \ldots\ldots, \theta_{k-1};\ a,\ b) + k(k-1)\theta_1\theta_2\theta_3 a b^3 f_{k-4}(\theta_4, \theta_5, \ldots\ldots, \theta_{k-1};\ a,\ b)$$
$$+\cdots\ldots + k(k-1)\ldots(k-i+2)\theta_1\theta_2\ldots\theta_i a b^i f_{k-i-1}(\theta_{i+1}, \theta_{i+2}, \ldots\ldots, \theta_{k-1};\ a,\ b) + \cdots\ldots +$$
$$k(k-1)\ldots 4.\,\theta_1\theta_2\ldots\theta_{k-2} a b^{k-2} f_1(\theta_{k-1};\ a,\ b) + k(k-1)\ldots 3.\,\theta_1\theta_2\ldots\theta_{k-1} a b^{k-1}$$
$$+(k!)\theta_1\theta_2\ldots\theta_k b^k$$

Therefore the result holds for $k = k$.

Hence, by Mathematical induction, we have the property (iii).

Note : As a particular case of property(i),

$$f_k\left(1, \tfrac{1}{2}, \ldots\ldots, \tfrac{1}{k};\ a,\ b\right) = a^k + 1.\,k.\,b f_{k-1}\left(\tfrac{1}{2}, \ldots\ldots, \tfrac{1}{k};\ a,\ b\right),\ \forall k\ (>1) \in \mathbb{N},$$
$$\forall a, b \in \mathbb{R}.$$

i.e., $(a+b)^k = a^k + 1.\,k.\,b f_{k-1}\left(\tfrac{1}{2}, \ldots\ldots\ \tfrac{1}{k};\ a,\ b\right),\ \forall k\ (>1) \in \mathbb{N},\ \forall a, b \in \mathbb{R}.$

Therefore, $(a+b)^k = a^k + (a+b)^{k-1}$, in general.

(μ) We can think easily about a – free term ($a = 0$) for odd and even values of $k \in \mathbb{N}$ in the expression

of $f_k\left(\theta_1, \theta_2, \ldots\ldots, \theta_k;\ a, \tfrac{1}{a}\right)$ and $f_k\left(\theta_1, \theta_2, \ldots\ldots, \theta_k;\ \tfrac{1}{a}, a\right),\ a \in \mathbb{R},\ \theta_1, \theta_2, \ldots\ldots, \theta_k \in I$.

(ν) We can extend f_k $(k \in \mathbb{N})$ to a real valued function F_k as :

$F_k : \mathbb{R}^k \times \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by

$$F_k(\theta_1, \theta_2, \ldots\ldots, \theta_k;\ a,\ b) = a^k + \theta_1 k a^{k-1} b + \theta_1\theta_2 k(k-1) a^{k-2} b^2 + \cdots\ldots +$$
$$\theta_1\theta_2\ldots\ldots\theta_i\, k(k-1)\ldots\ldots(k-i+1) a^{k-i} b^i + \cdots\ldots +$$
$$\theta_1\theta_2\ldots\ldots\theta_k\,(k!)\,b^k,$$
$$\forall\, \theta_i \in \mathbb{R}\ \ (i = 1, 2, \ldots\ldots, k),\ \forall\, a, b \in \mathbb{R}.$$

Now we can go through the routine check that which properties , as stated in section (3), are satisfied by F_k and which are not.

(η) We can extend the domain of Orders of f_k and F_k from $\mathbb{N}$ to the set $\mathbb{Q}$ of all rational numbers as :

(i) For a given $k \in \mathbb{Q}$ (I) if $k \in \mathbb{N}$ then $f_k : I^k \times \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by

$$f_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ;\ a,\ b) = a^k + \theta_1\, k a^{k-1} b + \theta_1 \theta_2\, k(k-1) a^{k-2} b^2 + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_i\, k(k-1) \ldots\ldots (k-i+1) a^{k-i} b^i + \cdots\ldots + \theta_1 \theta_2 \ldots\ldots \theta_k\, (k!)\, b^k,$$

$\forall\, \theta_i \in I \ \ (i = 1, 2, \ldots, k),\ \forall\, a, b \in \mathbb{R}$.

(II) $f_0 : \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by $f_0(a,\ b) = 1,\ \forall\, a, b \in \mathbb{R}$

(III) if $k \in \mathbb{Q} - (\mathbb{N} \cup \{0\})$ then $f_k : I^\infty \times \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by

$$f_k((\theta_i)_{i \in \mathbb{N}} ;\ a,\ b) = a^k + \theta_1\, k a^{k-1} b + \theta_1 \theta_2\, k(k-1) a^{k-2} b^2 + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_j\, k(k-1) \ldots\ldots (k-j+1) a^{k-j} b^j + \cdots\ldots\ldots\ldots \infty,$$

$\forall\, \theta_i \in I \ \ (i \in \mathbb{N}),\ \forall\, a, b \in \mathbb{R}$.

(ii) For a given $k \in \mathbb{Q}$ (I) if $k \in \mathbb{N}$ then $F_k : \mathbb{R}^k \times \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by

$$F_k(\theta_1, \theta_2, \ldots\ldots, \theta_k ;\ a,\ b) = a^k + \theta_1\, k a^{k-1} b + \theta_1 \theta_2\, k(k-1) a^{k-2} b^2 + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_i\, k(k-1) \ldots\ldots (k-i+1) a^{k-i} b^i + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_k\, (k!)\, b^k,$$

$\forall\, \theta_i \in \mathbb{R} \ \ (i = 1, 2, \ldots, k),\ \forall\, a, b \in \mathbb{R}$.

(II) $F_0 : \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by $F_0(a,\ b) = 1,\ \forall\, a, b \in \mathbb{R}$

(III) if $k \in \mathbb{Q} - (\mathbb{N} \cup \{0\})$ then $F_k : \mathbb{R}^\infty \times \mathbb{R}^2 \longrightarrow \mathbb{R}$, defined by

$$f_k((\theta_i)_{i \in \mathbb{N}} ;\ a,\ b) = a^k + \theta_1\, k a^{k-1} b + \theta_1 \theta_2\, k(k-1) a^{k-2} b^2 + \cdots\ldots +$$
$$\theta_1 \theta_2 \ldots\ldots \theta_j\, k(k-1) \ldots\ldots (k-j+1) a^{k-j} b^j + \cdots\ldots\ldots\ldots \infty,$$

$\forall\, \theta_i \in \mathbb{R} \ \ (i \in \mathbb{N}),\ \forall\, a, b \in \mathbb{R}$.

Now we can go through the routine check that which properties , as stated in section (3), are satisfied by these extended f_k and F_k and which are not.

4. MAIN RESULT : EXPRESSION OF POWER OF 2, WHICH GENERATES A NON-TRIVIAL COLLATZ CYCLE, IN TERMS OF A GENERAL BINOMIAL FUNCTION

Let n be the minimum element of a non-trivial Collatz cycle of length $k \geq 1$ ($n \in \mathbb{N}, n \geq 1$), i.e., n is the smallest element in the non-trivial Collatz cycle n, $C(n)$, $C^2(n)$, ..., $C^{k-1}(n)$ of length k. It is obvious that $n = C(n) = C^2(n) = \ldots\ldots = C^{k-1}(n)$. Let $C^i(n)$ be the largest element in this cycle. Then $n < C^j(n)$ for $j = 1, 2, \ldots\ldots, k-1$ and $C^i(n) \geq C^j(n)$ for $j = 1, 2, \ldots\ldots, i-1, i+1, \ldots\ldots, k$.

Let

$C^j(n) = \frac{3C^{j-1}(n)-1}{2^{a_j}}$ where $a_j \in \mathbb{N}$ such that $\frac{3C^{j-1}(n)-1}{2^{a_j}}$ is an odd positive integer, for $j = 1, 2, \ldots, k$

Then by Lemma(3), we have

$2^{a_1+a_2+\cdots+a_k} = \left(3+\frac{1}{n}\right)\left(3+\frac{1}{C(n)}\right)\left(3+\frac{1}{C^2(n)}\right)\cdots\cdots\left(3+\frac{1}{C^{k-1}(n)}\right)$ ..... (4.1)

Now, $\left(3+\frac{1}{C^i(n)}\right)^k < \left(3+\frac{1}{n}\right)\left(3+\frac{1}{C(n)}\right)\left(3+\frac{1}{C^2(n)}\right)\cdots\cdots\left(3+\frac{1}{C^{k-1}(n)}\right) < \left(3+\frac{1}{n}\right)^k$

i.e., $\left(3+\frac{1}{C^i(n)}\right)^k < 2^{a_1+a_2+\cdots+a_k} < \left(3+\frac{1}{n}\right)^k$ ..... (4.2) (by (4.1)).

