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AFLATOXIN CONTAMINATION AND MOISTURE LEVELS IN SRI LANKAN MARKET RICE

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Abstract : Samples of raw and parboiled, milled rice collected from wet (Veyangoda), intermediate (Kurunegala) and dry (Anuradhapura) zones of Sri Lanka during the dry and rainy seasons of the year were analysed for the presence of moisture and aflatoxin B1. Rice contained a mean moisture content of 14.9 ± 1.4%. Although slightly higher average moisture levels were observed during the rainy season and in the wet zone, the differences in the moisture levels between the two seasons and among the three locations were not statistically significant. Of the 597 samples of rice tested, aflatoxin B1 was not detected in 525 samples (minimum detectable level 12 μg/kg). Aflatoxin B1 was present at 30 μg/kg level in 12 parboiled samples and 60 samples contained 12–30 μg/kg. Of the 60 samples 57 were parboiled rice. At present, rice does not appear to pose a danger through aflatoxin contamination as it is stored only for about a week at moisture levels around 15%. However, with self sufficiency in rice resulting in longer durations of storage, the parboiled rice could indicate a potential danger, if efficient drying methods are not practised.

1. Introduction

Aflatoxins are the most potent naturally occurring hepatocarcinogens in experimental animals.[6] The aflatoxin producing fungi *Aspergillus flavus* and *Aspergillus parasiticus* have been reported to grow and produce aflatoxins on many agricultural food commodities, specially under tropical climatic conditions and in the presence of moisture levels above 10 per cent. Under experimental conditions rice has been shown to be a good medium for production of aflatoxins.[2]

In rice, moisture levels above 10 per cent could occur due to underdrying of paddy (rough rice), especially during the processing of parboiled rice and also due to absorption of moisture from the atmosphere at high relative humidities during storage.

The growth of fungi in market rice is a common phenomenon and the presence of aflatoxins in rice has been observed in several South-East Asian countries.[5] There are reports of several incidents of human deaths suspected

to be due to aflatoxicosis caused by the consumption of aflatoxin contaminated rice.[3]

Rice is the staple diet of Sri Lankans. The contamination of rice by aflatoxins even at low levels could be critical as considerable quantities of rice are consumed by the average Sri Lankan.

In view of the relevance of the rice-aflatoxin problem to human health a survey of aflatoxin contamination in rice was carried out in three locations representing the three different climatic zones in Sri Lanka during dry and wet seasons of the year, to assess the extent of contamination and relate it to possible factors which could support fungal contamination and aflatoxin accumulation. In this survey, only aflatoxin B1, which is the most toxic and most widely spread in foods, was estimated.

2. Materials and Methods

2.1 Source of samples

Samples of all available types of rice, consisting of parboiled and raw milled rice of locally grown varieties were collected from more than 90 per cent of the market places in the three towns Anuradhapura (dry zone, annual rainfall < 2000 mm) Kurunegala (intermediate zone, annual rainfall 2000 — 2500 mm) and Veyangoda (wet zone, annual rain fall > 2500 mm). Samples were collected during the dry and rainy seasons of the year.

2.2 Sampling

When sampling was done from 1—5 bags, each containing upto 65 kg, several primary samples were withdrawn from three different levels in the bag using a probe grain sampler. A composite sample was prepared by pooling the three primary samples. A replicate fraction (125 g) from one-fourth of the composite sample was used for laboratory analysis. When the number of rice bags available for sampling was between 6—25, 5 bags were picked randomly and primary samples were withdrawn from them. When the number of bags was over 25, they were marked into lots of 5 bags each and a sample was obtained from one bag in each lot.

Both parboiled and raw rice were sampled for analysis. The samples were packed and sealed in polythene bags for transport and storage.

2.3 Estimation of moisture

The moisture content in the rice (wet weight basis) was estimated immediately on return to the laboratories (within 24 hours of collection) using a Kett PBTK moisture meter. The grain samples in sealed polythene bags were stored at $-20^{\circ}C$ pending assay for aflatoxins.

2.4 Estimation of aflatoxins

Rice grains were ground to a fine powder in a Stein Mill Model M2. The samples in duplicate (20 g) were extracted by the modified 70% aqueous acetone procedure.[4] The estimation of aflatoxin B1 was done on 250 nm silica gel G (Merck) thin layer chromatographic plates by visual comparison with standard samples of pure aflatoxin B1 (Makor, Jerusalem) in chloroform under UV light at 365 nm. The minimum detectable level under these conditions was 12 μg/kg (ppb).

Solvents used in developing the chromatograms were,
(a) methanol : chloroform (3 : 97) equilibrated, lined tank
(b) acetone : chloroform (1 : 9) unequilibrated, unlined tank

3. Results and discussion

3.1 Locations

In the selection of localities for the survey, three rice producing areas were identified for sampling, as our preliminary studies indicated that it was not possible to find the area of origin of the rice, with reasonable accuracy, if sampling was done in non-rice producing areas of the country. In the three areas surveyed, all rice samples were found to be produced and processed in the same locality. In 99 per cent of the samples, the storage period as market rice was found to be less than 7 days. Occasionally, samples stored for 14—21 days were present.

3.2 Moisture levels

The mean moisture levels observed for all the samples was 14.9 ± 1.4%. Lowest moisture levels were observed in rice during the dry season in the intermediate zone (Kurunegala) and highest during the rainy season in the dry zone (Anuradhapura) (Table 1).

In the dry and intermediate zones, the mean moisture content in rice appeared to be higher during the rainy season than during the dry season,

Table 1. Mean moisture content (% wet basis) observed in rice samples from Anuradhapura, Kurunegala and Veyangoda during dry and rainy seasons

Location – zone	dry		rainy	
	number of samples	mean	number of samples	mean
Anuradhapura – dry	101	14.2 ± 1.8	105	15.9 ± 1.3
Kurunegala – intermediate	101	13.8 ± 1.1	104	15.5 ± 1.0
Veyangoda – wet	86	15.1 ± 0.9	106	15.1 ± 1.2

Analysis of variance

	df	ss	ms	F
treatments	5	319.8280		
location	2	23.97059	11.985295	8.2640***
season	1	211.78474	211.78474	146.02822***
1 x s	2	83.84651	41.923255	28.9066***
error	597	865.8280	1.45030	
total	602	1185.6600		

whereas no differences in the mean moisture content was observed during the dry and rainy seasons in the wet zone.

The distribution pattern of moisture levels in rice (Table 2) showed an increase in the number of samples containing higher moisture levels during the rainy season in all three localities. The differences were marked in the dry zone.

However, the analysis of variance (Table 1) did not show any significant differences between moisture levels during dry and rainy seasons and among the three locations. On the whole the average moisture content appeared to be sufficient for slow growth of fungi in rice.

Table 2. Distribution pattern of rice samples from Anuradhapura, Kurunegala and Veyangoda during dry and rainy seasons by the moisture percentage (wet basis).

Moisture Percentage	area sampled					
	Anuradhapura		Kurunegala		Veyangoda	
	dry	rainy	dry	rainy	dry	rainy
< 11.9	1	0	2	0	0	0
12.0 – 13.9	43	14	52	3	8	15
14.0 – 15.9	51	44	45	73	62	66
16.0 – 17.9	6	29	2	26	16	25
> 18.0	0	18	0	2	0	0
Total	101	105	101	104	86	106

3.3 Aflatoxin content

Of 597 samples of rice tested for the presence of aflatoxins none of the samples contained aflatoxin B1 above the UNICEF/WHO/FAO maximum permissible level of 30 μg/kg (ppb) for foods for human consumption. Aflatoxin B1 was not detected in 525 samples (minimum detectable level 12 μg/kg). Of the balance there were 12 parboiled rice samples containing 30 μg/kg of aflatoxin B1. Among the 60 samples which contained less than 30 μg/kg, 57 were parboiled rice and 3 were raw rice (Table 3).

Table 3. Aflatoxin B1 (g/kg) in raw and parboiled rice in relation to geographic zones and seasons in Sri Lanka.
(Figures refer to number of samples)

Location-zone	dry					wet				
	Type and number tested	n.d.	Number ⩽ 30 μg/kg	Number with 30 μg/kg	maximum aflatoxin content observed (μg/kg)	Type and number tested	n.d.	Number ⩽ 30 μg/kg	Number with 30 μg/kg	maximum aflatoxin content (μg/kg)
Anuradhapura	parboiled 87	79	8	0	12 – 30*	parboiled 94	83	11	0	4**
– dry	raw 14	14	0	0	–	raw 11	8	3	0	3**
Kurunegala	parboiled 95	77	15	3	30**	parboiled 98	93	5	0	5**
– intermediate	raw 6	6	0	0	–	raw 0	0	0	0	–
Veyangoda	parboiled 84	64	11	9	30**	parboiled 104	97	7	0	3**
– wet	raw 2	2	0	0	–	raw 2	2	0	0	–

* minimum detectable level 12 μg/kg; ** minimum detectable level 2μg/kg n.d. = not detected

Parboiled rice is more likely to be colonized by the fungi due to high moisture levels in them caused by improper drying, after parboiling. The gelatinized starch of the parboiled rice endosperm may also be more susceptible to fungal attack than the native starch of the raw endosperm.

As regards the susceptibility of raw rice, the number of samples analysed is not sufficient for a final conclusion. It is therefore not possible to conclude whether the parboiled rice is more susceptible to aflatoxin accumulation.

The aflatoxin B1 levels observed in the rice samples did not show any pattern of association with the different agro-climatic zones or the seasons indicating that the observed mean moisture levels up to 16% do not influence aflatoxin accumulation in rice during storage.

Under the present conditions where rice is not stored for periods above 7 days at moisture content above 16%, rice does not appear to pose a danger through aflatoxin contamination. However, the low aflatoxin levels detected in parboiled rice could indicate a potential danger specially if efficient drying is not practised after parboiling and prior to storage.

At present rice is a fast selling commodity in the market as Sri Lanka has not reached self sufficiency in rice. However, with self sufficiency in rice, which is expected in the near future, we may face problems of aflatoxin accumulation associated with longer periods of storage and at higher moisture levels as observed with prolonged storage of improperly dried rough rice.[1]

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THRESHOLD DENSITY OF *ECHINOCLOA CRUSGALLI (L)* BEAUV. IN RICE WEED COMPETITION

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Abstract : Experiments were conducted to determine the threshold *Echinocloa crusgalli* (L) Beauv. density in transplanted rice at the Regional Research Station, Angunukolapalessa, during the seasons Maha 1984/85 and Yala 1985. The soil of the experimental site was Ranna series of Reddish Brown Earths. The data indicated that the threshold *E. crusgalli* density at season long interspecific competition with rice was five plants per square meter. The decrease in yield at this weed density level was 8 – 17 per cent. The sensitive yield components of rice for the interspecific competition with *E. crusgalli* were the panicle number and the spikelet number per panicle. Projecting the data for broadcast rice the threshold level of *E. crusgalli* density was 6% of the total density. Weed dry weight gave highly significant negative correlation with grain yield and paddy straw weight.

1. Introduction

One of the major problems that reduces the rice yields world over is weed competition. Many weed species, vary from area to area and compete with rice in the field. Economics and statistics of the United States, Department of Agriculture (U.S.D.A)[12] reported that worldwide losses from weeds in rice was 33 m. metric tons annually valued at 12 billion dollars. Datta[3] reported that annual and perennial weed infestation reduced rice grain yields 20–80% depending on the method of establishment and in transplanted rice 11% reduction was observed.

In Sri Lanka, both annual and perennial weeds compete with lowland rice reducing the rice grain yields drastically. *Echinocloa crusgalli* (l) Beauv. among large number of genera in *Echinocloa* spp., is one of the most noxious weeds. *Echinocloa colonum* is second in importance.[5] Smith[10] reported that in the U.S.A. season long competition from *E. crusgalli* reduced grain yield by 25–70% and at a density of 11 plants/m^2 reduced grain yields inversely to rice stands. Chang[2] indicated that weed species at different densities vary in their competitiveness with rice and *E. crusgalli* at densities 100–200 plants/m^2 reduced rice yields 86–91%, respectively.

The method of land preparation and fertiliser practice also encourages the growth of weed species. Arai and Matsunaka[1] reported that emergence of *E. crusgalli* in transplanted rice was greatly reduced, when the field was ploughed 15 — 18 cm deep. Smith *et al.*[11] showed that pre-planting application of phosphorous stimulated the growth of *E. crusgalli.* Kleining and Noble[6] indicated at moderate levels of N (140 kg/h) grain yield of rice was inversely proportional to the density of *E. crusgalli.* In the present study, threshold density of *E. crusgalli* in lowland transplanted rice was determined with recommended rates of fertiliser application.

2. Materials and Methods

The experiments were conducted at the Regional Research Station, Angunukolapalessa, during the wet season (Maha) 1984/85 and dry season (Yala) 1985. Both experiments, *viz*; wet season and dry season, were conducted under rainfed conditions with supplementary irrigation, because of exceptionally high rainfall during the dry season. During experimentation, total rainfall of 251 mm and 365 mm and average sunshine hours of 6.79 and 6.85 per day were received during Maha 1984/85 and Yala 1985 seasons, respectively. In both experiments, 6m x 3m plots were used in a randomized complete block design replicated three times. The soils of the experimental site belonged to Ranna series of Reddish Brown Earths in the intermediate land class.

Three-month age group rice cultivars Bg 276—5 (Maha season) and Bg 34—8 (Yala season) were used. Seven treatments 1,3,5,10,15 and 20 *E. crusgalli* plants/m^2 and a control were used during the Maha season.In the Yala experiment, an additional treatment with 25 *E. crusgalli* plants/m^2 was used. Wet bed nurseries of both rices and *E. crusgalli* were raised and the rice was transplanted at the age of 21 days at a spacing of 20 cm x 20 cm with 3 plants/hill. The required density of *E. crusgalli* was also transplanted along with the rice and was well spaced in the plot. Fertiliser application and pest and disease control was carried out as per recommendations of the Department of Agriculture.[4]

Plant height and flag leaf length at harvest were measured from 10 plants selected at random. Tiller count/m^2 at 30 days after transplanting (DAT) and at harvest, yield components *viz*; panicle number/m^2 1000 seed weight, percentage filled grain, spikelets/panicle and panicle length from 10 panicles selected at random, were measured from each treatment. Paddy was harvested and *Echinocloa* straw was separated from paddy straw before threshing and weighed. After threshing and winnowing, grain weight and paddy straw weight were recorded.

Table 1. Grain yield and percentage yield loss of Bg 276–5, Maha 1984/85 season

Treatment	Grain yield mt/ha	% yield loss over control
Control	4.95 a	–
1 weed/m^2	4.79 ab	3.23
3 weed/m^2	4.35 abc	12.12
5 weed/m^2	4.11 abc	16.96
10 weed/m^2	3.93 bc	20.80
15 weed/m^2	3.52 c	24.03
20 weed/m^2	3.78 c	23.63

Any two means followed by the same letter is not significantly different at 5% probability level.

3. Results

Table 1 shows the results of the Maha 84/85 season experiment. In this experiment, yield component data were not taken except the panicles/m^2 and rice grain yields. Rice grain yield decreased with an increase in density of *Echinocloa* and was significant when it exceeds 5 plants/m^2.

Results of Yala season are presented in Tables 2 and 3. The plant height and flag leaf length showed no significant differences, whereas a significant drop in tiller count was observed at *Echinocloa* density of 25 plants/m^2. Among the other yield components, panicles/m^2 indicated a significant drop when the *Echinocloa* density exceeds 10 plants/m^2 (Table 2). Effect of competition on spikelet number/panicle shows that there were no significant differences among the treatments, but the treatments were significant compared to the control. Rest of the yield components do not show significant differences. Grain yield data indicate that weed density exceeding 5 plants/m^2 results in a significant drop (Table 3). Further, the rice straw yield also decreased with increase in *Echinocloa* density.

Correlation analysis of weed straw weight with grain yield and rice straw yield of dry season data shows a highly significant negative correlation, −0.97 and −0.91, respectively.

Table 2. Tiller count at 35 DAT, panicle number and spikelet number per panicle at harvest of Bg 34–8, Yala 1985

Treatment	Tillers/m^2	Panicles/m^2	Spikelets per panicles
Control	297.5 a	247.5 a	225.4 a
1 weed/m^2	307.3 a	227.5 ab	196.6 b
3 weed/m^2	295.0 a	237.5 ab	187.9 b
5 weed/m^2	315.0 a	210.0 ab	187.5 b
10 weed/m^2	292.5 a	225.0 ab	197.1 b
15 weed/m^2	277.5 a	192.5 b	174.8 b
20 weed/m^2	265.0 a	197.5 b	178.3 b
25 weed/m^2	197.5 b	165.0 c	185.9 b

Any two means followed by the same letter is not significantly different at 5% probability level.

Table 3. Grain yield, straw and weed weight at different densities of *Echinocloa*, Yala 1985.

Treatment	Grain yield mt/ha	Straw weight mt/ha	Weed Dry weight mt/ha	% yield loss over control
Control	4.68 a	4.50 a	–	–
1 weed/m^2	4.20 ab	3.84 abc	0.57 d	10.25
3 weed/m^2	4.37 a	3.92 ab	1.43 c	6.62
5 weed/m^2	4.29 ab	2.91 cd	1.01 d	8.33
10 weed/m^2	3.41 b	2.78 d	3.18 b	27.13
15 weed/m^2	3.43 b	2.85 c	2.94 c	26.70
20 weed/m^2	3.04 c	2.47 d	4.24 b	35.04
25 weed/m^2	1.94 d	1.51 c	5.65 a	58.54
Correlation Coefficient Weed weight	– 0.97	– 0.91	–	–

Any two means followed by the same letter is not significantly different at 5% probability level.

4. Discussion and Conclusions

Plant height at harvest in both seasons and flag leaf length during dry season showed no significant differences among treatments even though there were slight differences in height with increased *Echinocloa* density.

Tillers/m^2 at 35 DAT indicated that there appears to be no effect (Table 2) on increase in density of *Echinocloa* upto 20 plants/m^2. However equal number of hills planted to rice and *E. crusgalli* (25 plants/m^2) indicated a 33% drop in tiller number which was significant. On the other hand, panicle number at harvest, which is also the effective tiller number, showed significant differences among treatments and a decreasing trend with the increase in *E. crusgalli* density/m^2. This also indicates that increased interspecific competition with increased weed density rendered a greater number of tillers ineffective, thus bringing about significant differences in panicle number among treatments. Noda[9] also indicated that *E. crusgalli* competition during maximum tillering reduces the panicle number, while during early ripening reduces grain yield and quality. Data further indicated that the spikelet number/panicle was highly sensitive to interspecific competition rendered by *E. crusgalli* and gave a significant drop even at a density of 1 weed/m^2. Further significant decrease in spikelet number, was not observed even if the *E. crusgalli* density increased to 25 plants/m^2. The percentage filled grain and 1000 seed weight did not show significant differences among treatments.

Grain yield in both seasons with season long interspecific weed competition decreased with the increase in *E. crusgalli* density and was significant when it exceeds 5 plants/m^2. At this threshold level, the decrease in grain yield over the zero weed density was 8.33% and 16.9% during Yala and Maha seasons respectively. Datta[3] also indicated season long competition by mixed annual and perennial weeds reduced grain yield by 11% in transplanted rice. The threshold level as a percentage of total density (75 rice plants and 5 *E. crusgalli* plants) was 6.2%. Thus if we were to project this data for broadcast sown rice, we can safely conclude that a significant drop in grain yield could be expected if the *E. crusgalli* density exceeds 6%. Further, increasing the *E. crusgalli* density to 25 plants/m^2, a 58.5% drop in grain yield was observed and at this density all the planting hills were shared by 3 rice plants and a single weed. Smith[10] indicated similar results where season long competition at density levels, 3 rice and 25 *E. crusgalli* plants/ft^2 and 31 rice and 25 *E. crusgalli* plants/ft^2 resulted in 79% and 95% rice yield reduction, respectively. Noda[9] also indicated that competition at early ripening stage reduced rice grain yield and quality. Lubigan and Vega[7] showed that *E. crusgalli* density of 20 plants/m^2 reduced grain yield by 20%.

These data indicate that *E. crusgalli* at very low densities renders a very high competition to rice. The highly competitive nature of this weed could be attributed to its physiological superiority, being a plant following C_4 photosynthetic pathway. C_4 plants normally have a very high growth rate and a high dry matter production, as reflected in these experiments. At the highest weed density level (25 plants/m^2), while competing with 3 rice plants at the same hill, gave 5.65 mt/ha dry matter production whereas the control treatment (25 hills/m^2, with 3 rice plants per hill) with only intra-specific competition rice plants gave 4.50 mt/ha (Table 3).

Correlation analysis showed a very high significant negative correlation between weed dry weight and grain yield. Significant negative correlation between weed dry weight and rice straw indicate an increase in weed dry matter with the increase in weed density and a corresponding decrease in rice straw weight due to increasing intensity of inter-specific competition (Table 3). Matsunaka[8] indicated that a linear relationship existed between weed density and yield loss, at *E. crusgalli* densities normally encountered in the field. At threshold population level decrease in rice straw weight due to interspecific competition was 35.3% even though the decrease in grain yield was 8.33%.

In conclusion, the threshold *E. crusgalli* density with season long inter-specific competition was 5 plants/m^2 and the decrease in yield was 8.33 — 16.9%. The major contributory yield components decreasing grain yield with the increase in weed population were panicles/m^2 and the spikelet number/panicle. Significant negative correlation between weed dry weight with grain yield and rice straw weight was also observed.

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A RAPID METHOD FOR THE ESTIMATION OF CAERULOPLASMIN LEVELS IN HUMAN SERUM, THE ESTABLISHMENT OF THE CLINICAL NORM AND THE STUDIES OF THE CAERULOPLASMIN LEVELS IN VARIOUS CONDITIONS

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Abstract : A rapid method was developed for the estimation of caeruloplasmin levels in human subjects and the reliability of the method established by carrying out optimal condition and routine condition variance tests. The caeruloplasmin levels in 94 Sri Lankans were investigated and the clinical norm established. The serum caeruloplasmin levels lie between the range 1.19 – 2.78 μmoles/1 (18–42 mg%) with the mean of 1.98 μmoles/1 (29.95 mg%) and the standard deviation of 0.38 μmoles/1 (5.72 mg%). The caeruloplasmin levels were also investigated in persons suffering from liver diseases and in pregnant mothers. The values were raised in viral hepatitis, and in pregnancy but decreased in Wilson's disease and alcoholic cirrhosis.

1. Introduction

Copper is found in serum as metaloproteins. About 96% of the plasma copper is firmly bound to the α_2 — globulin and known as caeruloplasmin. Most of the non-caeruloplasmin bound copper, is attached to the serum albumin. It is believed that copper performs catalytic functions in the living organism. Copper influences erythropoiesis and its deficiency impairs absorption and transport of iron and decreases haemoglobin synthesis.

Caeruloplasmin is estimated by several methods,[1,2,3,4,5,6] but the best and the most convenient method is to measure the intensity of the purple coloured complex formed by the oxidation of para phenyl diamine (PPD) to give quinone types of compounds. This complex has a characteristic absorbance at 525 nm or 530 nm. Henry *et al*[5] recorded the optical density at 10 minutes and 40 minutes incubation at 37°C, after the addition of serum. The difference between the two figures was a measure of the

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serum oxidase activity and normal sera produced a different optical density of 0.28 to 0.57 at 530 nm. They used an empirical figure of 5000 to convert the optical density at 530 nm per minute at 25°C, to mg caeruloplasmin per 100 ml of serum. Conversion factor in this case became 5000 x Tf x 1/30 where Tf is the temperature correction. Houchin[6] however, recorded the optical density at 525 nm after 15 minutes incubation at 37°C and reported the activity as difference in optical density or mg of caeruloplasmin per 100 ml, by means of the formula 150 x OD_{525} 1.7 where OD_{525} is optical density at 525 nm. The conversion to international units was calculated as follows —

$$\frac{\text{mg\\% x 10 x 1000}}{151{,}000} = \text{Moles/1}$$

151,000 is the molecular weight of caeruloplasmin.

This procedure cannot be adopted as a routine method in most Sri Lankan laboratories because of the non-availability of visible spectrophotometers. It was decided to estimate the colour intensity both colorimetrically and spectrophotometrically in order to correlate the findings between the two readings.

In the recent past, there have been very many requests for the estimation of caeruloplasmin levels in patients suffering from Wilson's disease. But no data is available on caeruloplasmin levels in Sri Lankans. This and the non-availability of a rapid method, prompted modification of the existing method using a colorimeter which could then be used in most provincial laboratories.

2. Materials and Methods

2.1 Reagents

Paraphenylene diamine dihydrochloride powder obtained from BDH Chemicals Ltd, was brown in colour because of its auto-oxidative properties. It was therefore recrystallized using ethanol and water. After recrystalisation it was a colourless compound having a melting point of 140°C. It is necessary to store this compound in an amber coloured bottle in a dessicator to prevent oxidation.

Sodium azide (a white powder) was also obtained from BDH Chemicals Ltd.

The buffer used for the assay was a glacial acetic acid sodium acetate buffer of molarity 0.55 M and pH 5.2. (20 ml glacial acetic acid and 98.3g anhydrous sodium acetate dissolved in 1 litre). The pH was checked by a pH

meter, PYE UNICAM Model 291 mk 2.

To establish clinical norms, the blood samples from normal donors were obtained from the Blood Bank, General Hospital, Colombo. All samples were screened by the Blood Bank for haemoglobin, Wasserman reaction, total proteins and for malarial parasites.

2.2 Pathological specimens

Samples of blood received by the Biochemistry Department of the Medical Research Institute from several institutions in the country for routine assay for hepatitis, cirrhosis, Wilson's disease and other abnormalities, were used to estimate the caeruloplasmin levels. Samples of the blood from women in various stages of pregnancy were obtained from the Castle Street Hospital for Women, Borella.

2.3 Quality Control Serum

The pooled serum used for the estimation of optimal condition variance (OCV) and routine condition variance (RCV) was from samples of serum received for routine biochemical estimations. This serum was dispensed in 1 ml portions, in sterile bottles and stored at $-20^{\circ}C$.

2.4 Assay of caeruloplasmin activity

One ml of buffered substrate (0.1% w/v paraphenylene diamine dihydrochloride in acetate buffer, freshly prepared) was pipetted into each of two tubes, test and blank, covered with liquid paraffin, and pre-incubated for 5 min to attain $37^{\circ}C$, using a water bath. To the test, 0.1 ml of serum was added and incubated for exactly 15 minutes more. Then 5 ml of azide solution (0.2% w/v) was added both to the blank and to the test to inhibit the enzymic reaction and 0.1 ml of serum added to the control and the purple colour was measured using Klett Summerson colorimeter with filter No.54 (green), and Corning –253 spectrophotometer at 525 nm against the blank as zero.

Total protein was determined by the Biuret method,[7] albumin by the bromocresol green binding method,[8] bilirubin by treatment with diazotised sulphanilic acid,[9] serum glutamate oxalocetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) by the 2,4 dinitro phenyl hydrazine method[10] and alkaline phosphatase by the antipyrine method.[11]

3. Results and Discussion

3.1 Correlation between colorimetry and spectrophotometry

The relationship between the colorimetric and spectrometric readings is shown in Figure 1. It was interesting to note that the colorimetric readings were found to be 500 times that of the spectrometric readings (525 nm). This was so in almost all the readings. Therefore the empirical formula of Houchin[6] becomes (0.3 x colorimetric reading) – 1.7 mg of caeruloplasmin per 100 ml of serum.

3.2 Quality control

Before the introduction of any method of assay for routine use the WHO has recommended[11] that optimal condition variance (OCV) should be determined by analysing 20 replicate speciments. The results of such analysis of the OCV, given in Figure 2, show that the variation did not exceed ± 2 SD (standard deviation) of the mean. In this case the value of the SD was 0.099 μ moles/1 (1.49 mg%). OCV was calculated by the equation $SD/\bar{x} \times 100$ where SD is standard deviation and $\bar{x}$ is the arithmetic mean. It gave a value of 4.76%. OCV varies from estimation to estimation and the values of enzymes such as amylase, SGPT and Lactate dehydrogenase are 6, 7 & 8 respectively.[8] Therefore, the value 4.76% obtained by the present method seems satisfactory.

After OCV was established for the method, the routine condition variance (RCV) was also established over a period of 20 days, using the same sample under identical conditions. During RCV estimation, at least two random serum samples were included in each batch of assay. The RCV, shown in Figure 3, was 8.11% which seems satisfactory.

3.3 Clinical Norms

The clinical norm was established on 85 males and 9 females between the ages of 20 and 60. The frequency of distribution of the caeruloplasmin levels in Sri Lanka is shown in the form of a histogram in Figure 4. The standard deviation was 0.379 μ moles/1 (5.72 mg%) and arithmetic mean was 1.98 μ moles/1 (29.95 mg%) of serum. The lowest value was 1.20 μ moles/1 (18.1 mg%) and the highest value 2.74 μ moles/1 (41.5 mg%) of serum. The normal range was from 1.19 to 2.78 μ moles/1 (18 to 42 mg%) of serum.

Statistical normality of the sampling was established by plotting the results on a normal probability paper, as shown in Figure 5. The resulting straight line confirmed the sampling as satisfactory and the results as

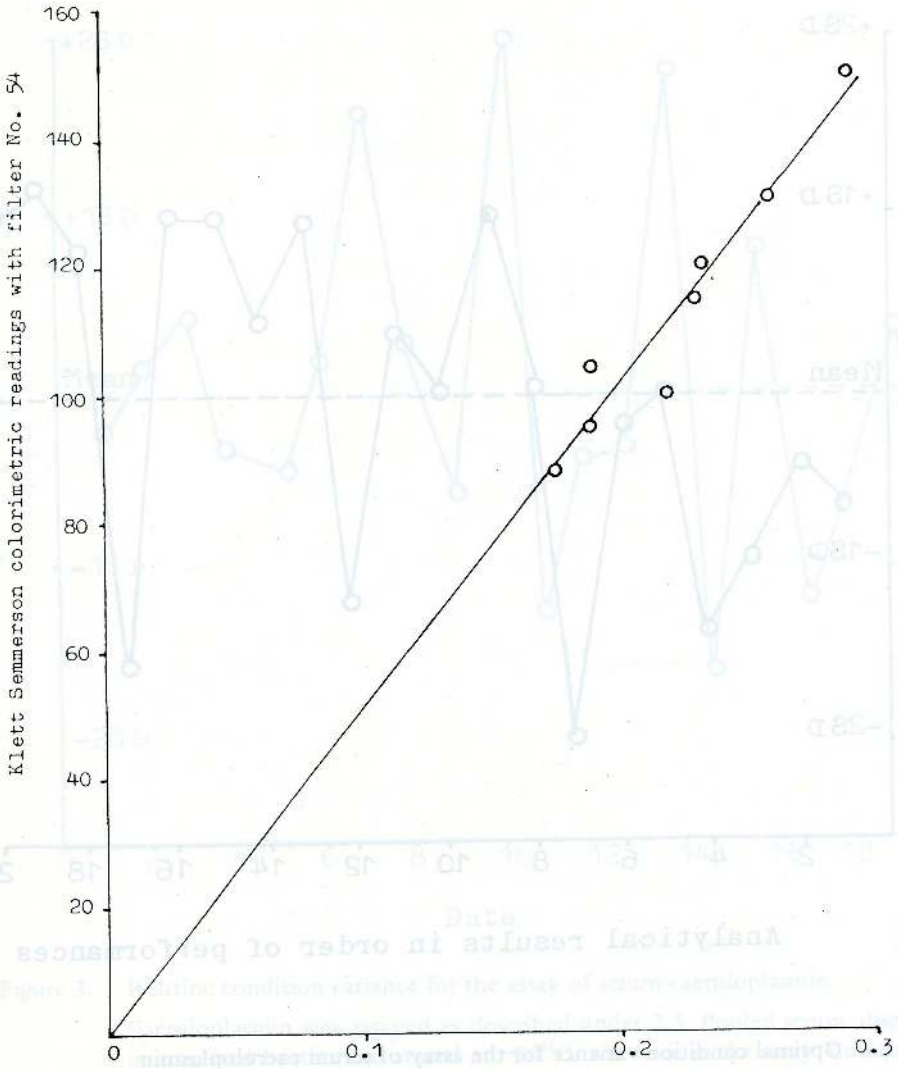

Figure 1. Relationship between colorimetric and spectrophotometric readings

Caeruloplasmin was assayed as described under 2.5. The intensity of the colour was measured using Klett Summerson colorimeter with filter No.54 and spectrometric readings recorded on a Corning–253 spectrometer against the blank at zero.

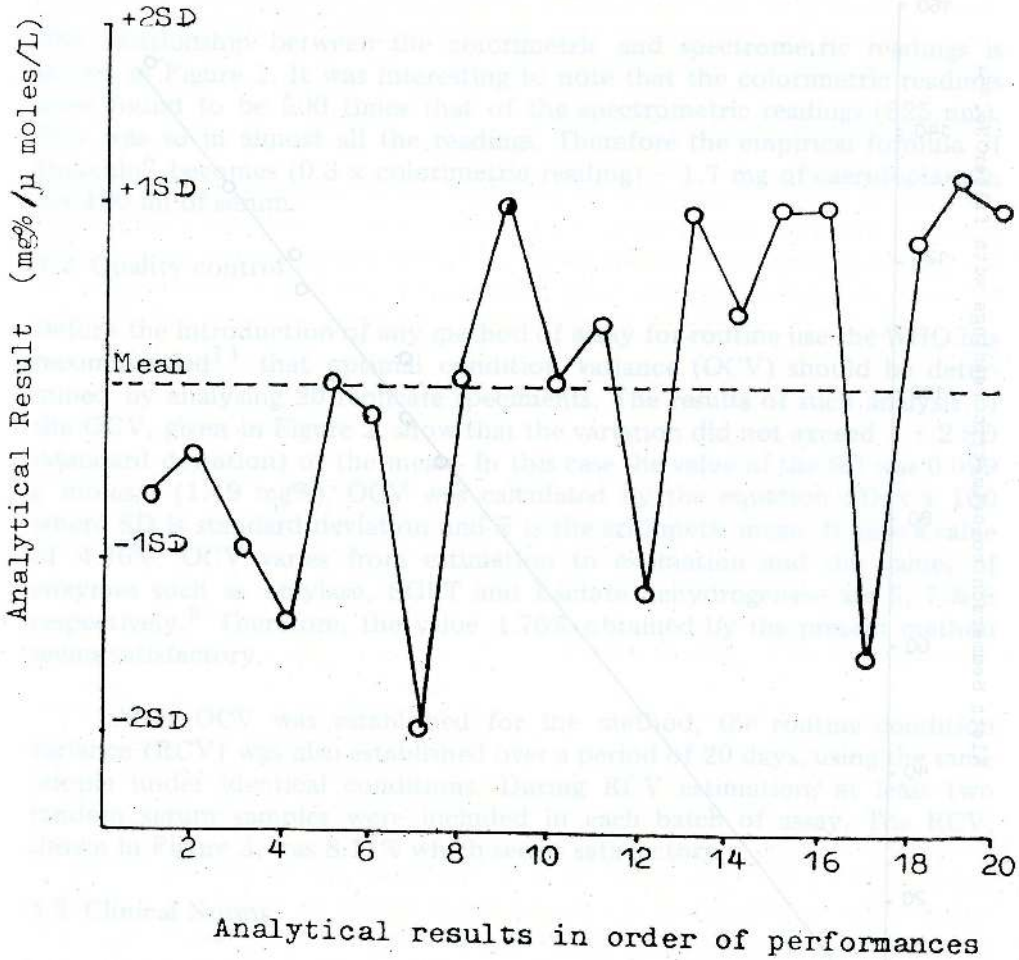

Figure 2. Optimal condition variance for the assay of serum caeruloplasmin

Caeruloplasmin was assayed as described under 2.5. Tests were carried out on pooled serum on 20 replicate specimens at the same time under optimal conditions. Colorimetric readings were recorded on a Klett Summerson using filter No. 54. SD = Standard deviation.