From (4.2) we have $2^{a_1+a_2+\cdots+a_k} = \left(3+\frac{1}{C^i(n)}\right)^k + \theta_1\left(\left(3+\frac{1}{n}\right)^k - \left(3+\frac{1}{C^i(n)}\right)^k\right)$,

for some $\theta_1 \in (0,1)\cap\mathbb{Q}$ ..... (4.3)

Now

$$\left(3+\frac{1}{n}\right)^k - \left(3+\frac{1}{C^i(n)}\right)^k = \frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{n}\right)^{k-1} + \left(3+\frac{1}{n}\right)^{k-2}\left(3+\frac{1}{C^i(n)}\right) + \cdots\cdots + \left(3+\frac{1}{C^i(n)}\right)^{k-1}\right\} \ldots (4.4)$$

Again

$$k\left(3+\frac{1}{C^i(n)}\right)^{k-1} < \left(3+\frac{1}{n}\right)^{k-1} + \left(3+\frac{1}{n}\right)^{k-2}\left(3+\frac{1}{C^i(n)}\right) + \cdots\cdots + \left(3+\frac{1}{C^i(n)}\right)^{k-1} < k\left(3+\frac{1}{n}\right)^{k-1}$$

Therefore $\left(3+\frac{1}{n}\right)^{k-1} + \left(3+\frac{1}{n}\right)^{k-2}\left(3+\frac{1}{C^i(n)}\right) + \cdots\cdots + \left(3+\frac{1}{C^i(n)}\right)^{k-1} =$

$$k\left(3+\frac{1}{C^i(n)}\right)^{k-1} + \theta_2\left\{k\left(3+\frac{1}{n}\right)^{k-1} - k\left(3+\frac{1}{C^i(n)}\right)^{k-1}\right\},$$

for some $\theta_2 \in (0,1)\cap\mathbb{Q}$ ..... (4.5)

Therefore (4.4) becomes

$$\left(3+\frac{1}{n}\right)^k - \left(3+\frac{1}{C^i(n)}\right)^k = k\frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{C^i(n)}\right)^{k-1} + \theta_2\left\{\left(3+\frac{1}{n}\right)^{k-1} - \left(3+\frac{1}{C^i(n)}\right)^{k-1}\right\}\right\}$$

(by (4.5)) ..... (4.6)

Therefore (4.3) becomes

$$2^{a_1+a_2+\cdots+a_k} = \left(3+\frac{1}{C^i(n)}\right)^k + \theta_1 k\frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{C^i(n)}\right)^{k-1} + \theta_2\left\{\left(3+\frac{1}{n}\right)^{k-1} - \left(3+\frac{1}{C^i(n)}\right)^{k-1}\right\}\right\}$$

(by (4.6))

$$= \left(3+\frac{1}{C^i(n)}\right)^k - \theta_1 k\frac{C^i(n)-n}{nC^i(n)}\left(3+\frac{1}{C^i(n)}\right)^{k-1} + \theta_1\theta_2 k\frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{n}\right)^{k-1} - \left(3+\frac{1}{C^i(n)}\right)^{k-1}\right\}$$

..... (4.7)

Replacing k by $k-1$ in (4.6), we get

$$\left(3+\frac{1}{n}\right)^{k-1}-\left(3+\frac{1}{C^i(n)}\right)^{k-1}$$
$$=(k-1)\frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{C^i(n)}\right)^{k-2}+\theta_3\left\{\left(3+\frac{1}{n}\right)^{k-2}-\left(3+\frac{1}{C^i(n)}\right)^{k-2}\right\}\right\}$$

for some $\theta_3 \in (0,1)\cap\mathbb{Q}$ ..... (4.8)

Using (4.8) in (4.7), we get

$$2^{a_1+a_2+\cdots+a_k}=\left(3+\frac{1}{C^i(n)}\right)^k+\theta_1 k\frac{C^i(n)-n}{nC^i(n)}\left(3+\frac{1}{C^i(n)}\right)^{k-1}+$$
$$\theta_1\theta_2 k\frac{C^i(n)-n}{nC^i(n)}(k-1)\frac{C^i(n)-n}{nC^i(n)}\left\{\left(3+\frac{1}{C^i(n)}\right)^{k-2}+\theta_3\left\{\left(3+\frac{1}{n}\right)^{k-2}-\left(3+\frac{1}{C^i(n)}\right)^{k-2}\right\}\right\}$$

or, $2^{a_1+a_2+\cdots+a_k}=\left(3+\frac{1}{C^i(n)}\right)^k+\theta_1 k\frac{C^i(n)-n}{nC^i(n)}\left(3+\frac{1}{C^i(n)}\right)^{k-1}+\theta_1\theta_2 k(k-1)\left(\frac{C^i(n)-n}{nC^i(n)}\right)^2\left(3+\frac{1}{C^i(n)}\right)^{k-2}+$

$$\theta_1\theta_2\theta_3 k(k-1)\left(\frac{C^i(n)-n}{nC^i(n)}\right)^2\left\{\left(3+\frac{1}{n}\right)^{k-2}-\left(3+\frac{1}{C^i(n)}\right)^{k-2}\right\} \quad \text{..... (4.9)}$$

Proceeding in this way, finally we get

$$2^{a_1+a_2+\cdots+a_k}=\left(3+\frac{1}{C^i(n)}\right)^k+\theta_1 k\frac{C^i(n)-n}{nC^i(n)}\left(3+\frac{1}{C^i(n)}\right)^{k-1}+\theta_1\theta_2 k(k-1)\left(\frac{C^i(n)-n}{nC^i(n)}\right)^2\left(3+\frac{1}{C^i(n)}\right)^{k-2}+$$

$$\theta_1\theta_2\ldots\ldots\theta_j k(k-1)\ldots\ldots(k-j+1)\left(\frac{C^i(n)-n}{nC^i(n)}\right)^j\left(3+\frac{1}{C^i(n)}\right)^{k-j}+\cdots\ldots\ldots\ldots\ldots+$$

$$\theta_1\theta_2\ldots\ldots\theta_k(k!)\left(\frac{C^i(n)-n}{nC^i(n)}\right)^k, \text{ for some } \theta_j\in(0,1)\cap\mathbb{Q}\ (j=1,2,\ldots,k) \quad \text{..... (4.10)}$$

From (4.10), we have

$2^{a_1+a_2+\cdots+a_k}=f_k\left(\theta_1,\theta_2,\ldots\ldots,\theta_k\ ;\ 3+\frac{1}{C^i(n)},\ \frac{C^i(n)-n}{nC^i(n)}\right)$ ..... (4.11), which is our desired expression.

REMARK : From (4.11), we can state a conjecture as :

If for $n\in\mathbb{N}', k\in\mathbb{N}$, $n, C(n), C^2(n),\ldots\ldots, C^{k-1}(n)$ be a Collatz cycle, then

$2^{a_1+a_2+\cdots+a_k}=f_k\left(\theta_1,\theta_2,\ldots\ldots,\theta_k\ ;\ 3+\frac{1}{C^i(n)},\ 0\right)$.

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Characterization and Calibration of a Nuclear Track Detector (NTD) for Rare Event Search in Cosmic Rays

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A commercially available polymer was investigated for its suitability as a nuclear track detector (NTD) in the search for rare events (e.g. strangelets) in cosmic rays at very high mountain altitudes. The material was identified as polyethylene terephthalate (PET) and was found to have a much higher detection threshold compared to many other widely used NTDs making it ideally suited to rare event search in cosmic rays by eliminating the dominant low Z background. Systematic studies were carried out with PET to determine the ideal etching condition. Also the charge response of PET was studied using various ion beams from accelerators. The results of such studies firmly establishes PET as a detector with good energy and charge resolution particularly suited to the search for high Z particles against a dominant low Z-background.

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I. INTRODUCTION: Ever since the discovery of cosmic rays more than a century ago, scientists have time and again turned their attention to these cosmic messengers in their quest for unravelling some of the deepest mysteries of the universe. Especially the search for exotic particles (e.g strangelets) in cosmic rays, predicted by thoery but yet to be produced or detected in laboratories, has always been an active field of research [1]. Strangelets are nuggets of a hypothetical state of matter called Strange Quark Mat-ter (SQM) containing almost equal numbers of up, down and strange quarks which, according to many theorists, constitute the true ground state of Quantum Chromodynamics [2, 3]. SQM if stable, is almost inevitably present in the core of dense steller objects called Neutron stars which often form part of binary systems. When such stars in a binary system collide leading to their breakup, strangelets can be produced; which are then accelerated just like other cosmic ray particles. The strangelets are expected to have a small positive charge. So the key experimental signature for such strangelets will be a highly unusual charge to mass ratio (Z/A□1/2) compared to ordinary baryons. According to one model of strangelet propagation through the earth's atmosphere [4], an initially small strangelet arriving at the top of the atmosphere will grow in size by preferentially absorbing neutrons over protons (protons being Coulomb repelled) from atmospheric nuclei as it propagates through the atmosphere. At the same time it will lose energy by ionization, but will be left with enough energy for them to be detected at high mountain altitudes, albeit with a very low flux. To investigate such rare events, one clearly requires a large area detector located at high mountain altitude. An ideal choice of detectors for setting up of large area arrays at remote locations are Nuclear Track Detectors (NTDs) as they are robust, easy to handle and do not require power for their operations. Also they have good charge resolutions and have natural thresholds of registration. We plan to set up NTD arrays at high mountain altitudes to look for strangelets in cosmic rays. Use of NTDs for charged particle detection is a well established method [5, 6]. NTDs are dielectric materials in which damage trails are produced

when subjected to energetic charged particles. For a given NTD, detection of a charged particle is possible only if its Z/β is above a certain value, known as the detection threshold of that NTD. Widely used NTDs e.g. CR-39, Makrofol, Lexan etc. due to their very low detection thresholds, will record huge low Z background, predominantly protons in cosmic rays, which is most undesirable for the present experiment. We have investigated a commercially available polymer (Century de'Smart, India) identified to be polyethylene terephthalate (PET) by chemical analysis and FTIR spectroscopy [7] [8] with chemical formula $(C_{10}H_8O_4)_n$ and found that it has a much higher detection threshold ($Z/\beta \approx 120$) than any other widely used NTDs. This makes it particularly suitable for rare event search in cosmic rays in presence of a low Z background. Before a new detector material is employed, it needs to be properly characterized and calibrated. With that aim, systematic studies were carried out with PET to determine the ideal etching condition, its charge response characteristics to various ions and also its energy and charge resolution. This review article summarizes the key findings of those studies.