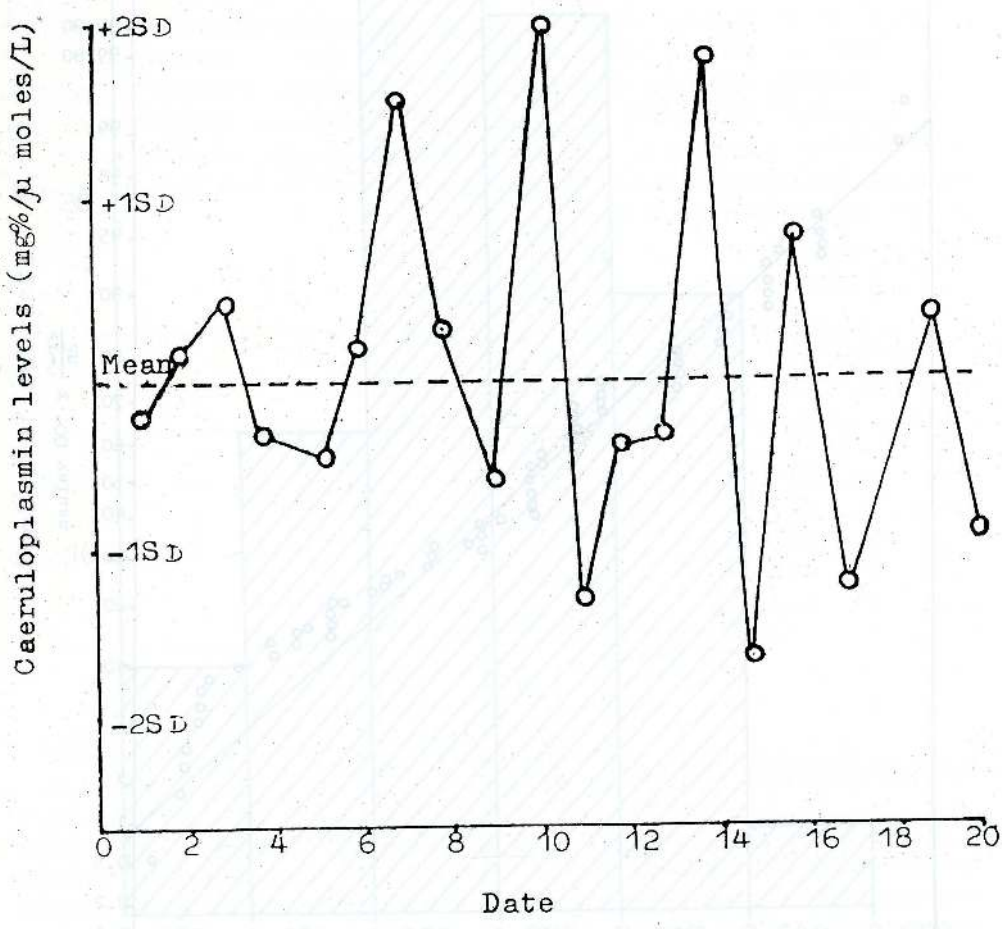

Figure 3. Routine condition variance for the assay of serum caeruloplasmin

Caeruloplasmin was assayed as described under 2.5. Pooled serum, dispensed in sterilized bottles and stored at $-20^{\circ}C$, was used in the assay. Colorimetric readings were recorded on a Klett Summerson using filter No. 54.
SD = Standard Deviation.

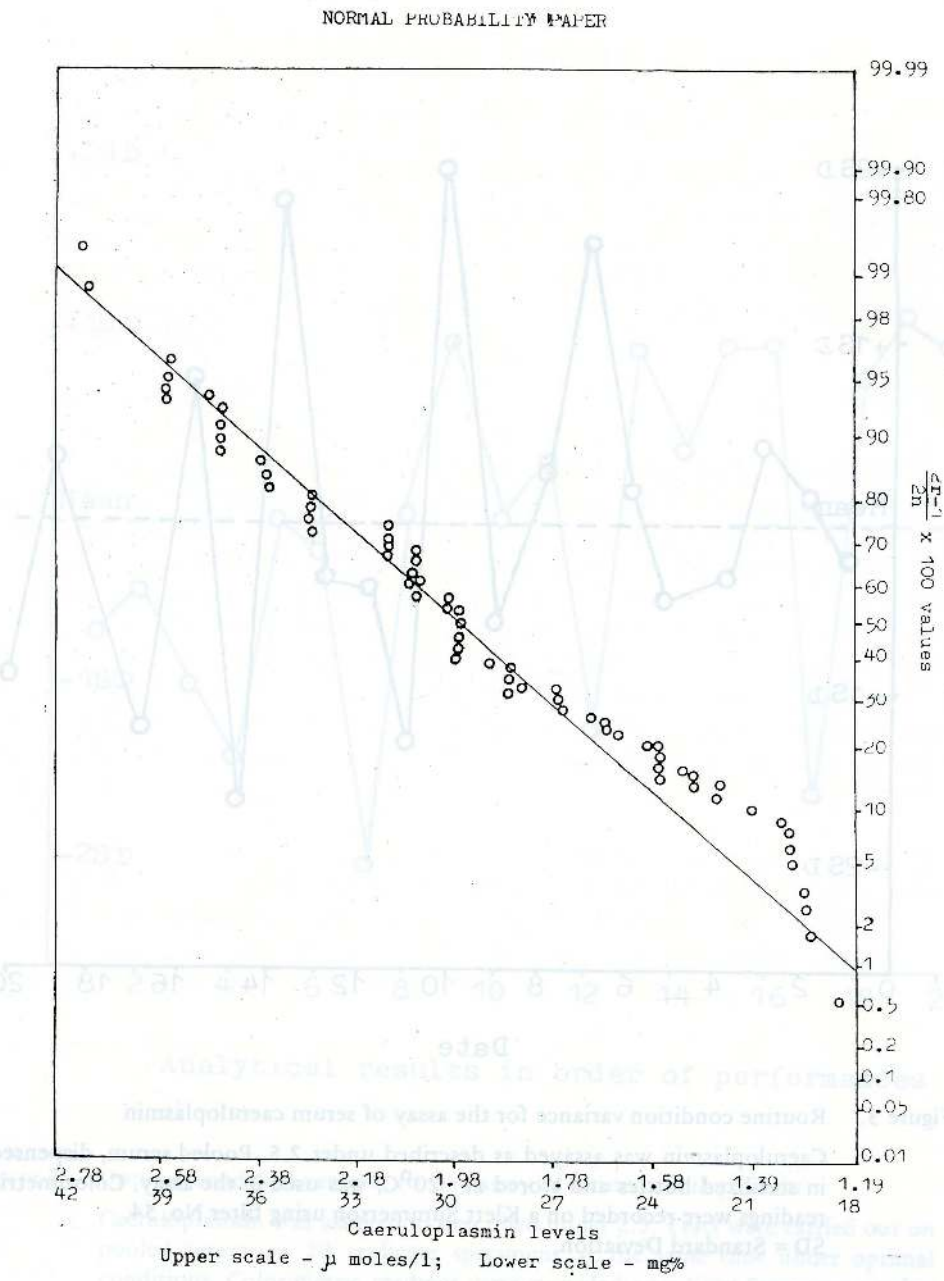

Figure 4. Distribution of caeruloplasmin activity in Sri Lankans

Caeruloplasmin was assayed as described under. 2.5. The intensity of the colour was measured using a Klett Summerson colorimeter, with filter No. 54 against the blank as zero.

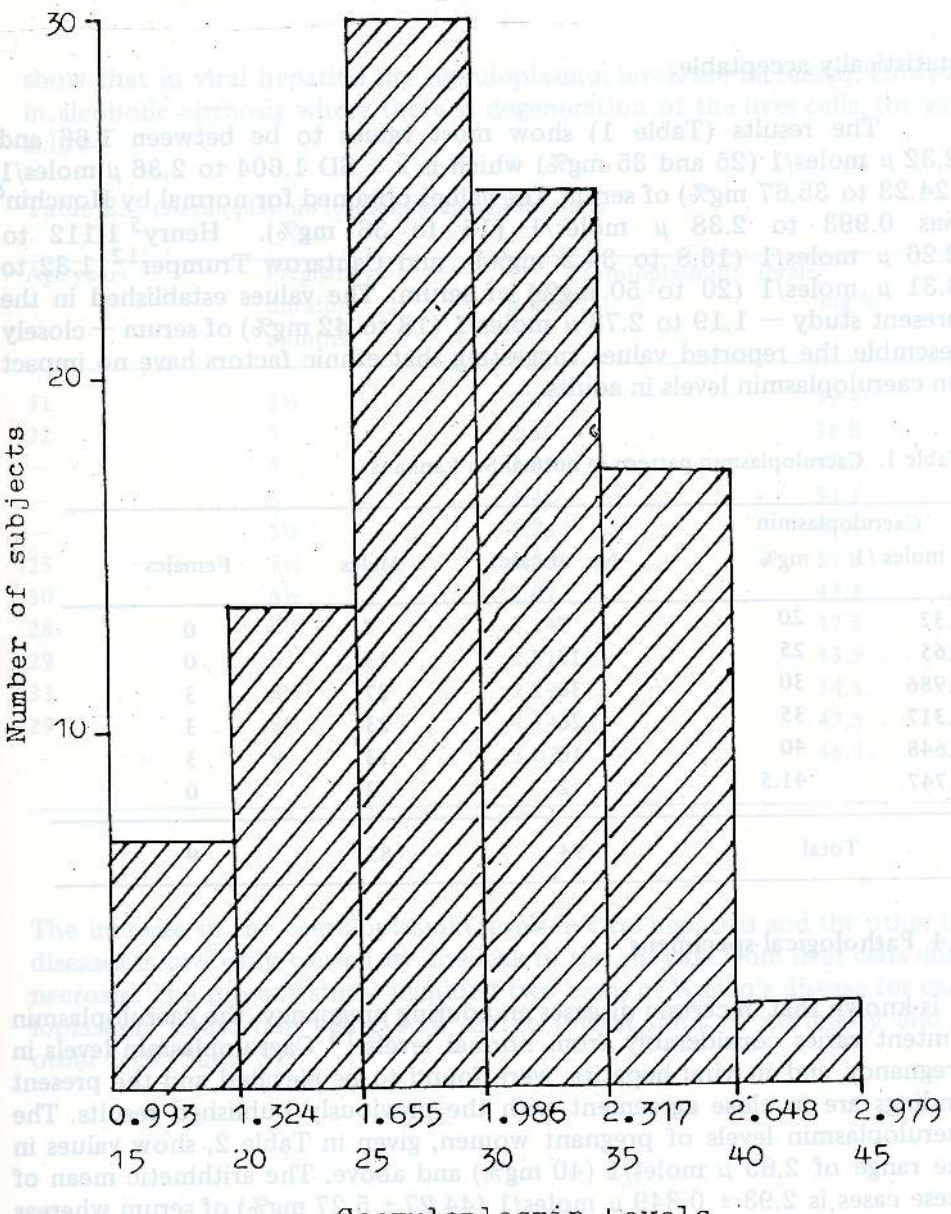

Figure 5. Normal probability distribution

Caeruloplasmin was determined as described under. 2.5. $\frac{2r-1}{2n} \times 100$ was calculated where n is the sample size (94) and r is the number is ascending order of the values. The graph was plotted on a normal probability paper. $\frac{2r-1}{2n} \times 100$ figures on the vertical axis and caeruloplasmin levels on the X axis.

statistically acceptable.

The results (Table 1) show most values to be between 1.66 and 2.32 μ moles/1 (25 and 35 mg%) which is $\bar{x}$ ± SD 1.604 to 2.36 μ moles/1 (24.23 to 35.67 mg%) of serum. The values obtained for normal by Houchin[6] was 0.993 to 2.38 μ moles/1 (15 to 36 mg%). Henry[5] 1.112 to 2.26 μ moles/1 (16.8 to 34.2 mg%) and Cantarow Trumper[12] 1.32 to 3.31 μ moles/1 (20 to 50 mg%) of serum. The values established in the present study — 1.19 to 2.78 μ moles/1 (18 to 42 mg%) of serum — closely resemble the reported values suggesting that ethnic factors have no impact on caeruloplasmin levels in adults.

Table 1. Caeruloplasmin pattern in normal Sri Lankans

Caeruloplasmin μ moles /1	mg%	No. of cases	Males	Females
1.32	20	7	7	0
1.65	25	13	13	0
1.986	30	30	27	3
2.317	35	26	23	3
2.648	40	16	13	3
2.747	41.5	2	2	0
Total		94	85	9

3.4 Pathological specimens

It is known that in certain diseases and during pregnancy, the caeruloplasmin content varies considerably from normal levels.[13] Caeruloplasmin levels in pregnancy and in viral hepatitis were found to be elevated and the present findings are in close agreement with the previously published results. The caeruloplasmin levels of pregnant women, given in Table 2, show values in the range of 2.65 μ moles/1 (40 mg%) and above. The arithmetic mean of these cases is 2.93 ± 0.349 μ moles/1 (44.27 ± 5.27 mg%) of serum whereas the normal values was shown to be 1.98 ± 0.378 μ moles/1 (29.95 ± 5.72 mg%) of serum. These findings are in agreement with those reported earlier.[14] The pregnancy cases studied had caeruloplasmin levels above the normal mean + 2 SD 2.74 μ mol/1 (41.39 mg%). It is suggested that the raised caeruloplasmin levels in pregnancy reflect an adaptive increase in caeruloplasmin to meet the increasing demand for fetal haemoglobin synthesis. The caeruloplasmin levels of the pathological specimens (Table 3)

show that in viral hepatitis the caeruloplasmin levels are increased. However, in alcoholic cirrhosis where there is degeneration of the liver cells, the value is low.

Table 2. Caeruloplasmin levels in pregnant mothers

Age years	Pregnancy duration months	Caeruloplasmin levels μ moles/1	mg %
31	3½	2.61	39.4
22	5	2.57	38.8
—	5	2.87	43.3
—	5	3.42	51.7
—	5½	2.75	41.5
25	5½	3.56	53.8
30	6½	2.87	43.3
28	6½	3.14	47.5
29	8	2.91	43.9
33	8½	2.27	34.3
29	8½	3.14	47.5
—	9	3.07	46.3

The increase in the caeruloplasmin levels in viral hepatitis and the other liver diseases is probably caused by the leak of the enzyme from liver cells due to necrosis. The present study included two cases of Wilson's disease for caeruloplasmin assay, one being 55% of the lowest limit of normality and the other 28% (Table 3).

Table 3. Caeruloplasmin levels in different diseases

Disease	Sex	Age	Test and Normal Values								
			Total protein 63–79 g/l	Albumin 37–49 g/l	Globulin 26–30 g/l	Bilirubin 3–14 μmol/l	SGOT 2–20 Iu	SGPT 2–15 Iu	Alkaline phosphatase 3–13 K.A. Units	Caeruloplasmin 1. 19–2.78 μ mol/l	18–42 mg%
Cirrhosis	M	–	57	17.6	39.4	–	–	–	–	0.88	13.3
	M	–	–	–	–	205.7	–	48	–	0.28	4.3
	M	47	–	–	–	15.3	–	20	–	1.08	16.3
	M	57	–	–	–	188.7	–	30	–	0.09	1.3
	M	40	–	–		–	–	60	–	1.08	16.3
	M	55	–	–	–	6.8	–	10	–	1.09	16.4
	F	55	–	–	–	–	60	120	–	0.97	14.7
Nephrotic Syndrome	F	4	37.5	14	23.5	–	–	–	–	0.48	7.3
Hepatitis	M	–	–	–	–	8.5	18	8	–	2.77	41.8
	F	7	–	–	–	214.2	–	–	–	2.47	37.3
Viral Hepatitis	M	50	80.3	37.6	42.7	–	–	90	85	2.67	40.3
	–	–	–	–	–	135	–	600	–	1.87	28.3
Wilson's Disease	M	–	–	–	–	–	–	–	–	0.78	11.8
	M	25	–	–	–	–	–	–	–	0.34	5.1

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MECHANISM OF OXIDATIVE RING CONTRACTION OF MONOCYCLIC POLYOXOENEDIOLS BY ACTIVE MANGANESE DIOXIDE

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(Date of receipt : 28.11.84)
(Date of acceptance : 10.11.86)

Abstract : A new mechanism which involves a benzylic acid type of rearrangement of fully dehydrated triquinoyl is presented for the oxidative ring contraction reactions of monocyclic polyoxoenediols. Manganese dioxide is thought to merely act as an oxidising agent, oxidising these polyoxoenediols to triquinoyl and is not involved in the actual ring contraction reaction.

1. Introduction

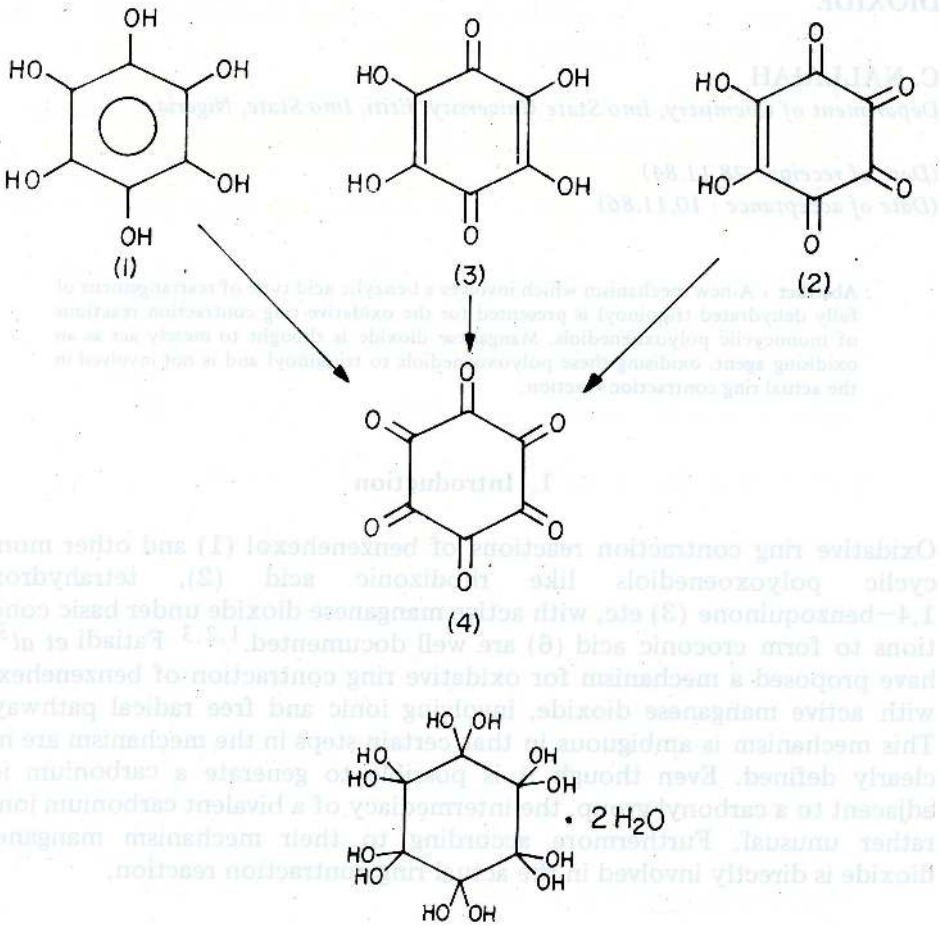
Oxidative ring contraction reactions of benzenehexol (1) and other monocyclic polyoxoenediols like rhodizonic acid (2), tetrahydroxy 1,4—benzoquinone (3) etc, with active manganese dioxide under basic conditions to form croconic acid (6) are well documented.[1,2,3] Fatiadi *et al*[3,4] have proposed a mechanism for oxidative ring contraction of benzenehexol with active manganese dioxide, involving ionic and free radical pathways. This mechanism is ambiguous in that certain steps in the mechanism are not clearly defined. Even though it is possible to generate a carbonium ion adjacent to a carbonyl group, the intermediacy of a bivalent carbonium ion is rather unusual. Furthermore according to their mechanism manganese dioxide is directly involved in the actual ring contraction reaction.

2. Results and Discussion

It is significant that compounds (1), (2) and (3), all of which form croconic acid in good yields can also be oxidised to triquinoyl (4) (Scheme—1).

The structure of triquinoyl has been controversial for many years and the absence of a characteristic absorption band in the carbonyl region of the infra red spectrum of this compound, has led to the proposal[7] that it has a

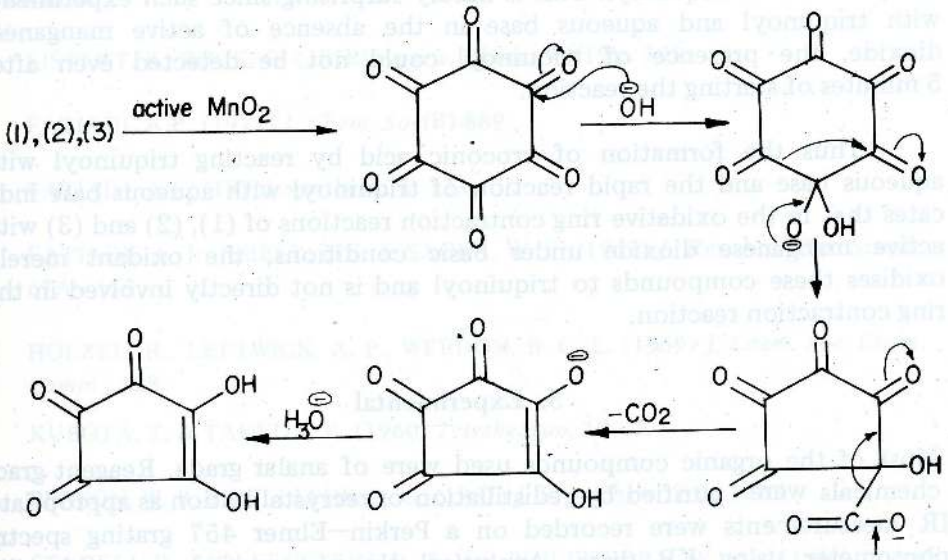
SCHEME - I



fully hydroxylated gemdiol structure (5). The ^{13}C-NMR Spectrum of triquinoyl in DMSO did not give a single peak for the six equivalent $C(OH)_2$ (or six equivalent C=O if present in the fully dehydrated form) but instead gave three peaks which were assigned to rhodizonic acid dihydrate.[8] The mass spectrum of triquinoyl octahydrate did not show a parent ion peak of either (5) (*m/e* 312) or (4) (*m/e* 168). Instead the mass spectrum revealed intense peaks at *m/e* 172 and *m/e* 170 due to (3) and (2) respectively.[1] Thus it is apparent that triquinoyl exists in the fully hydroxylated state (5) in the solid form and in solution it is in equilibrium with various species such as (2), (3), etc.

It has been reported[4] that triquinoyl can be oxidised to croconic acid by reacting it with active manganese dioxide under basic conditions. However, we have synthesised croconic acid in substantial yields (~50—60%) by refluxing triquinoyl in 50% aqueous solution of potassium hydroxide, for (30 — 45) minutes in the absence of active manganese dioxide. This indicates that the formation of croconic acid from (1), (2) and (3) could be via triquinoyl, active manganese dioxide merely acting as an oxidising agent, oxidising these compounds i.e.(1), (2) and (3) to triquinoyl. The mechanism that we propose for these transformations is depicted in Scheme — 2.

SCHEME - 2



Active manganese dioxide oxidises benzenehexol and other related compounds like (2) and (3) to (4), which under basic conditions undergoes a benzilic type of rearrangement to form the intermediate (7). This intermediate is a β-keto acid and undergoes ready decarboxylation to form croconic acid.

It is possible that in the absence of active manganese dioxide, triquinoyl (in form (5)) reverts to the fully dehydrated form (4) due to aerial oxidation. However when the reactions were carried out with triquinoyl and aqueous base in an inert atmosphere of nitrogen, the major product was again found to be croconic acid. Thus it appears that the fully hydroxylated form of triquinoyl (5) changes to the dehydrated form (4) on refluxing. In the oxidative ring contraction reactions of (1), (2) and (3) with active manganese dioxide, samples of the reaction mixture were withdrawn at intervals of 5 minutes. Analysis of these samples by tlc using silica gel and various mixtures of petroleum ether ($40^{\circ}C - 60^{\circ}C$)/ethyl acetate (10:1, 8:1, 5:1) with an authentic sample of triquinoyl as reference did not reveal the presence of triquinoyl. This is hardly surprising since such experiments with triquinoyl and aqueous base in the absence of active manganese dioxide, the presence of triquinoyl could not be detected even after 5 minutes of starting the reaction.

Thus the formation of croconic acid by reacting triquinoyl with aqueous base and the rapid reaction of triquinoyl with aqueous base indicates that in the oxidative ring contraction reactions of (1), (2) and (3) with active manganese dioxide under basic conditions, the oxidant merely oxidises these compounds to triquinoyl and is not directly involved in the ring contraction reaction.

3. Experimental

Most of the organic compounds used were of analar grade. Reagent grade chemicals were purified by redistillation or recrystallisation as appropriate. IR measurements were recorded on a Perkin—Elmer 457 grating spectrophotometer using KB discs. Analytical tlc was carried out with silica gel F_{254} (0.25 mm) deposited on plastic sheets (commercially available), and developed using various mixtures of petroleum ether ($40—60^{\circ}C$) and ethylacetate.

Triquinoyl[1] and active manganese dioxide[2] were prepared according to the methods given in literature.

Reaction of Triquinoyl with Base

Triquinoyl (1.5g) was mixed with water (50 cm^3), ethyl alcohol (25 cm^3) and 50% aqueous potassium hydroxide (15 cm^3). The mixture was refluxed for 45 minutes and filtered immediately. The filtrate was concentrated and on cooling in an ice bath, yielded bright yellow crystals of potassium croconate. A further crop of potassium croconate was obtained by concentrating the filtrate 0.789 g (52%), m.pt 360°C, IR (KBr) identical to that of an authentic sample of potassium croconate.

Acknowledgements

The author would like to thank Professor. L. Crombie for generating interest in cyclic polyoxoenediols.

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Li^+ ION CONDUCTION IN COBALTOUS COBALTICYANIDE DOPED WITH LITHIUM CHLORIDE

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Abstract : Cobaltous cobalticyanide (a Prussian Blue type of solid with large interstitial cavities) doped with lithium chloride is found to exhibit Li^+ ion conduction. The data on temperature variation of conductivity at different concentrations of LiCl are presented.

1. Introduction

Prussian Blue ($Fe_4(Fe(CN)_6)_3$) and related heavy metal hexacyanides form a class of crystalline solids with complex ions whose structure is well understood.[1,2,3] They have similar face-centered cubic arrangement of metal cations at the corners of unit cubes linked by cyanide ions placed along the edges. A peculiar property arising from this structure is that the unit cells are unusually large (lattice constant $\sim 10\ A^o$).[1,2,3] As the result the crystal can accommodate foreign molecules and ions as interstitial impurities.[1,2,3] We have noted that Prussian Blue type compounds doped with Li salts exhibit ionic conduction. Electronic conductivity (30°C) of Prussian Blue is $\sim 3 \times 10^{-6}\ \Omega^{-1}\ m^{-1}$. However, it was found that compound with identical structure cobaltous cobalticyanide ($Co_4(Co(CN)_6)_3$) has smaller electronic conductivity (30°C) $\sim 4 \times 10^{-8}$ and high stability towards thermal degradation. Consequently this material is more suitable for studying Li^+ ion transport in metal hexacyanides doped with lithium salts. In this note we report our observations on Li^+ ion conduction in $Co_4(Co(CN)_6)_3$ doped with LiCl.

2. Experimental

$Co_4(Co(CN)_6)_3$ was prepared by adding potassium cobalticyanide (Aldrich) solution (0.1 M) dropwise to a solution of cobalt nitrate (0.5 M). (Cobalt nitrate kept in excess to avoid formation of double salts containing potassium). The pink precipitate of $Co_4(Co(CN)_6)_3$ separated by filteration was washed with distilled water until the filterate is free from potassium. The powder was dried in vacuum at 140°C for several hours to remove water of hydration. (Anhydrous material has a deep blue colour). The doping with LiCl was done by the following method. $Co_4(Co(CN)_6)_3$ was mixed with the desired amount of LiCl, the mixture homogenized and then dried in vacuum at 140°C to remove all moisture. The dried powder was compacted between carbon electrodes in a glass tube (diameter $\sim$ 0.6 cm) to a pressure of 800 psi until a pellet (length $\sim$ 0.5 cm) was formed. Ends of the tube were sealed with epoxy resin, the sample immersed in a thermostatic oil bath and a.c. (40 Hz) conductivity measured. (Modified Electronic Instruments Conductivity Bridge Model MC — 1, operated at 9 V). The d.c. conductivity was also measured by the polarization (blocking electrode) method[4,5] and found to be of the same order as the a.c. values. The rapid decrease in conductivity with time approaching a limit comparable to intrinsic electronic conductivity of $Co_4(Co(CN)_6)_3$ clearly demonstrated that the charges carried are ionic.

3. Results and Discussion

Figure 1 gives a plot of $\ln \sigma$ vs T^{-1} for different concentrations of LiCl (c, measured as a percentage by wt). In each case the graph is a straight line showing that the relation,

$$\sigma = \sigma_\circ e^{-E/kT} \qquad (1)$$

is satisfied, both E and $\sigma_\circ$ are found to depend on the degree of doping (ie, c). The plots of E vs c and $\sigma_\circ$ vs c are shown in Figures 2 and 3. The conductivity was found to be maximum ($\sigma_{30} \simeq 2.5 \times 10^{-3}\ \Omega^{-1}m^{-1}$) when c $\simeq$ 34% wt (Figure 4) and minimum value of E also corresponds to this value of c. Again the minimum value of $\sigma_\circ$ happens occur when the level of doping is $\sim$ 34%. It is possible that the critical point occurs when the interstitial cavities are nearly filled with LiCl. A simple calculation based on estimate of the volume of an interstitial cavity (using following data: lattice constant, ionic radii of Co^{2+}, Co^{3+} and CN^-) and density of solid LiCl indicate that the cavities get completely filled when c $\simeq$ 41%. The Li^+ ion mobility probably results from ionization of LiCl into Li^+ and Cl^- ions by the crystal field of $Co_4(Co(CN)_6)_3$. The smaller Li^+ ion become mobile and move through the interstices. Cl^- ions could also have some mobility, but we did not succeed in detecting this experimentally.

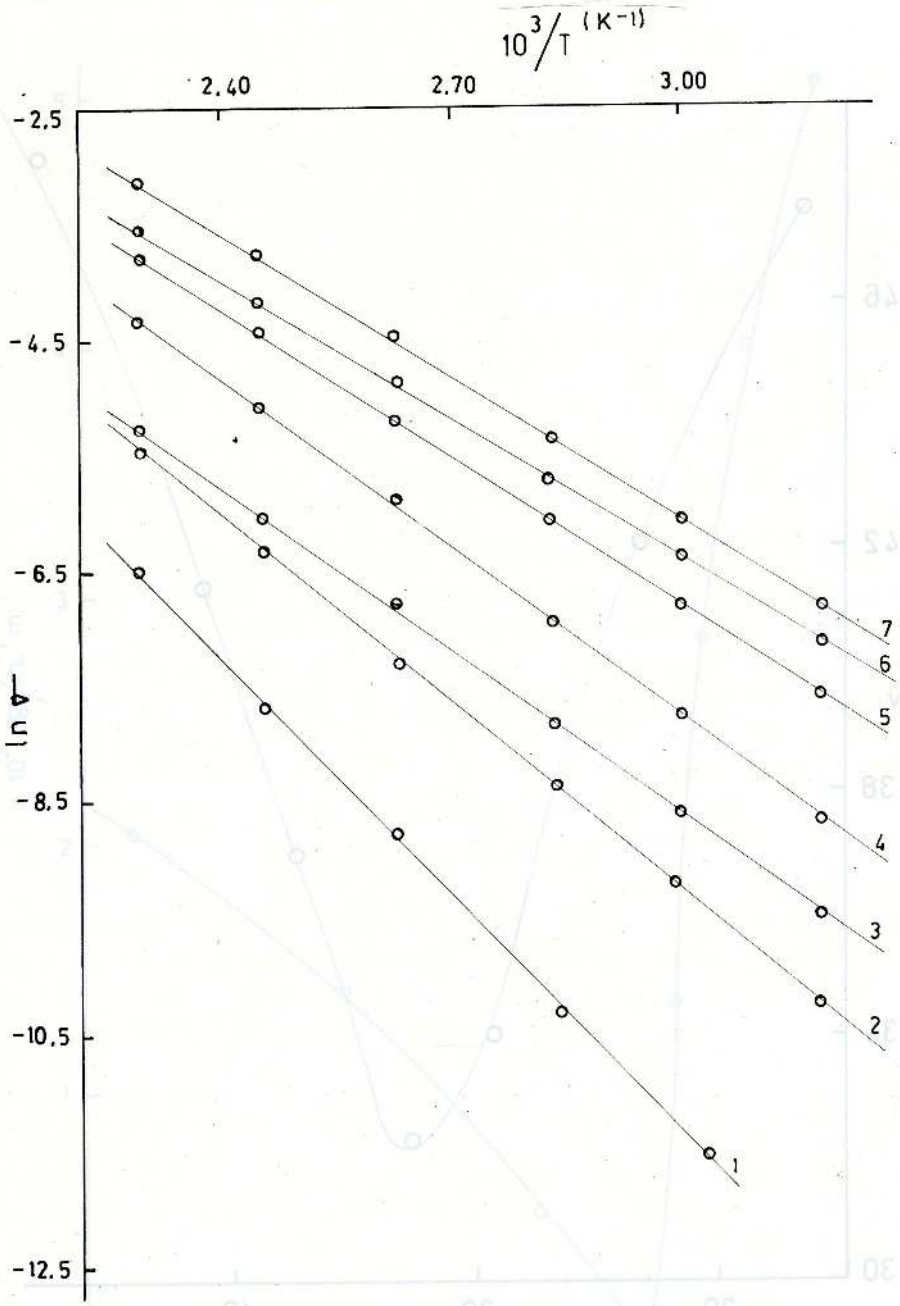

Figure 1. Plot of $\ln \sigma_{\bigcirc}$ vs T^{-1} (σ in $\Omega^{-1}\,m^{-1}$). Level of deoping (ie, % of LiCl by wt).

(1) 50 (2) 16.7 (3) 41.2
(4) 23.1 (5) 37.5 (6) 28.6 (7) 41.2

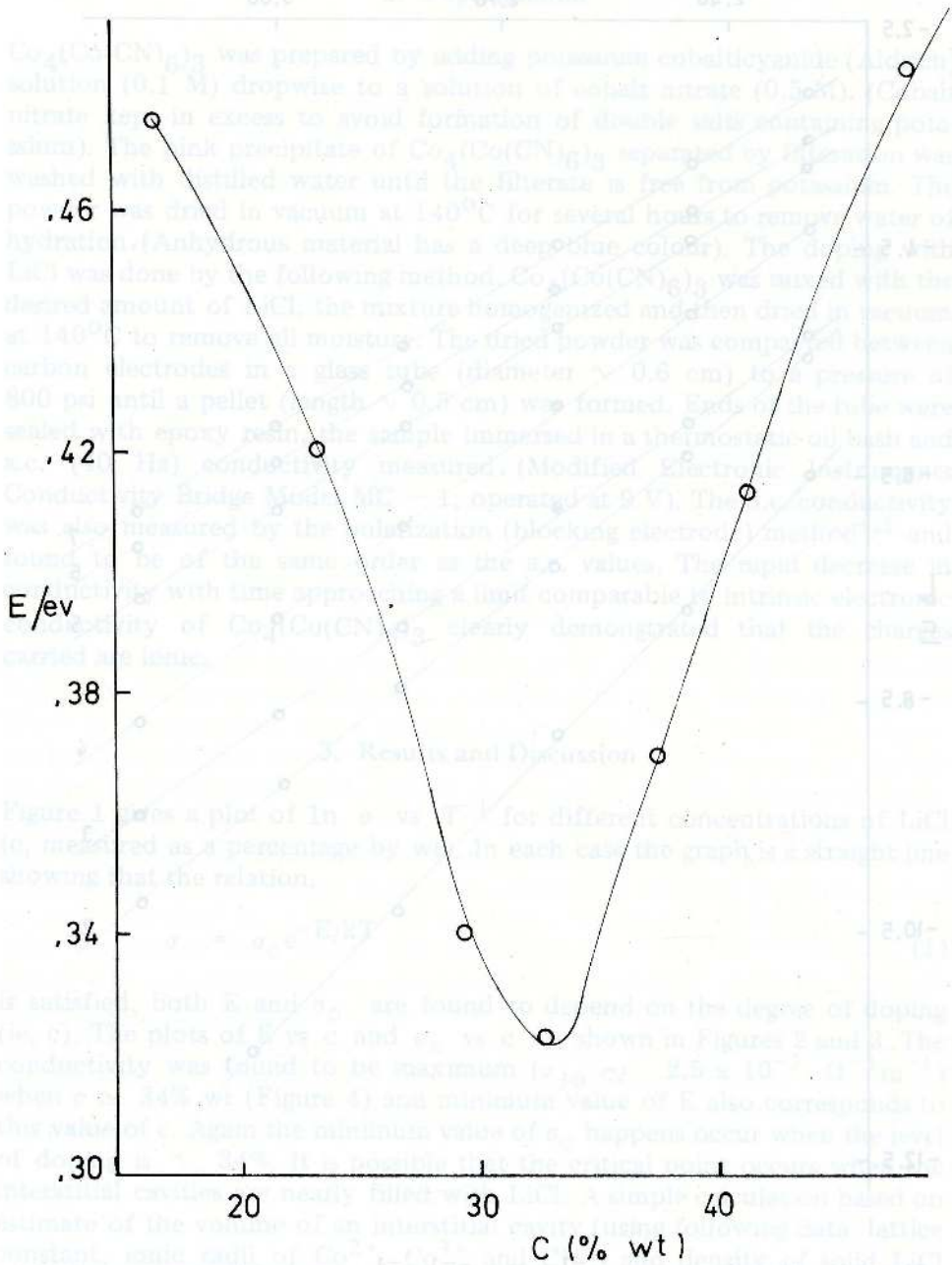

Figure 2. Plot of E vs C.