II. DETERMINATION OF IDEAL ETCHING CONDITION: As mentioned before, passage of charged particles through NTDs leave behind narrow damage trails. These damage trails are then enlarged and made visible through an optical microscope by treating the NTDs with properly chosen chemical reagents (etchant). During etching process the damaged regions are etched out at a faster rate, called track etch rate V_T, compared to the rate of etching, called bulk etch rate V_B of the undamaged bulk material, thereby forming etch pits. Such pits can be closely approximated by a geometrical cone with the damage trail along its axis. By studying the geometry of such etch pits viz., major and minor axes of etch pit openings and the depth of the tip of the etch cone beneath the detector surface one can determine the ratio of the track etch rate to the bulk etch rate V_T/V_B. This ratio, called the charge response of the detector, is the most important characteristic for NTDs, as it is crucial in identifying the particles forming tracks and it depends very sensitively on the etching condition.

Depending on the temperature, concentration of the etchant and the duration of etching, the etch pit geometry can vary significantly. It is essential to determine ideal etching condition so that for this particular combination of temperature and concentration of the etchant, the contours of etch pits formed are well defined and the V_T/V_B ratio is maximized. This will ensure that the errors in track parameter measurements are minimized.

In order to determine the ideal etching condition, we used PET samples that were exposed to cosmic rays at high mountain altitude (Darjeeling in Eastern Himalayas, elevation 2.2 km) for one year. These samples were cleaned by ultrasonic cleaner and were then etched in NaOH solution (a commonly used etchant for NTDs) of three different concentrations 5.0 N, 6.25 N and 7.5 N. For each of these concentrations three different temperatures 45°C, 55°C and 70°C were tried. The etching tank (Julabo, Germany) was fitted with an automated temperature controller and temperatures were maintained to within ± 0.5°C. To maintain uniformity of temperature and concentration within the bath it was continuously stirred by a motorized stirrer. For every combination of temperature and concentration, the PET samples were etched for three different durations: 2 hr, 3 hr and 4 hr. So a total of twenty seven combinations were tried.

The most easily measurable parameters of an etched track are the major and minor axes D_a and D_b respectively of the etch pit opening and depth of the tip of the cone from the surface after etching Z_{obs}. It is customary to relate V_T/V_B to these track parameters. V_T/V_B can be calculated from the following equation:

$$V_T/V_B = Z_{obs}/[V_B.t.\ \cos(i)] + 1/\cos(i) \qquad (1)$$

where i is the angle of incidence, t is the duration of etching process and $\cos(i) = D_b/D_a$. The track parameters viz., D_a, D_b and Z_{obs} were measured under the ×100 dry objective of a digital optical microscope (Leica DM 4000M, Germany), interfaced with a computer preloaded with an image

analysis software. Resolution of the microscope was 0.15 μm in the image plane (X-Y direction) while for the depth determination (Z-direction) it was 0.1 μm. The ratio V_T/V_B was calculated by the above equation. For every individual condition several observations were made to evaluate the ratio and the associated statistical error.

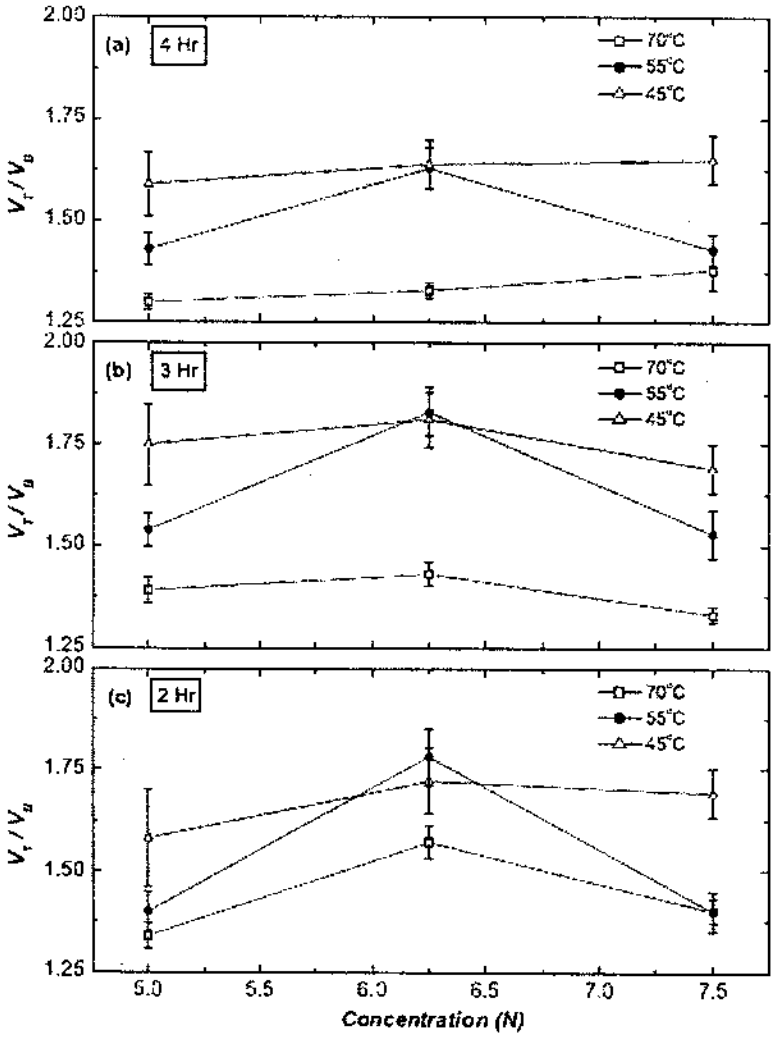

FIG. 1: Variation of charge response (V_T/V_B) with concentration and temperature after 2hr, 3hr, and 4hr etching. Lines are drawn to guide the eye.

The results of such studies are given in Fig. 1. As can be seen from the figures the ratio V_T/V_B is showing a tendency to get maximized when the concentration of NaOH solution is 6.25 N and the temperature is 55°C for different etching durations. The track quality was also good for this particular condition but it deteriorates for other etching conditions. So for all subsequent etching processes of PET detector, we have used 6.25 N NaOH solution at 55°C as the ideal etching condition.

The bulk etch rate (V_B) was also separately studied by two different methods, by weight loss method and by measuring the thickness of the PET samples before and after etching with a micrometer screw

gauge with least count of 0.5 μm. The bulk etch rate was found to vary with temperature and concentration and was measured to be (0.85±0.05) μm/hr for unexposed samples under the ideal etching condition.

III. STUDY OF CHARGE RESPONSE OF PET : The charge response of NTD (V_T/V_B) depends on the specific energy loss (dE/dx) of the incident charged particle and hence is related to its (Z/β) by the Bethe equation [9]. The ratio V_T/V_B as well as the range R of the particle inside the detector are the two most important parameters which helps in identifying the particle forming the track. In order to study the charge response of PET, detectors films were exposed to different ion beams from particle accelerators. Ion beams and their energies were chosen in such a way that it covered a wide range of (Z/β) values. Another consideration for minimum acceptable beam energy was that the incident ion on PET must have energy higher than the Bragg peak energy for that particular ion on PET. This is because for energies lower than that of the Bragg peak, the Bethe equation relating dE/dx with (Z/β) starts to break down due to charge neutralization. Beam current was chosen by the following consideration: Typical diameters of etch-pit openings are □ 10 μm, consequently maximum number of etch pits that can be resolved with small probability of overlapping is about □ 10^5/cm^2. Beam current was fixed so that the number of ions impinging on the detector lies in the range □ $10^4 - 10^5$ /cm^2, which is optimal for the data analysis process.