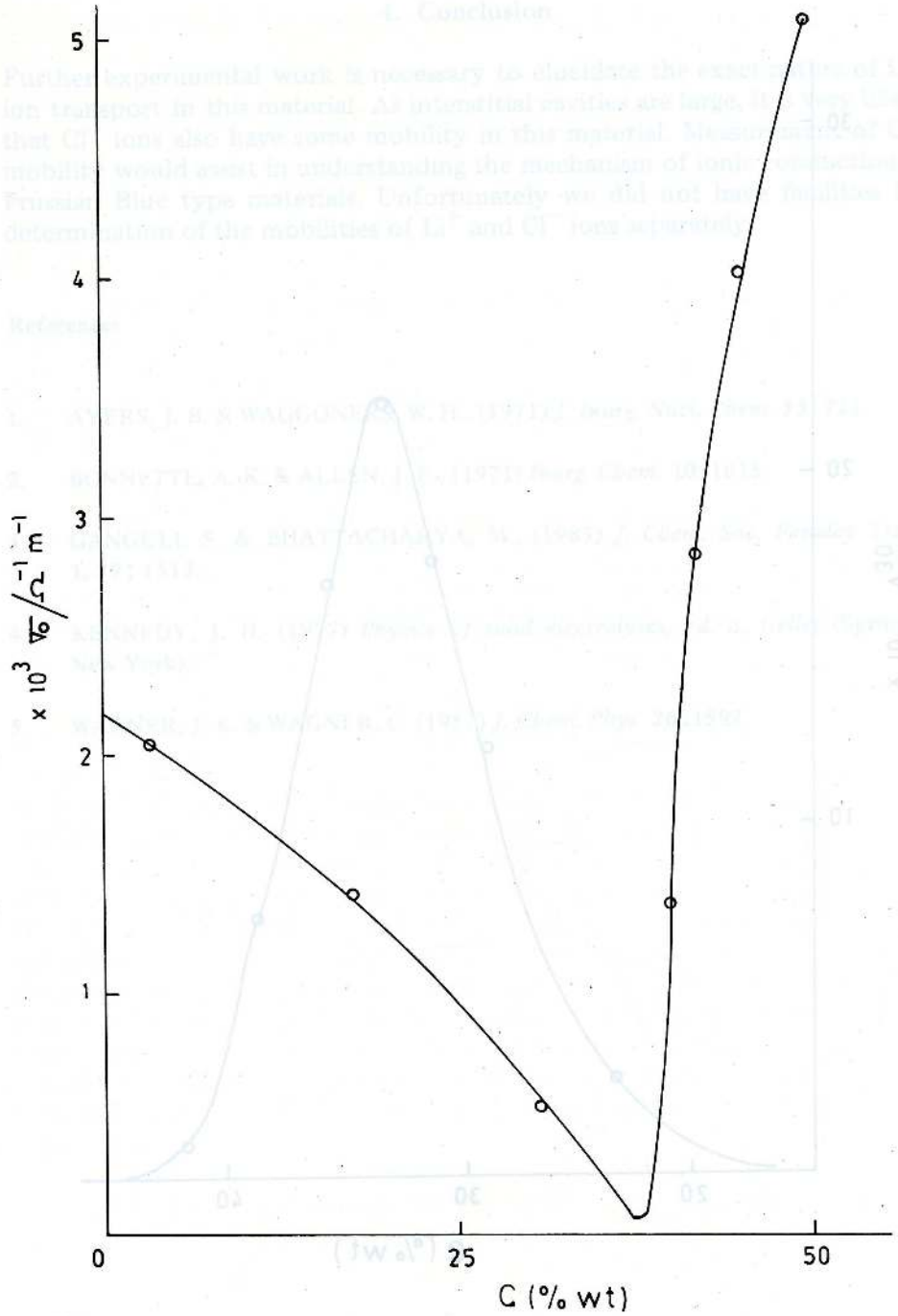

Figure 3. Plot of $\sigma_\circ$ ($\Omega^{-1}m^{-1}$) vs c.

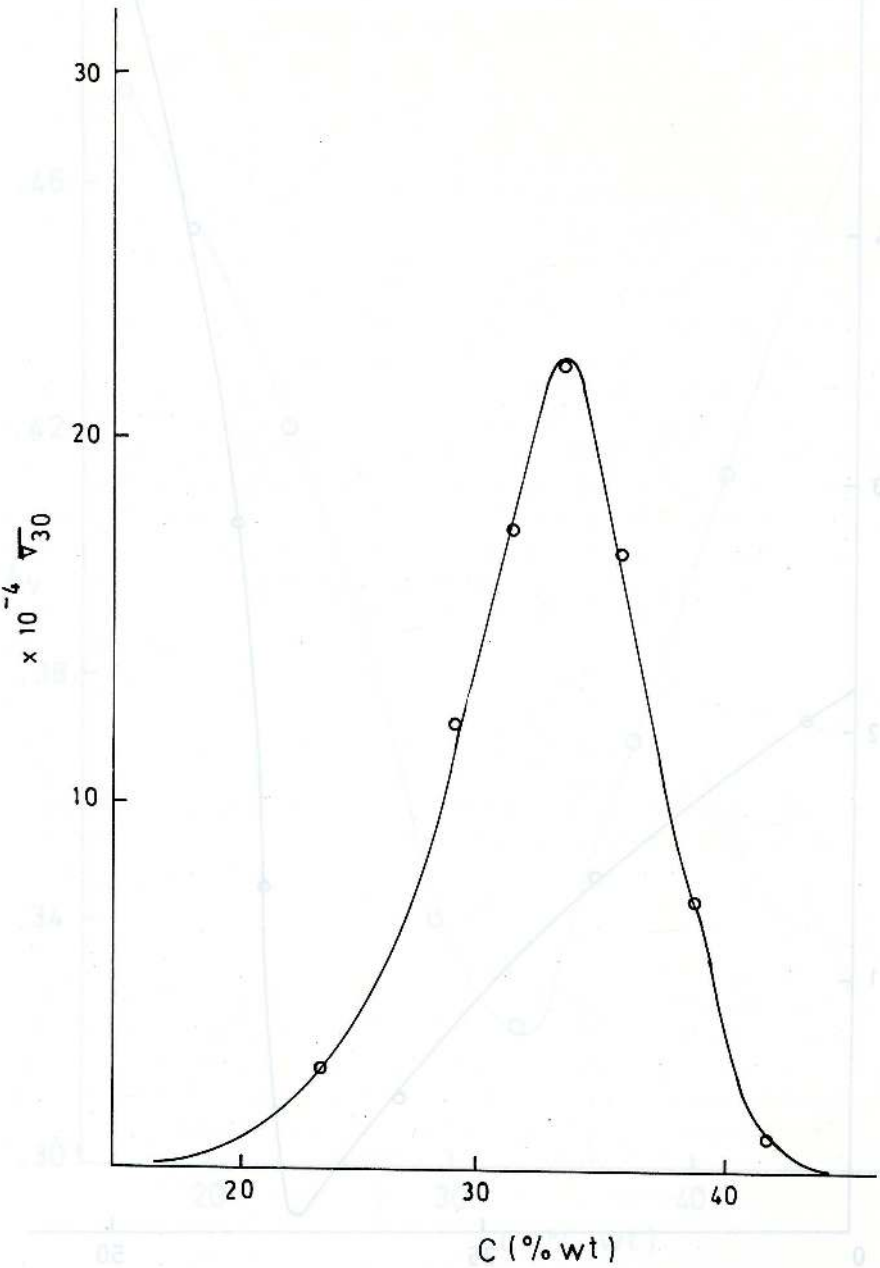

Figure 4. Plot of σ_{30} ($\Omega^{-1}m^{-1}$) vs c.

4. Conclusion

Further experimental work is necessary to elucidate the exact nature of Li^+ ion transport in this material. As interstitial cavities are large, it is very likely that Cl^- ions also have some mobility in this material. Measurement of Cl^- mobility would assist in understanding the mechanism of ionic conduction in Prussian Blue type materials. Unfortunately we did not have facilities for determination of the mobilities of Li^+ and Cl^- ions separately.

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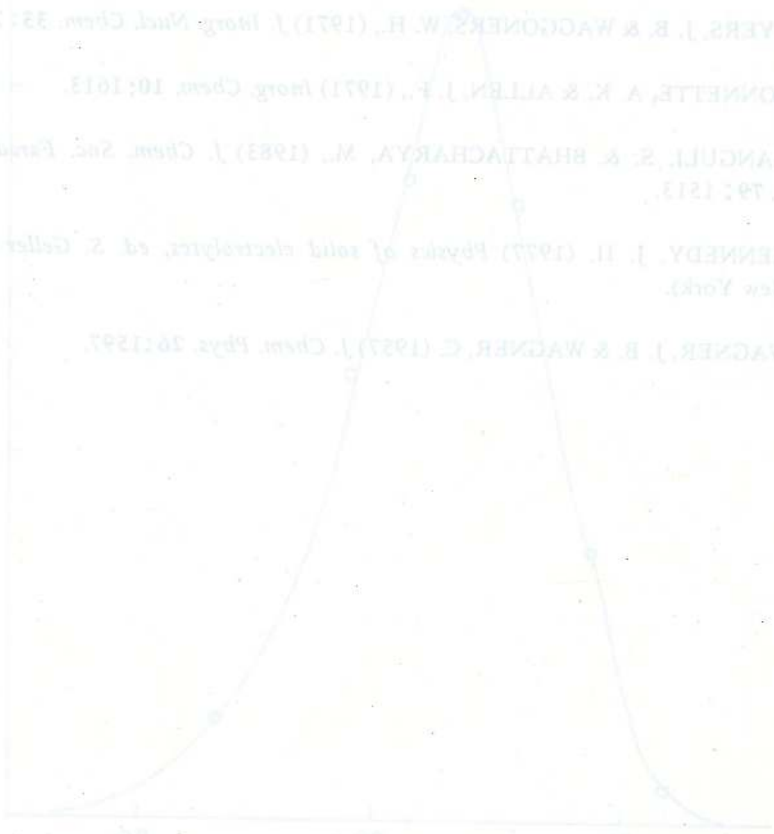

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SENSITIZATION OF PHOTOELECTROCHEMICAL CELLS BY RESONANT ENERGY TRANSFER BETWEEN TWO DYES

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Abstract : It is found that when a semiconductor electrode surface in a photoelectrochemical cell is coated with a dye that can transfer a charge carrier upon excitation into an energy band and if a layer of a second dye that readily transfers the energy of photon excitation into an inner one by resonance is deposited on top of the first dye, then stability and quantum efficiency of photocurrent are both improved. Observations based on dye coated CuCNS photocathodes are presented to illustrate the phenomenon. Photochemical processes involved are discussed.

1. Introduction

It is well known that the spectral response of photoelectrochemical cells (PECs) based on high band gap semiconducting materials can be extended to the visible region by deposition of suitable dyes on the surface.[1,2,3,4,5] In addition to adjustability of the spectral response, dye sensitized PECs have the following advantages. (1) The DS photocurrent is rather insensitive to impurities and defects in the semiconductor,[6] polycrystalline and amorphous materials when sensitized yield large photocurrents. (2) When dyes with intense absorption bands are deposited, the light absorption at a sensitized surface becomes much higher than that at a bare semiconductor surface. (3) High band gap materials are generally photocorrosion resistant. It is also known that deposition of dyes enhance the photostability of a semiconductor surface.

The chief disadvantages of DS systems are, (1) low energy and quantum conversion efficiencies, (2) photodegradation of the dye even in

the presence of a redox couple. Low conversion efficiency results from poor absorption of the dye at the semiconductor surface, high electrical resistivity of the dye and concentration quenching.[1,2,3,4,5] Recently one of the authors[8] and collaborators have found that cuprous thiocyanate (CuCNS is a p—type semiconductor of band gap $\simeq$ 3.6 eV[7]) readily absorbs thiocyanates of cationic dyes (cationic dyes whose anionic ligand CNS, the method of preparation is described.[8]) The simple method of depositing CuCNS on copper plates and the ability of these surfaces to absorb dyes readily and the fact that absorbed dyes do not pass into the electrolyte, makes CuCNS photocathodes ideal for study of dye sensitization. In this work we report our observations on the behaviour of a PEC where a thin outer layer of a dye D_2 is deposited on the first layer of a dye D_1 absorbed on CuCNS substrate. If D_2 is a fluorescent dye absorbing at a shorter wave length than D_1, the photocurrent spectrum of the system is found to be strongest in the overlap region of absorption spectrum of D_1 and fluorescence spectrum of D_2. The photocurrent quantum efficiency and stability are also found to be higher in the composite system.

2. Experimental

CuCNS was deposited on 3 x 3 cm copper plates electrochemically, by the method described by Tsubomura *et al.*[8] They were coated with thiocyanates of Methyl Violet (M) and Acridine Orange (A) by keeping immersed in the dye solution (0.01 g l^{-1}), the dyes get well absorbed within few minutes. The surface concentration of the dye was determined by noting the depletion of the dye concentration in the solution spectrophotometrically. The deposition of a layer of a second dye (e.g. A on M) over the one that is absorbed into the CuCNS substrate is a more difficult process. To achieve this the plate coated with the first dye was immersed in a solution containing the thiocyanate of the second dye (0.01 g l^{-1}) for about 6 h.

The electrolyte was a 0.1 M solution of KCNS. A platinum foil was used as the counter—electrode and the photocathode was biased (−0.40 V vs SCE) to give zero dark voltage (Hokuto Denko HA — 301 potentiostat was used). Light intensities were measured with an International Light IL 700 radiometer. The photocurrent spectra were ascertained with an Applied Photophysics Monochromator and 100 W tungsten filament lamp. Absorption spectra of dye solutions were measured with a Hitachi U — 3200 spectrophotometer.

3. Results and Discussion

CuCNS/Dye/Pt Cell : Firstly a cell sensitized with one dye (M or A) is considered. The redox action of KCNS solution depends on existence CNS^- as well $(CNS)_2^-$ ions[8] (atmospheric oxygen generate small quantities of $(CNS)_2^-$ ions in a KCNS solution[8]). The chemical reactions occuring near the electrodes are summarized below.

Photocathode

$$D + h\nu \rightarrow D^*$$

$$D^* \rightarrow D^- + hole^+$$

$$D^- + (CNS)_2^- \rightarrow 2CNS^- + D$$

Here D denotes a dye molecule, D* an excited dye molecule and D^- a dye molecule that has accepted one electron. The hole generated is transferred into the valence band of CuCNS.

Anode

At the anode CNS^- ions discharge electrons yielding CNS free radicals, that combine with CNS^- in the electrolyte regenerating $(CNS)_2^-$, ie,

$$CNS^- - e \rightarrow CNS \qquad (2)$$

$$CNS^- + CNS \rightarrow (CNS)_2^-$$

Action spectra of this system for A and M are presented in Figure 1. Photocurrent quantum efficiencies are given in Table 1. The cell performs best when the dye layer is 8 — 12 monolayers thick.

Photodegradation of the dye could result from following reactions. Hydrogen ions in the solution can accept electrons from D^- to yield atomic hydrogen, which reacts with unexcited or excited dye molecules in the neighbourhood, ie,

$$D + H \rightarrow \text{reduction products of the dye} \qquad (3)$$

$$D^* + H \rightarrow \text{reduction products of the dye} \qquad (4)$$

Chemical analysis indicates that the photodegraded dye consists mainly of the reduced lecuobase. As the reaction (4) is faster than (3), it is

probably the predominant mode of photodegradation. Since the semiconductor is photostable and the solute is regenerative, the decay of the photocurrent results almost entirely from degradation of the dye. The time development of the photocurrent in cells sensitized A and M are shown in Figure 2.

CuCNS/D_1 D_2/pt Cell: The above cell with a deposit of D_1 = Methyl violet (5—10 monolayers) on CuCNS and on top of D_1 a dye D_2 = Acridine Orange, (3 — 6 monolayers) has different characteristics. The photocurrent spectrum is shown in Figure 1. It is seen that the photoresponse is strongest in the overlap region of absorption spectra of A and M. For comparison Figure 1 also gives the photocurrent spectrum of a cell sensitized with a mixture two days A and M. Here in contrast to the previous case two peak positions in the spectrum corresponding to individual dyes are clearly evident. Quantum conversion efficiencies of the single and two dye systems are shown in Table 1. It is seen that quantum conversion efficiency at the peak spectral region is higher in the M/A (inner dye M outer dye A) system. Figure 2 compares the stability of different systems.

Table 1. Photocurrent quantum efficiencies of CuCNS sensitized with different dye systems. In the M/A system the wavelengths 516, 489, 580 nm indicated within the brackets corresponds to peak positions in the photocurrent spectrum of M/A, absorption spectrum of A and absorption spectrum of M respectively.

System	Quantum Efficiency %
M/A (516 nm)	1.24
M/A (489 nm)	1.20
M/A (580 nm)	0.92
M (580 nm)	1.15
A (489 nm)	0.47

A dye that shows strong fluorescence in aqueous solution (e.g. Rhodamine or Acridine Orange) when deposited as a single layer is found to degrade more rapidly than a non—fluorescent dye. However, in a two dye system much better stability and conversion efficiencies were obtained when the outer layer is a fluorescent dye. The photochemical mechanisms discussed below explain the above observations.