EXPERIMENT:

A. Charge response study with ^{16}O ions: In this experiment small (3.5×3.5 cm^2) PET detector films of thickness 100 μm were exposed to ^{16}O beam from the pelletron at the Inter University Accelerator Centre (IUAC), New Delhi. Beam energy from the accelerator was 95 MeV. A gold foil of thickness 3.97 μm was used to diffuse the beam over a circular area of diameter 3 cm. Fig. 2 shows the experimental arrangement. The beam energy just outside the flange of the beam pipe was 53.6 MeV considering the loss of energy at the diffuser and other absorbers. The PET detectors were mounted on a remote controlled rotator for successive stopping of the detectors in front of the beam pipe. With this arrangement one could expose a large number of detectors in a short period of time. We exposed the detectors at varying distance from the flange to determine its charge response corresponding to different energies as the incident energy degraded in the air between the flange and the targets. Separation between the flange and the detector samples was varied in steps of 2 mm. The incident energy on PET detectors varied between 16.0 and 24.0 MeV. The widely used Monte Carlo code SRIM [10] was used to calculate the specific energy losses and to estimate the degraded energies of the incident ions at the detector surface.

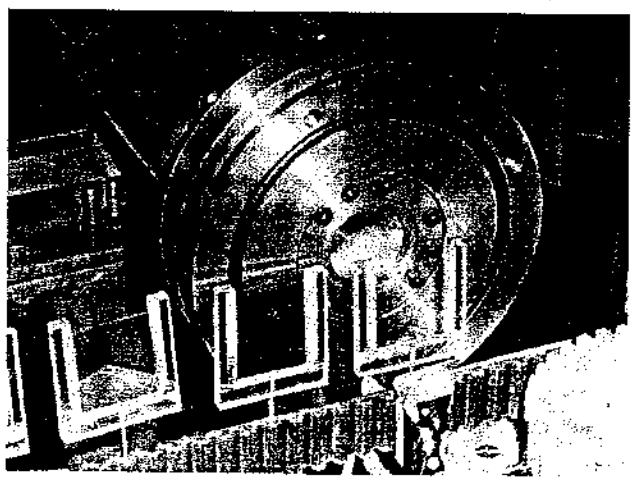

FIG. 2: Experimental arrangement for the ^{16}O exposure. The samples were mounted on a remote controlled rotator for successive stopping of the samples in front of the beam pipe.

B. Charge response study with ^{56}Fe and ^{32}S ions: Further charge response studies for PET were conducted by irradiating those detectors with 2.7 MeV/n ^{56}Fe and 3.9 MeV/n ^{32}S beams from the pelletron accelerator at IUAC, New Delhi, India. For this experiment a different setup was employed. Small (5 cm × 5 cm) pieces of PET were placed in aluminum holders which in turn were mounted on two movable arms inside a scattering chamber as shown in Fig. 3. A 250 μg/cm^2 gold foil was used as a scatterer. By moving the two arms inside the scattering chamber it was possible to vary the angles and hence the energies and also the fluxes with which the ions will be impinging on the PET films after being scattered by the gold foil.

For one set of aluminium holders placed on one of the arms the top was turned by an angle of 30° so that the scattered ions impinge on PET films at an 30° angle. This was done so that the conical profile of the tracks became clearly visible after etching, thereby enabling a more accurate determination of the track parameters. For the experiment the two arms were placed symmetrically making equal angles with the beam direction for each set of exposures. This was done to enable cross checking of the data obtained from detectors placed on two different arms.

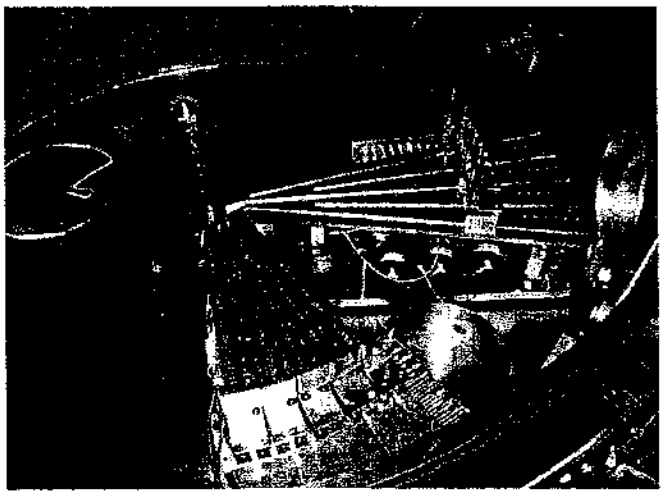

FIG. 3: Inside of the scattering chamber showing PET detectors mounted on aluminium holders and placed on the two movable arms. The target ladder with the gold foil (used as a scatterer) mounted on it can also be seen.

To enable us to develop a plan for the placement of the detectors a software code was written utilising the Rutherford scattering formula which gives the energy values and also the flux of the impinging ions at various angles and also at various distances from the scatterer after they scatter from the gold foil. The code also takes into account the loss of energy of ions inside the gold foil used as the scatterer and also their effective charges. It also gives the energies and fluxes of the scattered gold ions.

The beam current for the ^{56}Fe beam was 1 pnA. Three separate runs were used in this case. Twice with the arms in the forward direction with PET detectors making angles between 23°–47 ° and 35 ° –59 ° with respect to the beam direction and detector distances 50 cm from the scatterer and once with the arms in the backward direction with PET detectors making angles between 118 ° –142 ° with respect to the beam and detectors placed at distances of 25 cm from the scatterer. The exposure durations and distance of the detectors from the scatterer were chosen so as to keep the number of ions impinging on the detector within the limits stated before. The energies for incident ^{56}Fe ions consequently varied between 2.5 MeV/n to 0.9 MeV/n. The flux of the ^{79}Au ions coming from the scatterer was significant compared to the ^{56}Fe ions only for the detectors placed between 35 ° –59 °. But the tracks due to ^{79}Au ions are easily identifiable owing to the different sizes of their etch pit openings and also different

cone lengths. Since the energy of ^{79}Au ions (≈ 2–94 MeV) were significantly lower than their Bragg peak energy in PET (≈ 600 MeV) they were left out of the subsequent data analysis process.

For the experiment with ^{32}S, the setup was similar to that of Fe ion exposure described above. The beam energy was 3.9 MeV/n. The beam current was 1 pnA. In this case two separate runs were utilized. In the first case, the arms inside the scattering chamber were placed in the forward direction with detectors at angles between 23 ° – 47 ° with respect to the beam direction. Detectors were kept at a distance of 50 cm from the scatterer. In the second instance the angle of the detectors varied between 130 ° –154 °. The detector distance was 25 cm. The incident energies of the ions on PET were between 3.8 MeV/n to 2 MeV/n.

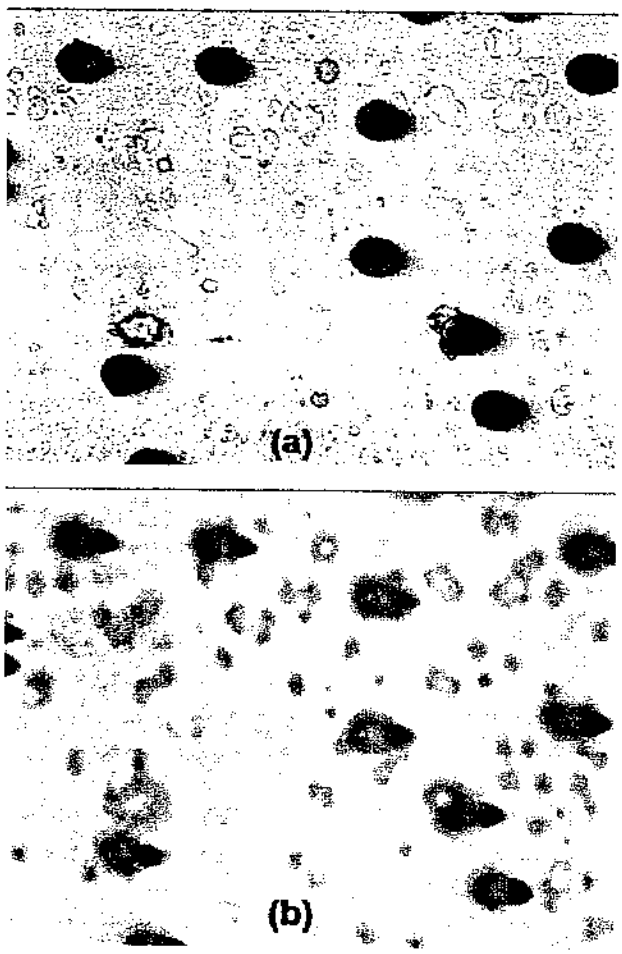

FIG. 4: Fe-tracks on PET after 3hr etching with the microscope focused (a) on the detector surface showing the openings of the etch pits, (b) at a depth of 9 μm showing the conical profile of the etch pits. Energy of the incident particles is 2.3 MeV/n and angle of incidence is 30 °.

All the exposed PET samples from the above experiments were etched in 6.25 N NaOH solution at 55.0±0.5°C for durations ranging from 1–3 hrs. Fig. 4 show some etch pits due to Fe-ions (incident energy 2.3 MeV/n) on PET after 3hr etching, once with the microscope focussed on the detector surface and once focussed at a depth of 9 μm clearly showing the conical profile of the etch pits.

C. Charge response study with ^{238}U ions: In a different experiment PET films were exposed to 11.1MeV/n ^{238}U ions from the accelerator at GSI, Darmstadt, Germany. In this case Aluminium foils of thickness 8.5 μm were used as energy degraders. To get three different incident energies on the PET targets, three separate runs were used, once without any Al foil, followed by two runs with four and eight Al foils respectively acting as degraders.

D. Charge response study with ^{129}Xe and ^{78}Kr ions: For these sets of exposures the 20° beam line of the REX-ISOLDE [11] facility at CERN, Switzerland was utilized. The 2.82 MeV/u ^{129}Xe and ^{78}Kr ion beams were produced in the ISOLDE GPS target and accelerated with the REX - ISOLDE linear accelerator. The ^{78}Kr beam contained an admixture of 2.82 MeV/u ^{49}Ti ions which were detected with PET and which provided an additional data point for calibration. The target chamber for carrying out the implantations is shown in Fig. 5.

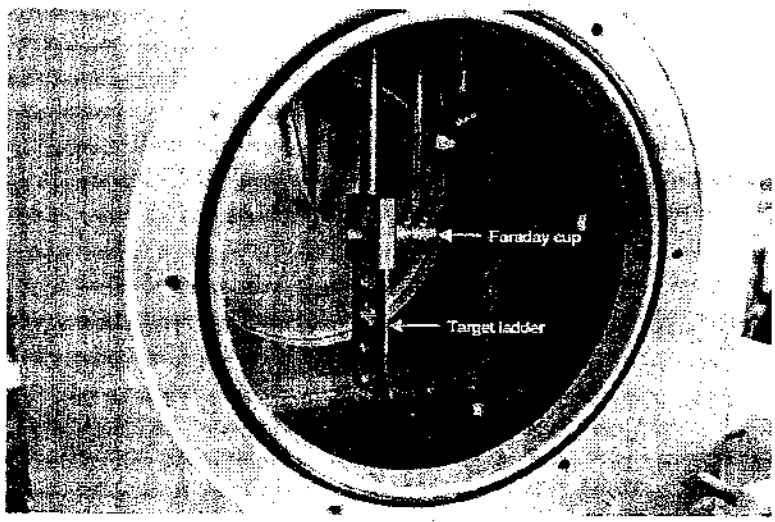

FIG. 5: Interior of the target chamber used in the experiment. The target ladder and the Faraday cup are indicated.

The target ladder seen in this figure has four circular apertures of 15 mm diameter which allows for mounting and exposure of the samples and a larger opening of diameter 30 mm behind which a Faraday cup could be mounted for monitoring beam current. The PET films were cut into rectangular strips (8.0 cm × 3.5 cm) so that it covered all four apertures and fixed onto the downstream side of the target ladder with screws. This ensured that the exposed areas were well defined. The target ladder and the Faraday cup were mounted on actuators. This allowed for the monitoring of the beam current as well as up to four different exposures of PET samples without venting the target chamber, by moving the ladder up or down. The actuator to which the target ladder was mounted could also be rotated. This enabled us to vary the angle of incidence of the ions on PET films. To ensure that the number of the impinging ions lie in the range mentioned before, beam currents of 1.5pA for ^{78}Kr and 3.1 pA for ^{129}Xe beams were first measured with the Faraday cup, because for too low beam currents direct measurements are not possible. The beam intensities were then attenuated by a factor of 0.005 by employing the REXTRAP beam gate. This made sure that for the exposure duration of 30 sec we got □ 5×10^4 particles/cm^2.

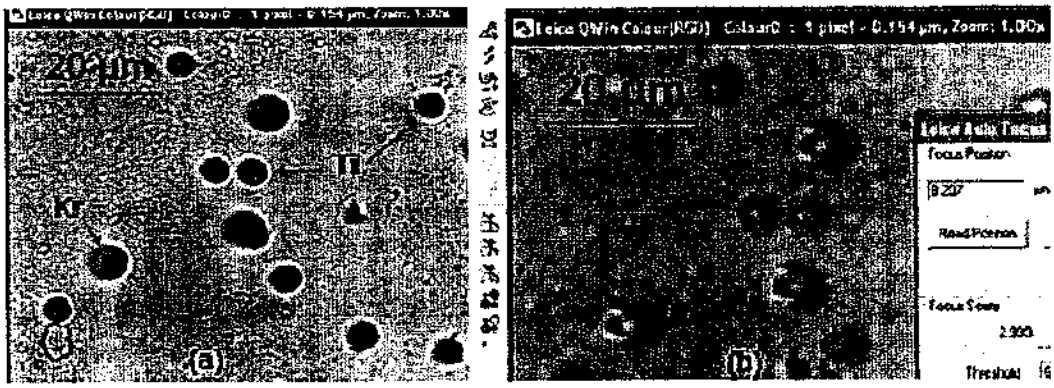

FIG. 6: The different track dimensions for ^{78}Kr and ^{49}Ti can be seen with the microscope focussed (a) on the surface and (b) at a depth of 8.2 μm.

In all 20 different exposures were carried out. For half of the exposures the angle of incidence was kept at 30° with respect to the beam. This was done to make sure that the conical profiles of the tracks are clearly visible after etching, thereby enabling a more accurate determination of track parameters. Typical pressure inside the target chamber during the experiment was kept at 10^{-5} mbar. The exposed PET samples were again etched in 6.25 N NaOH solution at 55.0±0.5°C. Fig. 6 shows track images due to ^{78}Kr as well as ^{49}Ti on PET after 2 hr etching. Different track diameters corresponding to the two ions are evident from Fig 6(a) with the microscope focussed on the detector surface. Fig 6(b) shows that the end of track is reached for ^{49}Ti ions but not for ^{78}Kr with the microscope focussed at a depth of 8.2 μm. This implies different track lengths for the two ions.

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RESULTS AND DISCUSSION : Figs. 7 - 10 show the charge response, (V_T/V_B), for PET detector plotted as a function of Z/β values of impinging nuclei. (V_T/V_B) values were obtained from track parameter measurements for ^{16}O, ^{56}Fe, ^{32}S and ^{238}U nuclei respectively.

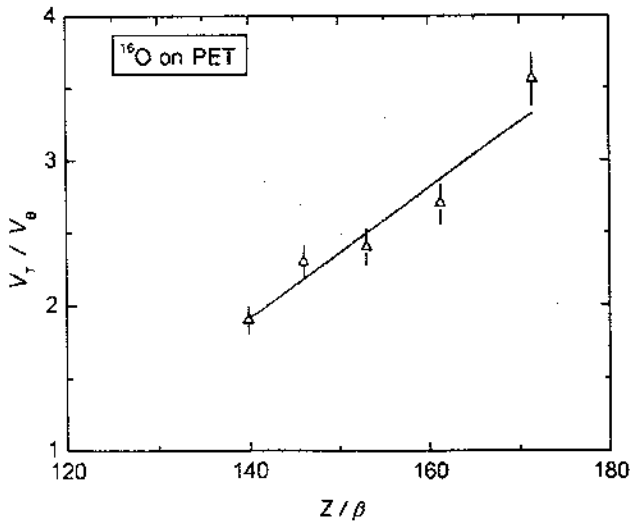

FIG. 7: Charge response characteristic for ^{16}O ions in PET. It can be seen that Z/β has a value of *about* 120 when V_T/V_B is 1.0. The solid line is a linear fit.

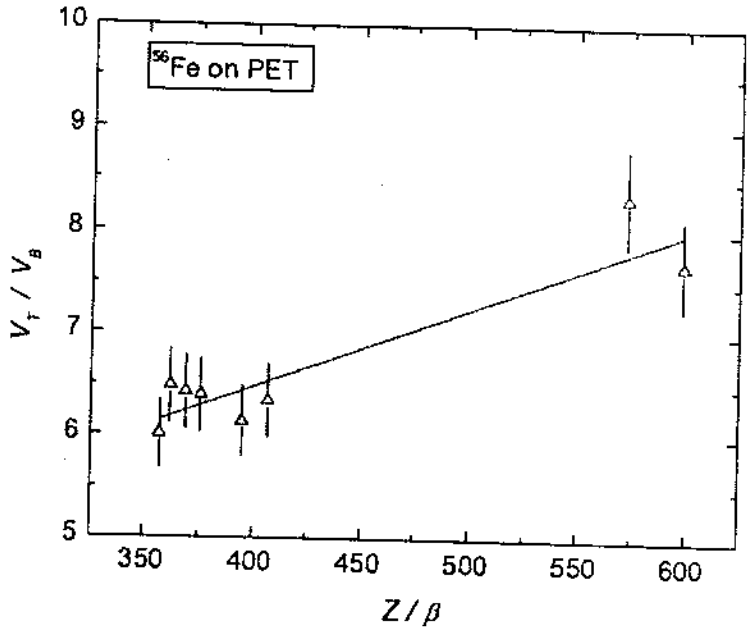

FIG. 8: Charge response characteristic for ^{56}Fe ions in PET. The solid line is a linear fit.