Light absorbed excite dye molecules D_2 in the outer layer,

$$H\nu + D_2 \rightarrow D_2^*$$

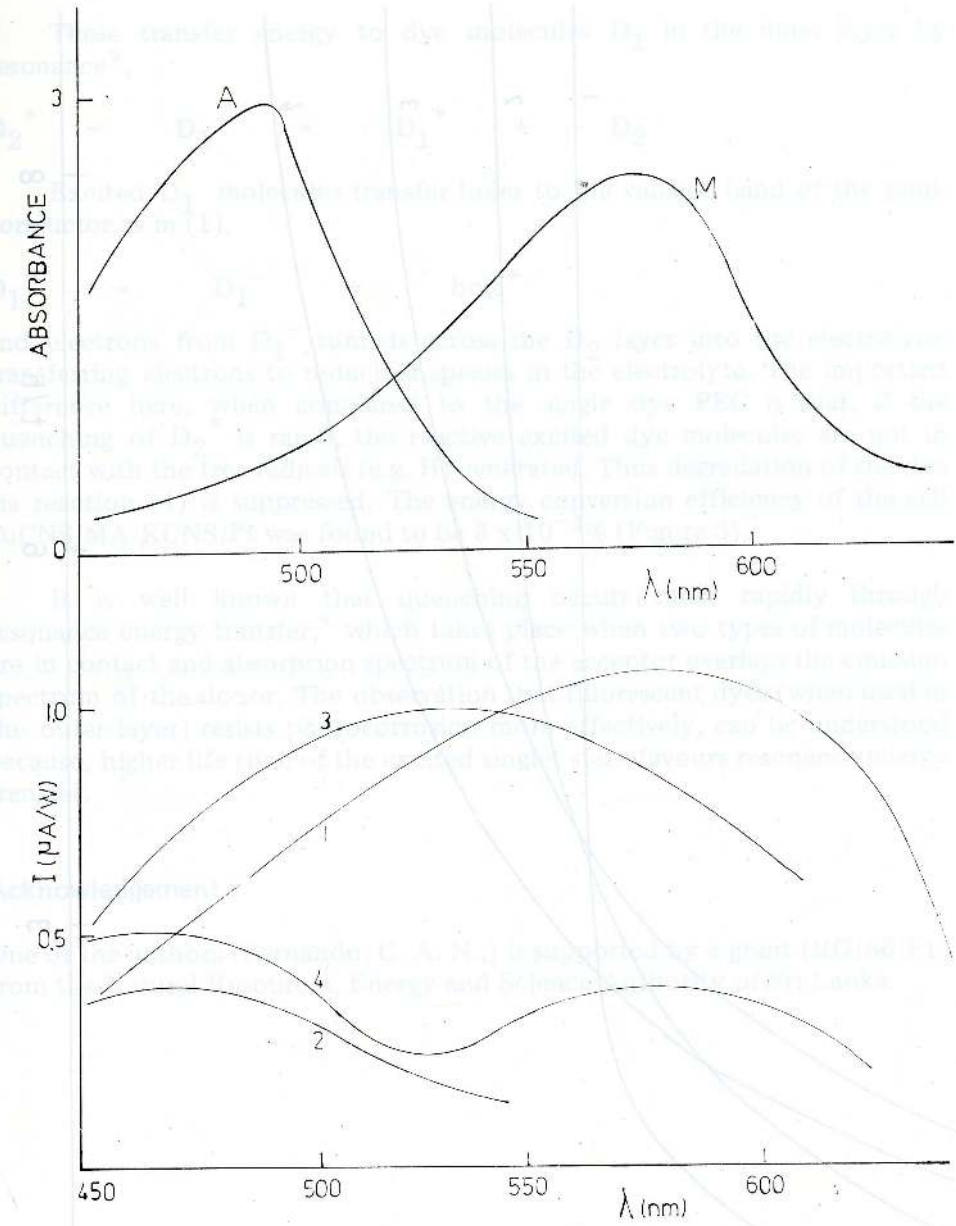

Figure 1. Photocurrent spectrum of cells sensitized with
(1) Methyl Violet
(2) Acridine Orange
(3) Acridine Orange on Methyl Violet
(4) Mixture of Acridine Orange and Methyl Violet for comparison the fluorescence spectrum of Acridine Orange and the absorption spectrum of Methyl Violet are indicated above.

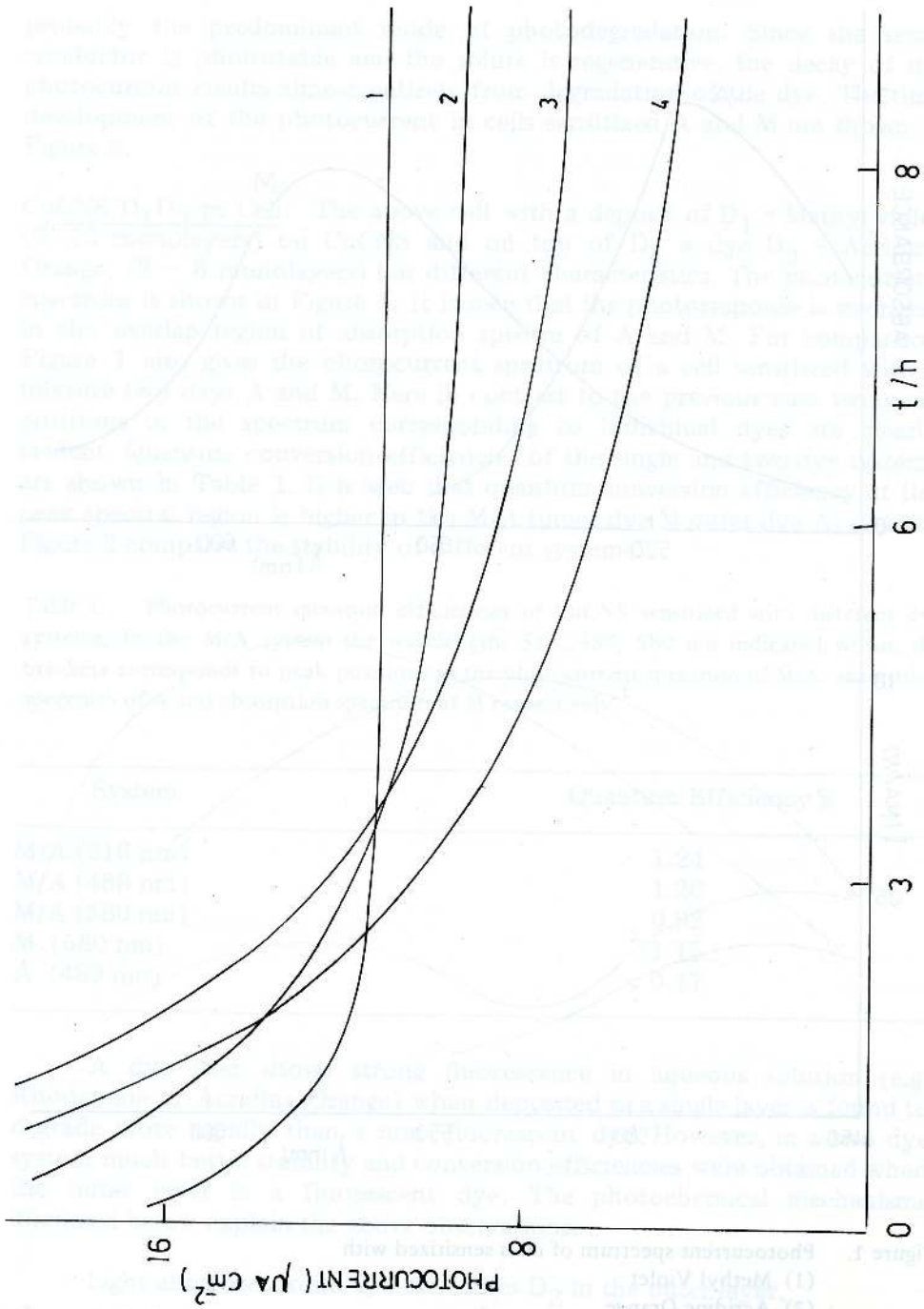

Figure 2. Time development of the photocurrent in CuCNS photocathodes sensitized with
(1) Acridine Orange on Methyl Violet
(2) Mixture of Acridine Orange and Methyl Violet
(3) Acridine Orange
(4) Methyl Violet on Acridine Orange

These transfer energy to dye molecules D_1 in the inner layer by resonance[9],

$$D_2^* + D_1 \rightarrow D_1^* + D_2$$

Excited D_1 molecules transfer holes to the valence band of the semiconductor as in (1),

$$D_1^* \rightarrow D_1^- + \text{hole}^+$$

and electrons from D_1^- tunnels across the D_2 layer into the electrolyte, transferring electrons to reducible species in the electrolyte. The important difference here, when compared to the single dye PEC is that, if the quenching of D_2^* is rapid, the reactive excited dye molecules are not in contact with the free radicals (e.g. H) generated. Thus degradation of the dye via reaction (4) is suppressed. The energy conversion efficiency of the cell CuCNS/MA/KCNS/Pt was found to be 3×10^{-3}% (Figure 3).

It is well known that quenching occurs most rapidly through resonance energy transfer,[9] which takes place when two types of molecules are in contact and absorption spectrum of the acceptor overlaps the emission spectrum of the donor. The observation that fluorescent dyes (when used as the outer layer) resists photocorrosion more effectively, can be understood because, higher life time of the excited singlet state favours resonance energy transfer.

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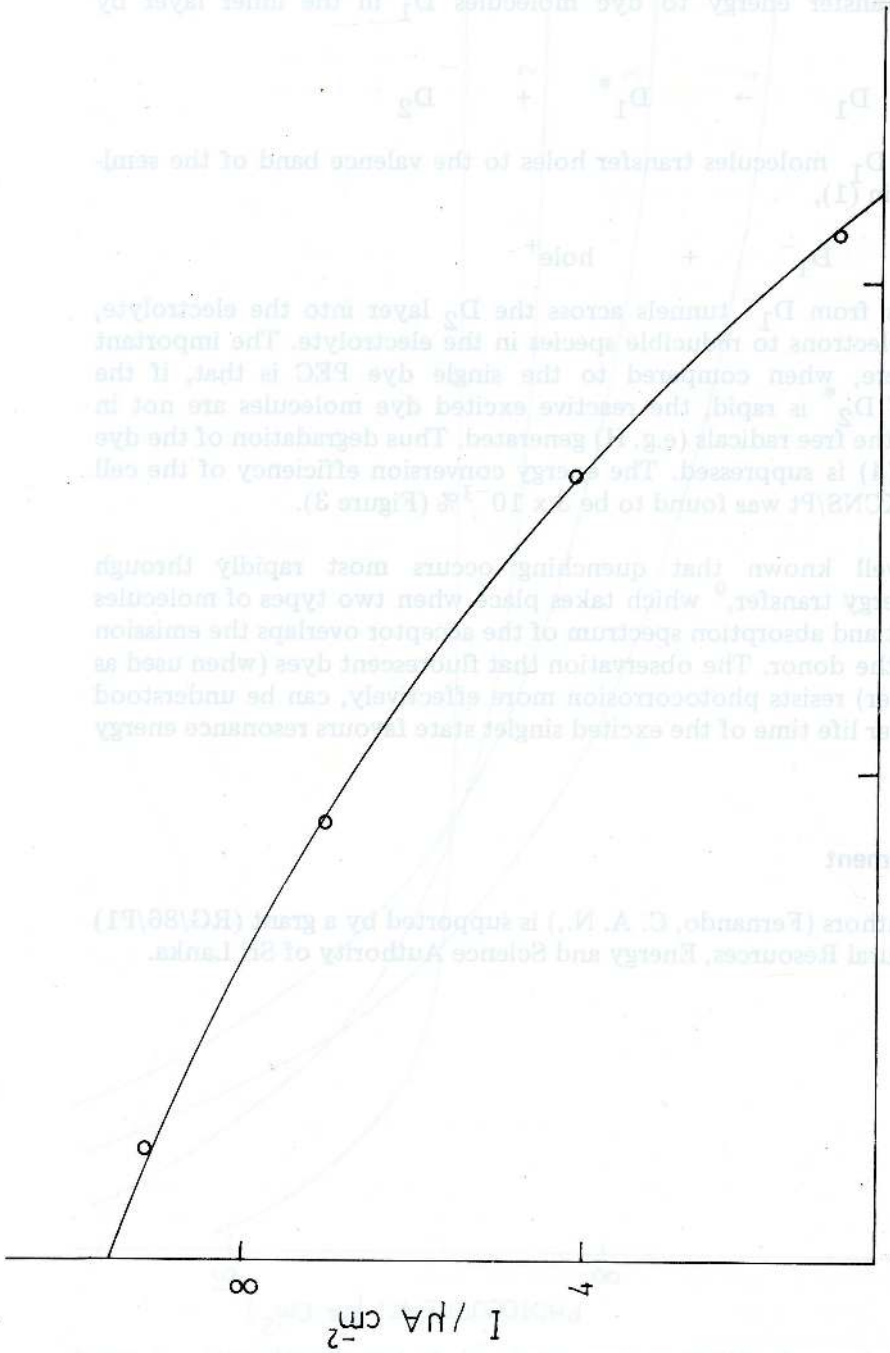

Figure 3. Plot of I vs V for the CuCNS/MA/KCNS/Pt Cell (Intensity of illumination 250 Wm^{-2} from a tungsten filament lamp).

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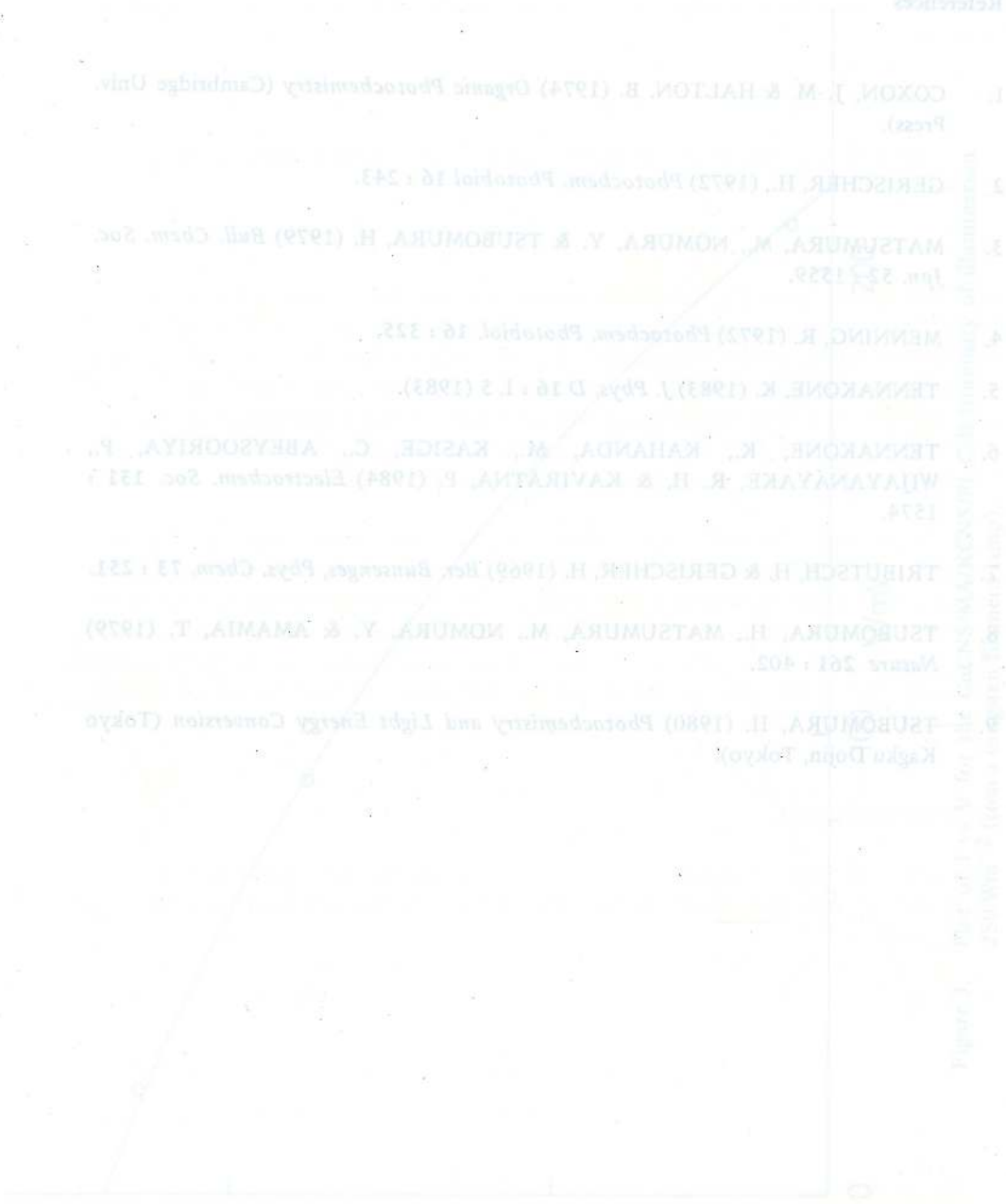

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A RESULT ON TWO-DIMENSIONAL POLAR LATTICES

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Abstract : Suppose P and P^o denote closed positive and open positive quadrants in R^2 respectively. Let Λ be any lattice in R^2 with polar lattice Λ^*. Let F be a convex and symmetric (with respect to the axes of coordinates) distance function with $F(1,0) = F(0,t) = 1$, where $t \in R$ and let μ = area $(\underline{x} \in R^2 / F(\underline{x}) \leqslant 1)$. For certain distance functions F, there exist non-zero $\underline{x} \in P \cap \Lambda$ and $\underline{y} \in P^o \cap \Lambda^*$ such that $\mu\, F(\underline{x})\, F(\underline{y}) \leqslant \gamma_t$, where γ_t is a constant depending on t and the distance function. There exist a lower bound $2(t + 1/t)$ and an upper bound $4(t + 1/t)$ for γ_t over all convex symmetric distance functions.

1. Introduction

Let P and P^o denote closed positive and open positive quadrants in R^2 respectively. Let Λ be any lattice in R^2 with polar lattice Λ^*. Let F be a convex and symmetric (with respect to the axes of coordinates) distance function with $F(1,0) = F(0,t) = 1$, where $t \in R$. Without loss of generality we can take $t \geqslant 1$. If $t \leqslant 1$, we have the same situation as in the case when $t \geqslant 1$ with the coordinate axes interchanged. Let $\mu = \text{area}\{\underline{x} \in R^2 / f(\underline{x}) \leqslant 1\}$.

Hossain and Worley[3] have shown that for certain distance functions F, there exist non-zero $\underline{x} \in P \cap \Lambda$ and $\underline{y} \in P^o \cap \Lambda^*$ such that

$$\mu F(\underline{x}) F(\underline{y}) \leqslant \gamma_t,$$

where γ_t is a constant depending on t and the distance function. In this note we show that γ_t has a lower bound and an upper bound over all the convex symmetric distance functions. In this note symmetric means the symmetricity with respect to the axes of coordinates.

2. Discussion

The following notations will be used frequently in this section.

$$F_1(\Lambda) = \inf\{F(\underline{x}) : \underline{x} \in \Lambda \cap P\}$$
$$F_2(\Lambda^*) = \inf\{F(\underline{x}^*) : \underline{x}^* \in \Lambda^* \cap P^o\},$$

where F is a distance function.

Theorem:

If F is any convex symmetric distance function with F(1,0)=F(0,t)=1, then

$$2(t+1/t) \leqslant \mu\, F_1(\Lambda)\, F_2(\Lambda^*) \leqslant 4(t+1/t)$$

for the lattice Λ with basis $\{(1,0), (0,t)\}$

The lower bound is best possible for the distance function $F(x_1, x_2) = |x_1| + 1/t\,|x_2|$ and the lattice Λ with a basis (1,0) and (0,t). The upper bound may not be best possible, but cannot be below 4t. In order to prove the theorem, we use the following lemmas.[2]

Lemma 1

Let Λ be the lattice with a basis $\{(1,0), (0,t)\}$. Then $\min_{\alpha} \mu F_1(\Lambda)\, F_2(\Lambda^*) = 2(t+1/t)$, for the convex symmetric polygonal distance function F given by

$$F(x_1,x_2) = \max\left\{\frac{1-\alpha}{\alpha}|x_1| + 1/t\,|x_2|, |x_1| + \frac{1-\alpha}{\alpha t}|x_2|\right\}$$

where $1/2 \leqslant \alpha \leqslant 1$.

(α has to satisfy the above conditions since F is convex and symmetric).

Lemma 2

Let Λ be a lattice with basis $\{(1,0),(0,t)\}$. Let F be the convex polygonal distance function, where $F(x_1, x_2) = 1$ has two more vertices at $(\alpha,\alpha t)$ and $(\beta,\beta/t)$ in P^o in addition to (1,0) and (0,t), where $1/2 \leqslant \alpha \leqslant 1$ and the limit of β depends on α.

$$\text{Then } \min_{\alpha,\beta} \mu\, F_1(\Lambda)\, F_2(\Lambda^*) \geqslant 2(t+1/t).$$

From Lemma 1 and Lemma 2, we can establish the left hand side of the inequality in the theorem.

Suppose $F(x_1,x_2) = 1$ intersects the

lines OL at B and OM at C respectively,

where L ≡ (1,t) and M = (1,1/t).

Let B ≡ $(\alpha,\alpha t)$ and C ≡ $(\beta,\beta/t)$.

The curve $F(x_1,x_2) = 1$ passes through

the points A ≡ (0,t) and D ≡ (1,0).

Then $F_1(\Lambda) = 1$.

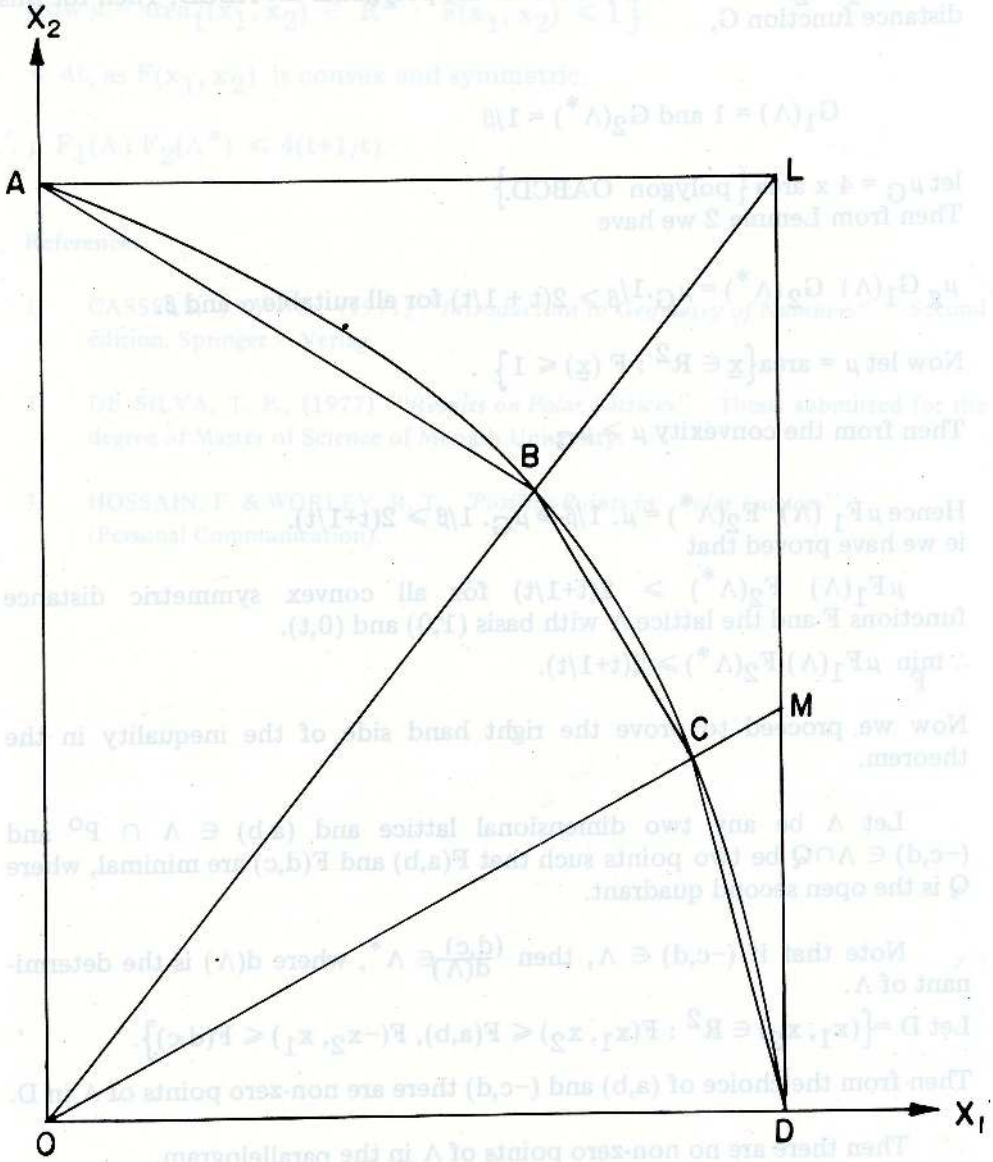

Figure 1. The curve $F(x_1,x_2) = 1$ passes through the points A = (0,t) and D = (1,0). It intersects the lines OL at B and OM at C respectively, where L = (1,t) and M = (1, 1/t).

and $F_2(\Lambda^*) = F(1,1/t) = 1/\beta\ F(\beta,\beta/t) = 1/\beta$.

Let $G(x_1, x_2) = 1$ be the equation of the polygonal arc ABCD. Then for this distance function G,

$$G_1(\Lambda) = 1 \text{ and } G_2(\Lambda^*) = 1/\beta$$

let $\mu_G = 4 \times \text{area}\{\text{polygon OABCD.}\}$
Then from Lemma 2 we have

$\mu_g\ G_1(\Lambda)\ G_2(\Lambda^*) = \mu_G . 1/\beta \geqslant 2(t + 1/t)$ for all suitable α and β.

Now let $\mu = \text{area}\{\underline{x} \in R^2 : F(\underline{x}) \leqslant 1\}$.

Then from the convexity $\mu \geqslant \mu_G$.

Hence $\mu F_1(\Lambda)\ F_2(\Lambda^*) = \mu . 1/\beta \geqslant \mu_G . 1/\beta \geqslant 2(t+1/t)$.
ie we have proved that

$\mu F_1(\Lambda)\ F_2(\Lambda^*) \geqslant 2(t+1/t)$ for all convex symmetric distance functions F and the lattice Λ with basis (1,0) and (0,t).

$\therefore \min_F \mu F_1(\Lambda)\ F_2(\Lambda^*) \geqslant 2(t+1/t)$.

Now we proceed to prove the right hand side of the inequality in the theorem.

Let Λ be any two dimensional lattice and $(a,b) \in \Lambda \cap P^o$ and $(-c,d) \in \Lambda \cap Q$ be two points such that F(a,b) and F(d,c) are minimal, where Q is the open second quadrant.

Note that if $(-c,d) \in \Lambda$, then $\frac{(d,c)}{d(\Lambda)} \in \Lambda^*$, where $d(\Lambda)$ is the determinant of Λ.

Let $D = \{(x_1, x_2) \in R^2 : F(x_1, x_2) \leqslant F(a,b), F(-x_2, x_1) \leqslant F(d,c)\}$.

Then from the choice of (a,b) and (−c,d) there are non-zero points of Λ in D.

Then there are no non-zero points of Λ in the parallelogram.
$\{(x_1, x_2) \in R^2 : |-x_1 + tx_2| \leqslant tF(d,c), |tx_1 + x_2| \leqslant t\,F(a,b)\}$
which lies entirely in D.

Hence by Minkowski's linear form theorem[1], we have

$t^2\ F(a,b)\ F(d,c) \leqslant (1+t^2)\ d(\Lambda)$.

$$\therefore F(a,b)\ \frac{F(d,c)}{d(\Lambda)} \leqslant 1 + 1/t^2.$$

Now μ = area $\{(x_1, x_2) \in R^2 : F(x_1, x_2) \leqslant 1\}$

$\leqslant 4t$, as $F(x_1, x_2)$ is convex and symmetric.

$$\therefore \mu\ F_1(\Lambda)\ F_2(\Lambda^*) \leqslant 4(t+1/t).$$

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CHARACTERIZATION OF SOIL PROFILES FOR NUMERICAL CLASSIFICATION

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Abstract : Soil individuals are three-dimensional natural bodies with their properties varying both in vertical and lateral directions. The tendency of certain soils to develop horizons, has influenced the collection of soil information and the soil profile is divided into a series of Master Horizons and each Master Horizon is sub-divided. Data on soil horizons are used to characterize soils for classification purposes. Numerical taxonomists have used various models of the soil profile in order to compute the relative similarity between soil individuals, but only a few attempts have been made to assess the effect of different soil profile models on the results of classification. In this study two soil profile models were compared and the effect of inter-attribute correlation on the inter-individual similarity was examined. It was demonstrated that it was not necessary to use data for a large number of depth levels or soil horizons because of the correlation between depth levels. It was also shown that the use of rigorous mathematical methods to characterize soil individuals only increases the computational load while similar results can be obtained from much simpler methods of soil profile description.

1. Introduction

Soils are anisotropic entities, the properties of which vary both in lateral and vertical dimensions. The lateral variation can be represented by a point sampling procedure whereas the vertical variation is represented by sampling from a series of depth levels. Although soils are three-dimensional bodies, the study of which has been based upon a two-dimensional entity known as the soil profile (a vertical cross-section through soil). The use of the soil profile as the basic unit of soil classification was first introduced by Dukuchaev in Russia in the 19th Century. This concept was introduced to America by Marbut[8] who claimed that soils at maturity developed a soil profile, the features of which could be used to characterize them. However, with the development of numerical taxonomic methods, it became necessary to find a suitable model of the soil profile that could be used as the basic taxonomic unit.

Conventional soil taxonomists have considered that the soil profile consists of a set of genetic horizons. Grouping of soil profiles according to

the nature and arrangement of soil horizons has gained a wide acceptance among soil taxonomists in almost all parts of the world. This is the basis of the definition of the Soil Series both in USA[9] and British[1] classifications. This method, despite being the most practical one, leads to subjectivity; and the Soil Series defined this way show a high degree of heterogeneity.[11]

Lance and Williams[3] have recognized four soil profile models that have been used in soil classification to characterize soils.

1. Depth levels as arrays of independent attributes,
2. Mean values of soil properties averaged over all depth levels (horizons),
3. A 'linked level system'; the similarity between soil profiles is defined as the average similarity between their corresponding horizons,
4. Parameters of a depth dependent function fitted to the soil properties.

Lance and Williams[3] reckon that the theoretically most sound model is the fourth one, despite the heavy computational load involved.

A fifth model, that can be used in numerical classification is the one which uses mean values of the attributes for the Master Horizons. This model does not discard too much information as the second model does and is not as complex as the fourth. However, it must be emphasized that the information loss due to averaging over all depth levels depends on the level of inter-attribute correlation. It has been demonstrated that depth levels are correlated[11] and elimination of the correlated attributes has no adverse effect on the classifications.[7] Therefore, the use of the first model seems to have been based on a faulty assumption. This investigation attempts to compare the fourth model and the three-horizon model.

2. Data and Methods

Data for 32 soil profiles were obtained from the published data of the United States Department of Agriculture.[9] The soil profiles used in this study were selected in such a way that they all have data at least for seven depth levels in order to fit a fifth degree polynomial. Moreover, the soil profiles chosen had data for all or most of the ten soil properties used in this study (Table 1). This list does not form an exhaustive set of soil properties, but it was chosen mainly because of the availability of quantitative data.

The two models that were used to characterize soils for numerical classification are:

(a) the orthogonal polynomial model,
(b) the three-horizon model.

Table 1. Soil properties used to characterize soil profiles

1. Percentage Silt
2. Percentage Clay
3. Percentage Organic Carbon
4. Percentage Dithionite Extractable Iron as Fe
5. pH (1:1 soil/water suspension)
6. Exchangeable Ca me/100g Soil
7. Exchangeable Mg me/100g Soil
8. Exchangeable Na me/100g Soil
9. Exchangeable K me/100g Soil
10. Cation Exchange Capacity (CEC) me/100g Soil

A fifth degree polynomial function of the following form was fitted to all ten soil properties.

$$Y_i = b_{0i} + b_{1i}X + b_{2i}X^2 + \ldots + b_{ki}X^k \ldots\ldots\ldots \quad (1)$$

where, Y_i — value of property i at depth X.

This model is flexible enough to fit a wide range of trends if a sufficiently large value is chosen for k. For soil properties k = 5 has been suggested by Colwell.[2] For statistical analysis a much more convenient form of this function can be obtained.

$$Y_{xi} = c_{0i}\,\ell_{y_{0x}} + c_{1i}\,\ell_{y_{1x}} + \ldots\ldots + c_{ki}\,\ell_{y_{kx}} \ldots\ldots\ldots \quad (2)$$

where, $\ell_{y_{kx}}$ is the value of orthogonal polynomial of degree k (k = 1, 2, . . . 5) at depth x. c_{ki} is the polynomial coefficient for the kth power of X.

The main advantage of this model is that each term of the polynomial function can be computed independent of others, and therefore it is not necessary to compute the whole function whenever the power of the polynomial function is changed. The original function was proposed for equally space X values (independent variable), but a modification has been described by Robson[6] and a computer programme for it has been written by Mather.[4] The coefficients c_{ki} can be used in numerical analysis in the place of original observations.

The second model was obtained by taking the mean values of ten soil properties for three Master Horizons, A, B and C. In cases where all three horizons were not present, only the available horizons (Master Horizons)

were used to compute the inter-individual similarity matrix. By this method, the number of attributes was reduced from 70 (10 soil properties for 7 depth levels) to 30. Product-moment correlation between the 30 attributes was computed and classification of all thirty attributes was done by average linkage method.

Two similarity measures (distance type) were used to generate inter-individual similarity matrices;

$$(a)\ d_{ij} = (1 - r_{ij})/2$$

where, d_{ij} — similarity between ith and jth individuals
r_{ij} — product-moment correlation between ith and jth individuals.

$$(b)\ d_{ij} = 1/p \sum_{k=1}^{p} (x_{ik} - x_{jk})^2$$

where, x — attribute values standardized to unit variance and zero mean.
p — number of attributes.

Euclidean distance requires the attribute vectors to be mutually independent (orthogonal). However, attributes of both models may not meet this requirement and therefore the possibility of masking certain attributes and its effect on the classifications should be examined. This is one of the objectives of this study. The relationship between the two soil profile models was also examined by product-moment correlation between inter-individual similarity matrices generated using the two models.

Classification of soil profiles was done by Ward's Error Sum-of-Squares (ESS) method.[10] This method was preferred to other agglomerative strategies because it tends to produce well defined clusters. Possible misclassifications by this method may be corrected by a suitable reallocation procedure if the hierarchy is not of interest. However, average linkage method was used to classify soil attributes since they very often show well defined clusters when depth levels are treated as arrays of independent attributes.[11]

3. Results and Discussion

Orthogonal polynomial coefficients were calculated for all ten soil properties; only in a few cases were poor fit recorded. A fifth degree polynomial seemed to be adequate to represent the vertical variation of soil properties considered in this study.

A series of inter-individual similarity matrices were calculated using similarity measure (a) and (b) described in Section 2.

The similarity matrices calculated for the two soil profile models using similarity measure (a) were classified by Ward's ESS method and two dendrograms were drawn to represent the classifications (Figure 1, a & b). Both dendrograms show well defined clusters with somewhat similar composition. The Group consisting of 1, 26, 28, 29 is common to both classifications; all soil profiles in this Group belong to Mollisols Order.[9] Again the Group consisting of 6, 7, 9, 10, 13, 14, 17, 30 can be identified from both dendrograms as a single group. Most soils in this Group belong to Alfisols Order. The other soil profiles have produced clusters which can only be described as broadly similar in the two dendrograms. However, it is worth noting that all soil profiles of any given order have not clustered together to form a single group.

Similarity between the classifications obtained for the two soil profile models confirm the findings of Moore, Russel and Ward[5] who concluded that no additional information could be gained by fitting mathematical functions to soil profile data.

Similarity between classifications produced for the two soil profile models can be traced back to the inter-individual similarity matrices. A sample of 30 similarity values was chosen randomly from one similarity matrix and plotted against 30 corresponding values taken from the other similarity matrix (Figure 2) and product-moment correlation between the two matrices was calculated. The scattergram (Figure 2) and product-moment correlation coefficient show a strong linear relationship ($r = 0.9$, $n = 30$). There is a slight scatter of the higher values, but it affects only those individuals which have a very low similarity to other individuals.

Further two classifications were obtained by Ward's ESS method using squared Euclidean distance (similarity measure b) as the similarity measure. The classifications are represented by two dendrograms (Figure 3, a & b). Although some groups (e.g. 2, 26, 28, 29 and 6, 9, 30) can still be identified from the two dendrograms, they are not very similar. This may well be due to the effect of inter-attribute correlation on the similarity measure. Since both classifications were obtained from the same clustering strategy, the difference between the classifications should be related to the nature of relative similarity between individuals. There are two possible explanations for this :

(a) difference between the soil profile models,
(b) the effect of inter-attribute correlation on the similarity measure.

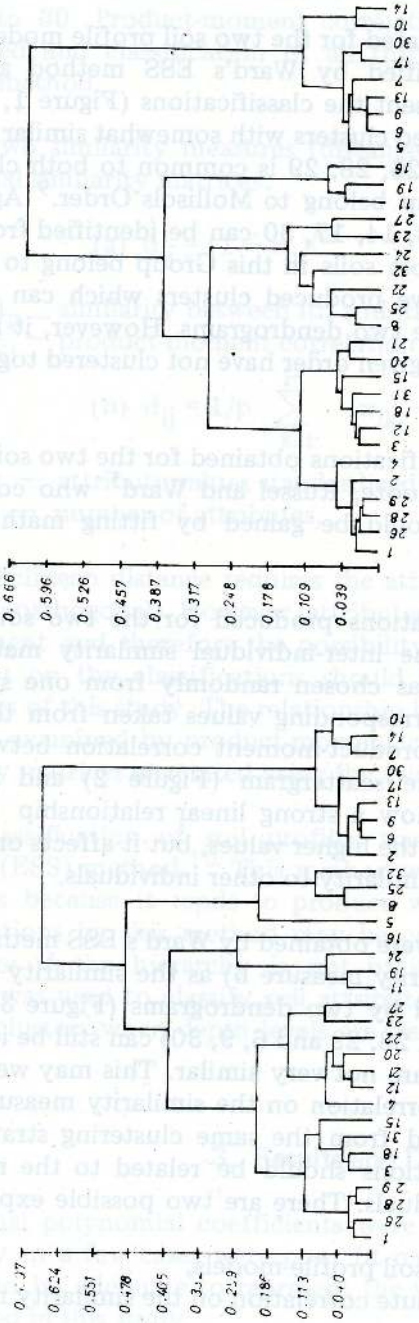

Figure 1. Classification of 32 soil profiles by Ward's method with $(1 - r_{ij})/2$ as the similarity measure, (a) three-horizon model (b) orthogonal polynomial model.