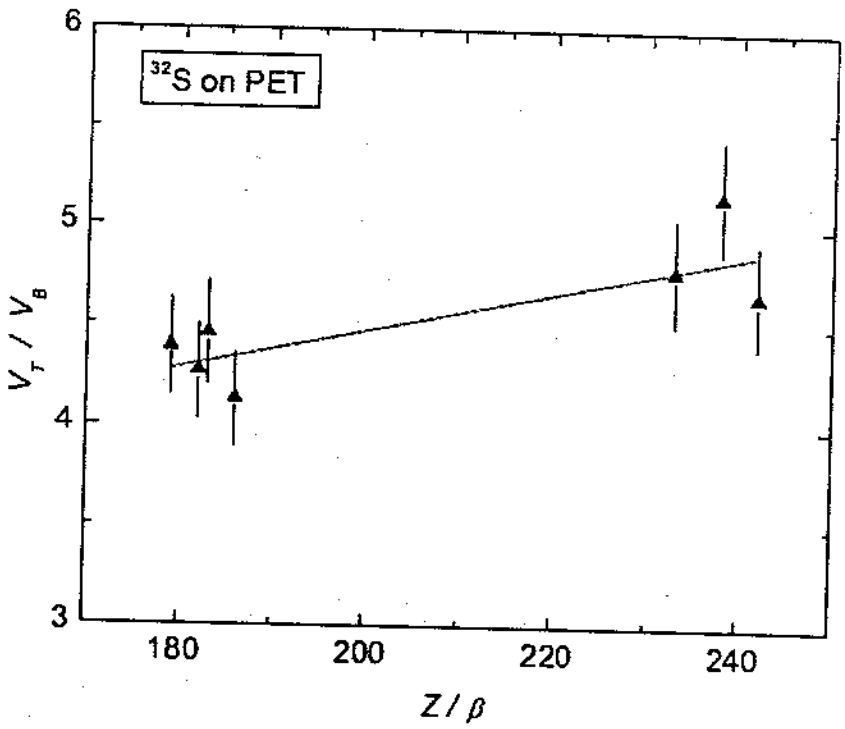

FIG. 9: Charge response characteristic for ^{32}S ions in PET. The solid line is a linear fit.

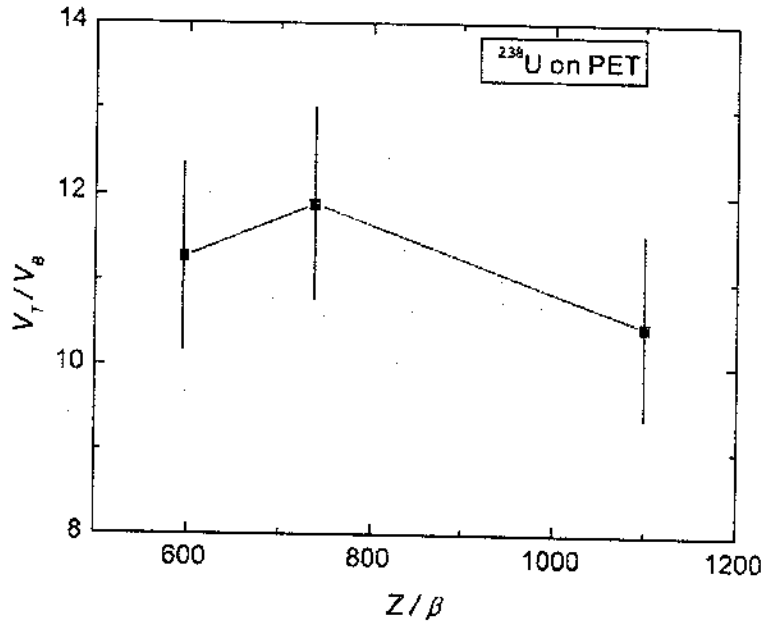

FIG. 10: Charge response characteristic for ^{238}U ions in PET. The solid line is meant to guide the eye.

From Figs. 7 - 9, where straight lines drawn are linear fits, it is evident that the V_T/V_B of PET increases with Z/β in the range shown for ^{16}O, ^{56}Fe and ^{32}S. But from Fig. 10 we find that the charge response of PET for ^{238}U increases with Z/β in the range 600-750 and then decreases with increasing Z/β. This can be understood in the following way. For ^{16}O, ^{56}Fe and ^{32}S all the energies of incidence ions are higher than the Bragg peak energies for the respective ions on PET, so that for those energies dE/dx increases with decreasing energy i.e., increasing Z/β. Since V_T/V_B increases with increasing specific energy loss dE/dx, we expect V_T/V_B to increase with increasing Z/β. On the other hand for ^{238}U ion data, the lowest energy data point (3.3 MeV/n) corresponding to the highest measured Z/β □1100 lies on the left side of the Bragg peak for which dE/dx decreases with decreasing energy, so V_T/V_B decreases with increasing Z/β . Ideally the energy values should be higher than the Bragg peak energy for proper detector response for reasons explained earlier. So the ^{238}U data point corresponding to Z/β ~1100 was not used while the overall calibration curve for PET was determined.

The calibration curve for ^{16}O as shown in Fig. 7 also indicates that the Z/β detection threshold for PET is ≈120. It is to be noted that for tracks to form in NTDs, the track etch rate V_T must be greater than the bulk etch rate V_B. At the threshold Z/β value V_T/V_B for the detector becomes 1.0 i.e., no track is formed. This supports our claim that PET has a much higher detection threshold than other commercially available NTDs.

Figs. 11 and 12 show the variation of track diameter and length with duration of etching, for tracks due to ^{129}Xe ions of energy 2.82 MeV/u. The monotonic rise with etching time of the track diameters and lengths implies a consistency in the rate of etching for PET which is a requisite quality for any good track etch detector.

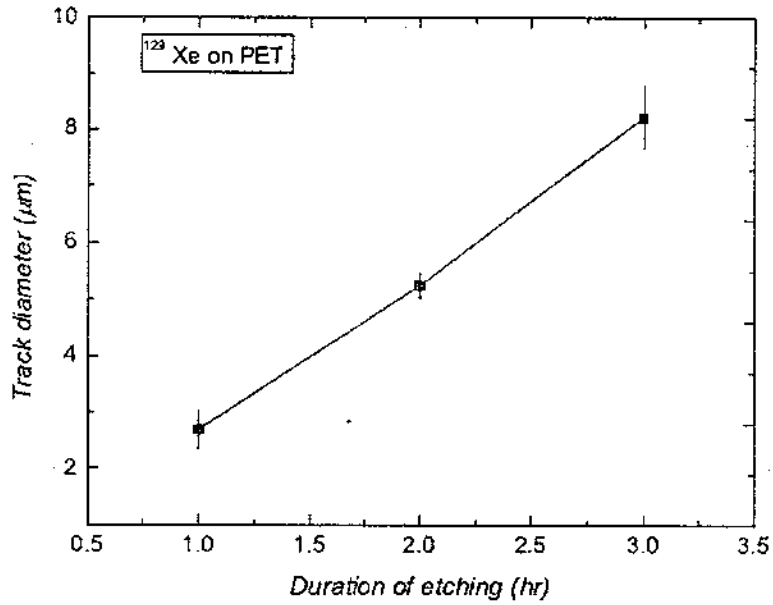

FIG. 11: The variation of track diameter for ^{129}Xe tracks in PET for different durations of etching. The line is only to guide the eye.

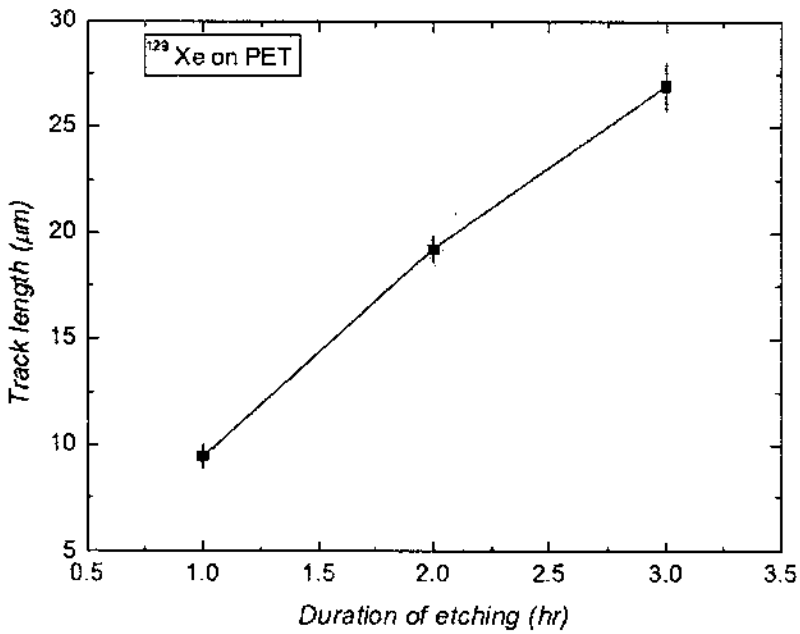

FIG. 12: The variation of track length for ^{129}Xe tracks on PET for different durations of etching. The line is only to guide the eye.

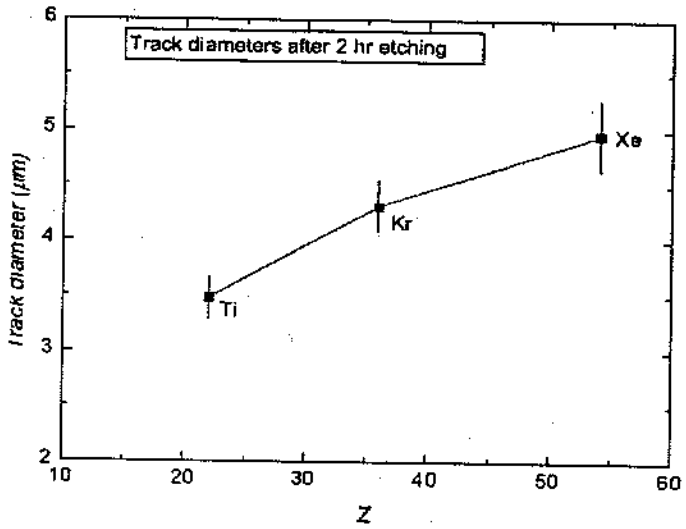

FIG. 13: Figure compares the diameters of ^{129}Xe, ^{78}Kr and ^{49}Ti tracks on PET after 2 hr etching. The line is only to guide the eye.

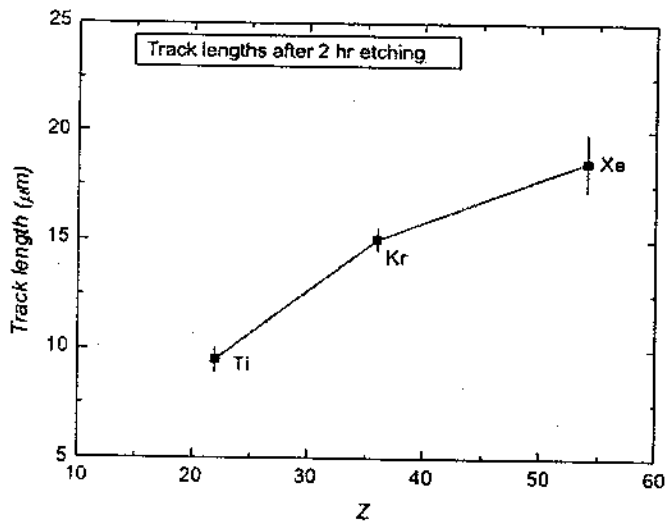

FIG. 14: Figure compares the track lengths of ^{129}Xe, ^{78}Kr and ^{49}Ti tracks on PET after 2 hr etching. The line is only to guide the eye.

Figs. 13 and 14 compare the track diameters and track lengths for the three different ions, all with energies of 2.82 MeV/u, for a fixed etching duration. The same energy per nucleon implies the same values for β, thus the differences seen in track diameters and lengths purely reflect the different Z values for the three ions. This can provide an alternative method of identifying incident particles when they have similar values for energy per nucleon.

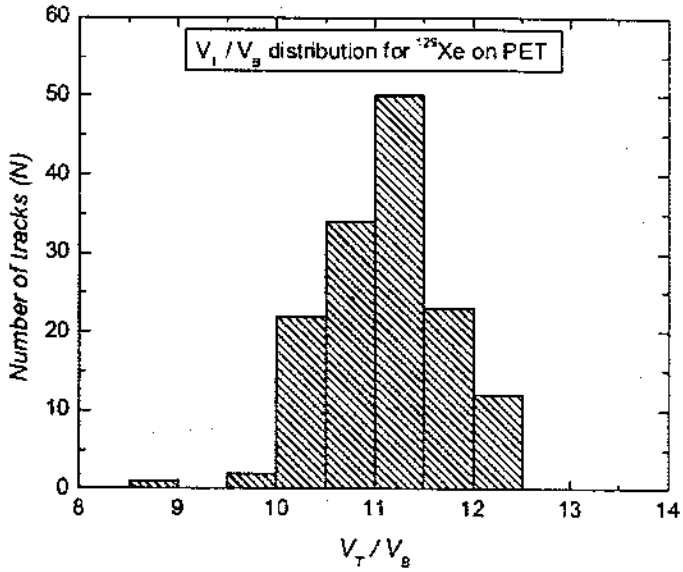

FIG. 15: Histogram showing the distribution of V_T/V_B values corresponding to ^{129}Xe tracks on a PET sample.

Fig. 15 shows the distribution of V_T/V_B values for ^{129}Xe obtained from track parameter measurements on a particular PET sample. The closely bunched values of V_T/V_B for ^{129}Xe show the consistency of the charge response of PET.

By combining the V_T/V_B values for ^{129}Xe, ^{78}Kr, ^{49}Ti as well as those for ^{16}O, ^{32}S, ^{56}Fe, ^{238}U ions, we can get a calibration curve for PET as shown in Fig. 16. The specific energy losses dE/dx of the incident ions at different energies were obtained using SRIM [10].

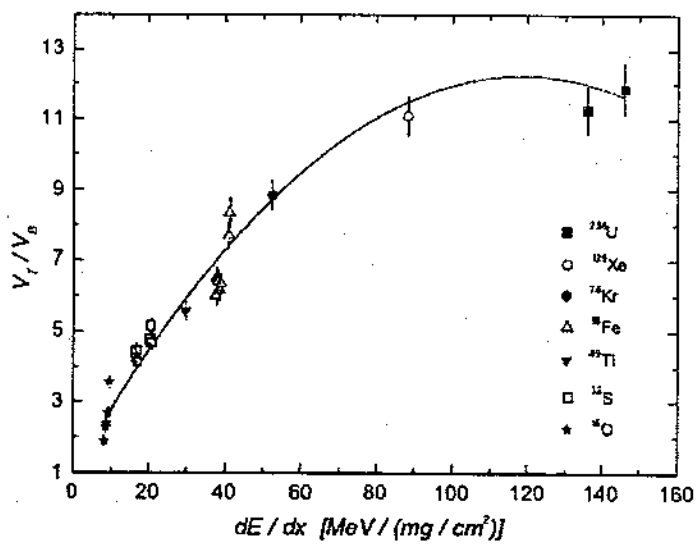

FIG. 16: Calibration curve for PET.

The nature of the calibration curve can be explained thus: High values of dE/dx leads to highly damaged polymer matrix along the latent track. But the etching process is limited by the transportation of etching solution and etching products in the narrow track channel [12]. So for higher dE/dx values,

large quantities of etching products cannot be removed from the track channel fast enough. So V_T starts to slow down, will reach a maximum and may then fall for any further increase in dE/dx. Consequently the ratio V_T/V_B is also expected to show similar behaviour. The fitted line is given by $y = a + bx + cx^2$, where $a = 0.96 \pm 0.08$, $b = 0.19 \pm 0.01$ and $c = (0.80 \pm 0.05) \times 10^{-3}$; where the parameter errors represent 95% confidence level. With this calibration curve one can find dE/dx of any charged particle impinging on PET. Along with the measured range inside the PET detector for a stopped charged particle, one can identify any charged particle with its charge, mass and energy.

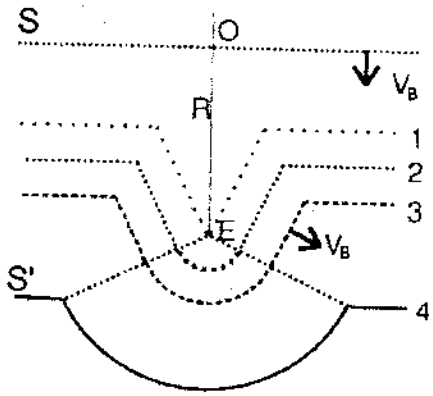

FIG. 17: The various stages of track development. S is the initial detector surface. O and E are the entrance and end points of the particle path, R is the particle range in the detector material. V_B is the bulk etch rate. Line 1 hows conical track. 2 and 3 show the gradual rounding off of the conical etch pit as the detector is over-etched. 4 shows the condition when the track is fully spherical.

IV. CHARGE AND ENERGY RESOLUTION OF PET: In order to determine the charge (Z) of the ions responsible for the tracks, theoretical plots for specific energy loss (dE/dx) vs range (R) for various ions were obtained using SRIM [10]. For an unknown charged particle its charge response (V_T/V_B) is obtained from track parameter measurements. The corresponding dE/dx values can then be determined from the calibration curve for that detector. Then the range of the particle in the detector is determined, if necessary, by repeating the etching process for short durations and carefully observing the resulting etch pits. The etching process proceeds initially at a faster rate V_T along the damage trail. But once the end of particle trajectory is reached, etching proceeds in all directions with the same bulk etch rate V_B and consequently the tip of the etch cone starts to become round and the corresponding track is over-etched. So by finding evidence of the beginning of such an over-etching process, the range R of the particle inside the detector can be determined. Fig. 17 illustrates various phases of track development. Once the dE/dx and R of an unknown particle is ascertained, its Z can be obtained from the above mentioned dE/dx vs. R plot for various ions for that detector. Also the energy can be estimated from the range of the ions in the detector.

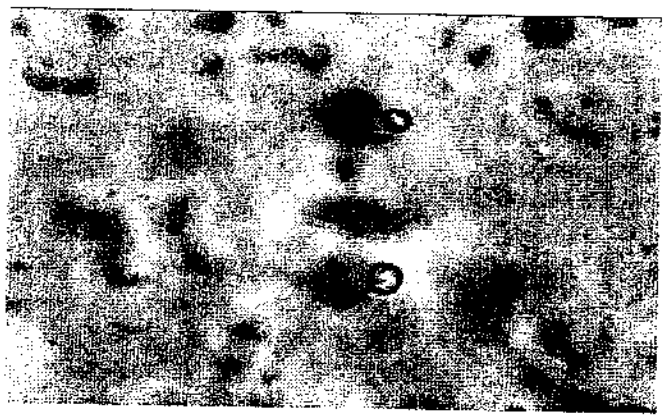

FIG. 18: The rounded off tips of the etch cones due to ^{49}Ti ions in PET after 4 h etching.

The tracks resulting from the impinging ^{49}Ti ions were used to get an idea of the charge and energy resolution of PET using the particle identification scheme described previously. From the measured V_T/V_B value for ^{49}Ti, the corresponding dE/dx value of 31±1.4 MeV/(mg/cm^2) was obtained from the calibration curve for PET without REX-ISOLDE data [13]. The etching process was continued in successive steps until the ends of the tracks were reached for ^{49}Ti ions as evidenced by the rounding off of the tip of the etch pits as shown in Fig. 18. This determines the range of the ions in PET. The range of ^{49}Ti ion in PET was measured to be 32 ± 3 μm. The experimental dE/dx and R values for ^{49}Ti were compared with the theoretical dE/dx vs. R plots of various ions on PET as shown in Fig. 19. The error bar of dE/dx shown in the figure is related to the error in V_T/V_B which in turn depends on the variation of the measured depth of the tip of the etched cones for a given type of ion of a given energy and the error in the measurement of the bulk etch rate. The error bar of R shown in the above figure reflects the variation in the measured range. From fig. 19 one concludes that the charge of the ion is either Z = 22 or Z = 23, as compared with Z = 22 for ^{49}Ti ion. So we could identify the charge Z of an ion with an accuracy of ±1. From range, the incident particle's energy was determined to be 128 ± 12 MeV i.e, 2.61 ± 0.24 MeV/u, as compared to the actual energy at incidence of 2.82 MeV/u. This indicates an accuracy of 10% in energy measurement.

It can also be seen from Fig. 19 that for particles of low energies (below Bragg peak) the curves due to various ions start to converge making identification of single particle charges difficult. This sets a lower energy threshold for particle identification with PET.

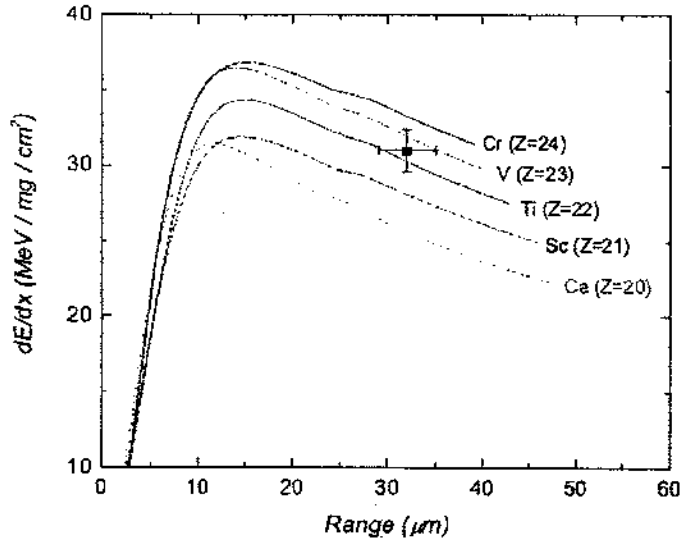

FIG. 19: Data point (black square) obtained from measurements on a ^{49}Ti track plotted on dE/dx vs R plots for various ions impinging on PET.

V. CONCLUSION:

Our work [13][14] clearly demonstrates that PET can be effectively used as a charged particle detector while detecting high Z particles against a low Z background. Another advantage for PET is its significantly low cost compared to other commercially available NTDs. Cost considerations become particularly important if one intends to set up large area passive detector arrays which we plan to do in future.

Acknowledgments

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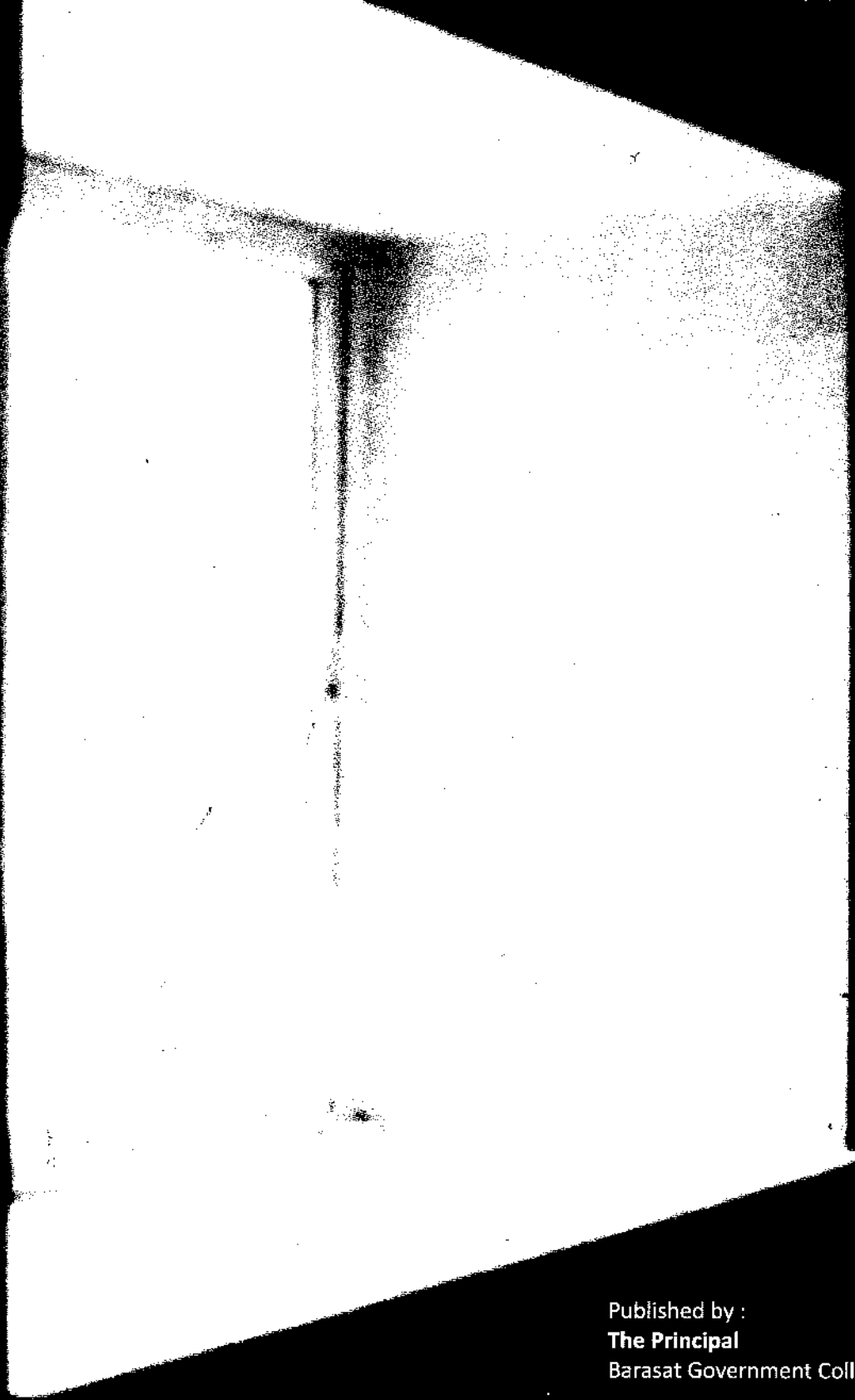

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