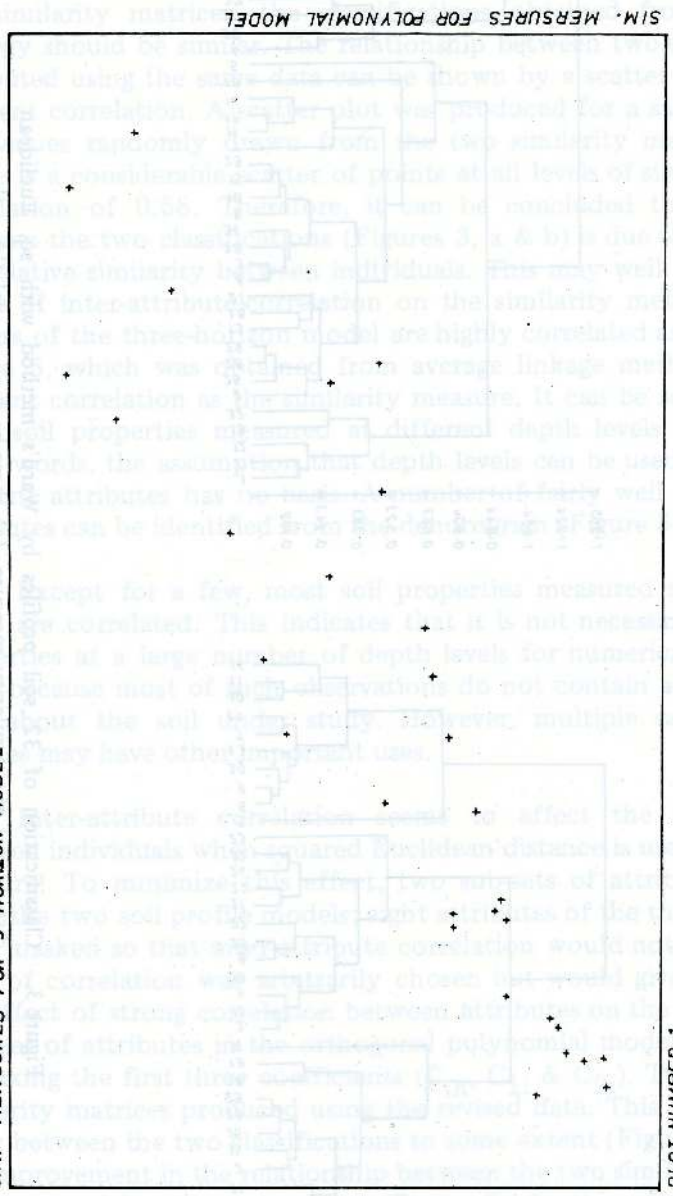

Figure 2. Relationship between inter-individual similarity $(1 - r_{ij})/2$ matrices calculated from the two soil profile models.

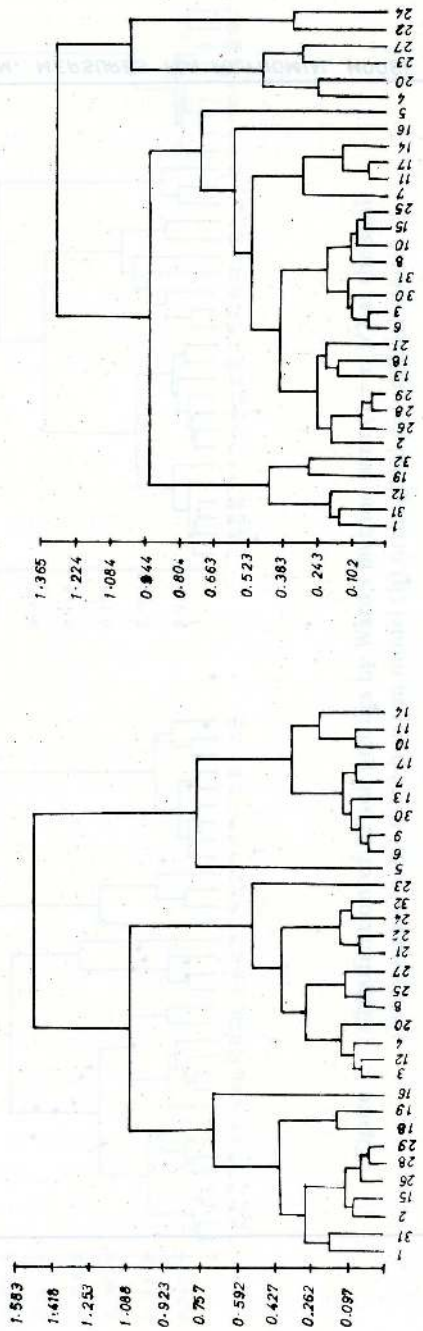

Figure 3. Classification of 32 soil profiles by Ward's method with sq. Euclidean distance as the similarity measure, (a) three-horizon model (b) orthogonal polynomial model.

It was shown earlier that when similarity measure (a) was used both models produced similar classifications. Therefore it was felt necessary to examine the relationship between the two similarity matrices calculated using similarity measure (a). If there is a strong linear relationship between the similarity matrices, the classifications obtained from any clustering strategy should be similar. The relationship between two similarity matrices computed using the same data can be shown by a scatter plot and product-moment correlation. A scatter plot was produced for a sample of 30 similarity values randomly drawn from the two similarity matrices (Figure 4). There is a considerable scatter of points at all levels of similarity with a low correlation of 0.58. Therefore, it can be concluded that the difference between the two classifications (Figures 3, a & b) is due to the difference in the relative similarity between individuals. This may well be due to unequal effect of inter-attribute-correlation on the similarity metric. The attribute vectors of the three-horizon model are highly correlated as can be seen from Figure 5, which was obtained from average linkage method with product-moment correlation as the similarity measure. It can be seen from Figure 5 most soil properties measured at different depth levels are correlated. In other words, the assumption that depth levels can be used as arrays of independent attributes has no basis. A number of fairly well defined groups of attributes can be identified from the dendrogram (Figure 5).

Except for a few, most soil properties measured at different depth levels are correlated. This indicates that it is not necessary to measure soil properties at a large number of depth levels for numerical classification of soils, because most of such observations do not contain additional information about the soil under study. However, multiple sampling from soil profiles may have other important uses.

Inter-attribute correlation seems to affect the relative similarity between individuals when squared Euclidean distance is used as the similarity measure. To minimize this effect, two sub-sets of attributes were chosen from the two soil profile models; eight attributes of the three-horizon model were masked so that inter-attribute correlation would not exceed 0.90. This level of correlation was arbitrarily chosen but would give some idea about the effect of strong correlation between attributes on the classification. The number of attributes in the orthogonal polynomial model was also reduced by taking the first three coefficients (C_{0i}, C_{1i} & C_{2i}). Two inter-individual similarity matrices produced using the revised data. This improved the similarity between the two classifications to some extent (Figure 6, a & b) due to the improvement in the relationship between the two similarity matrices as is demonstrated by the scatter plot (Figure 7). Scatter of points has reduced and correlation has increased from 0.58 to 0.79. This suggests that the relative similarity between individuals is similar for both soil profile models when inter-attribute correlation is eliminated.

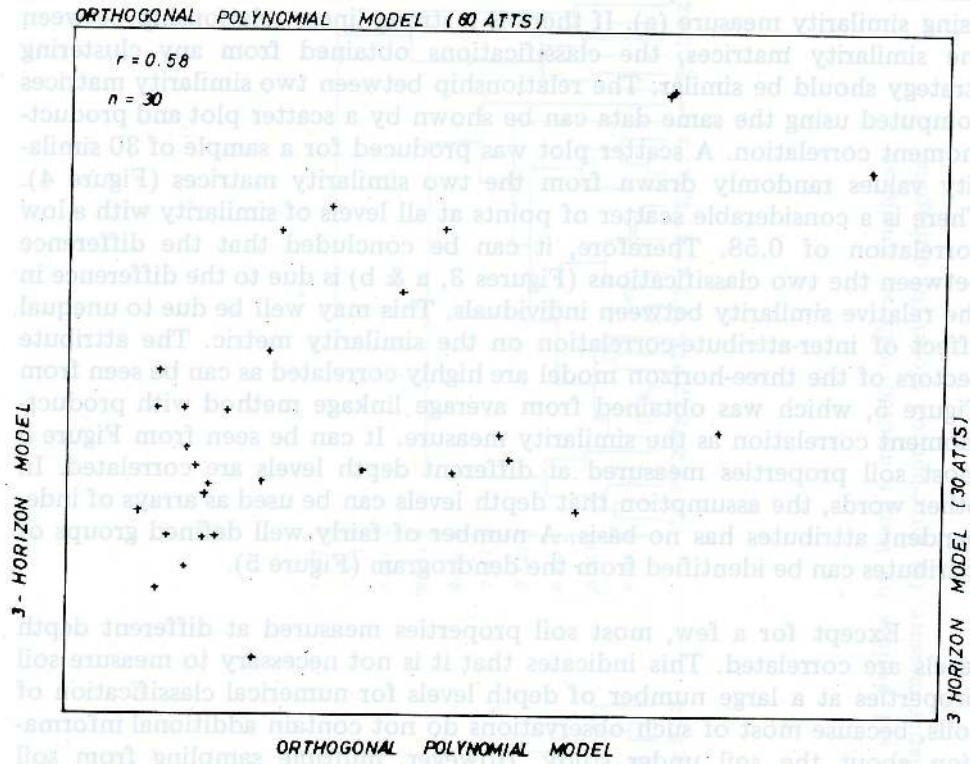

Figure 4. Relationship between the two inter-individual similarity (Euclidean distance) computed from the two soil profile models.

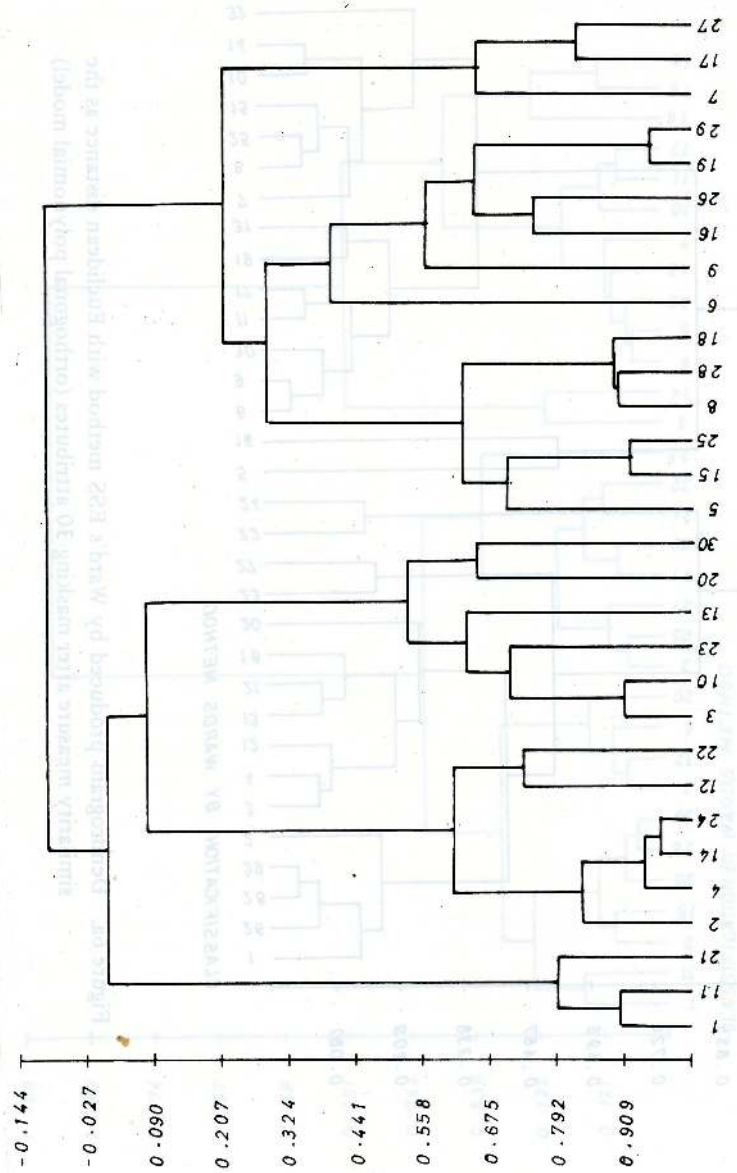

Figure 5. Classification of 30 soil attributes (10 soil properties for 3 horizons) by Average Linkage Method with product-moment correlation as the similarity measure.

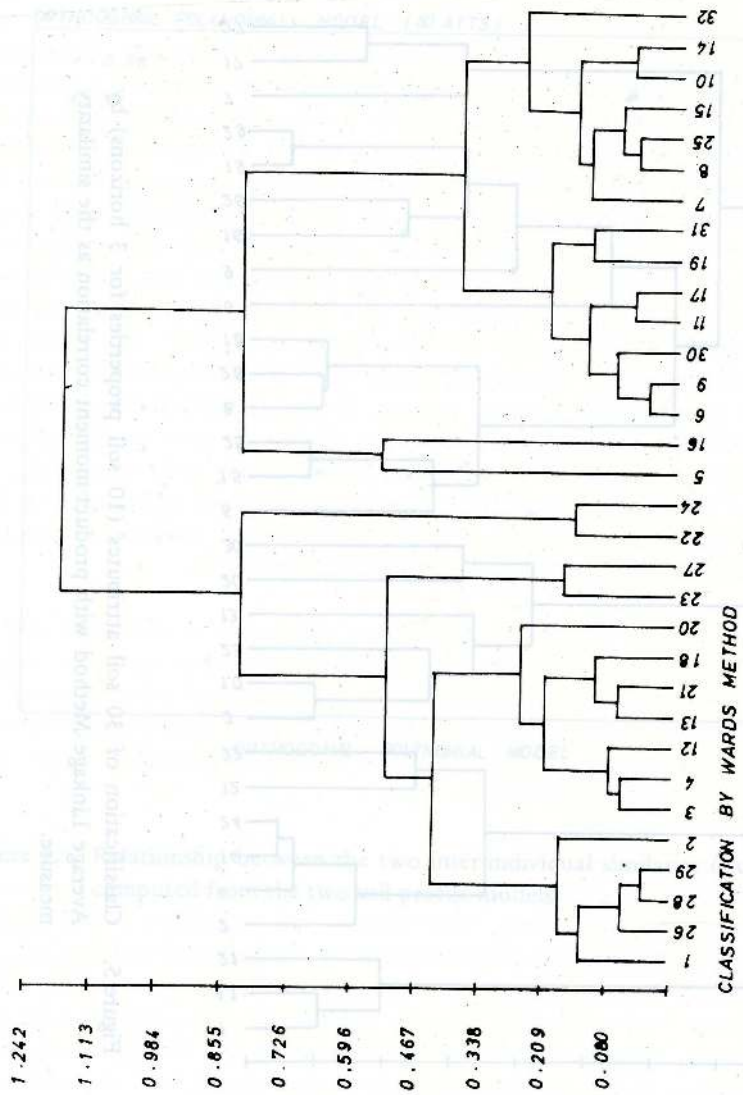

Figure 6a. Dendrogram produced by Ward's ESS method with Euclidean distance as the similarity measure after masking 30 attributes (orthogonal polynomial model)

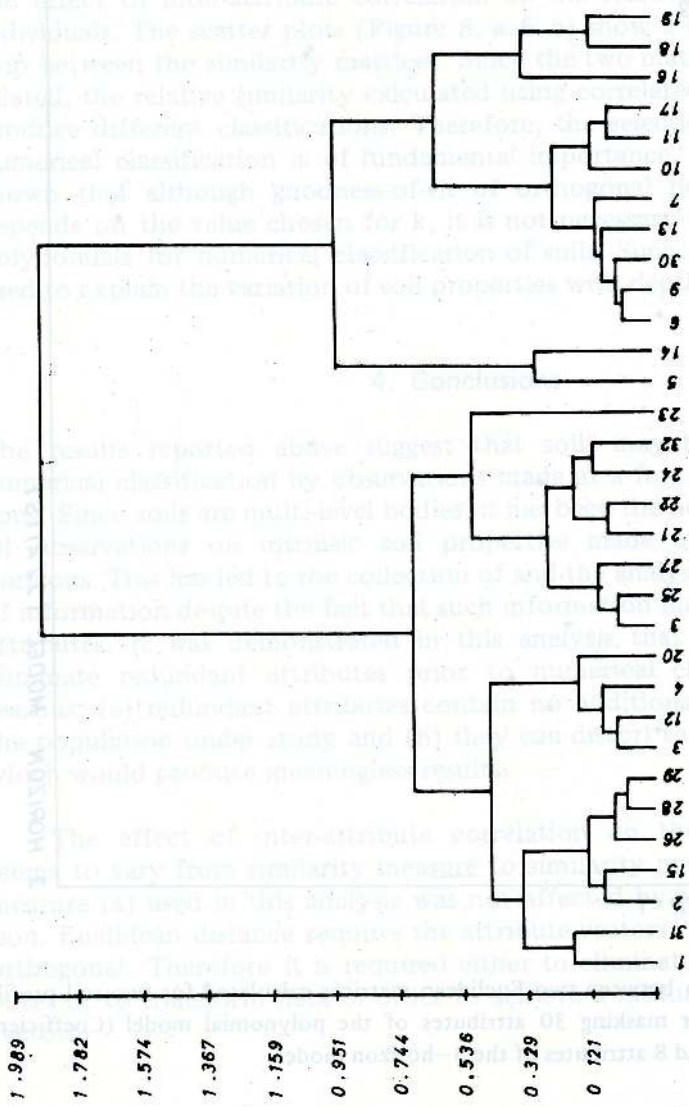

Figure 6b. Dendrogram produced by Ward's ESS method with Euclidean distance as the similarity measure after masking eight attributes (3–horizon model).

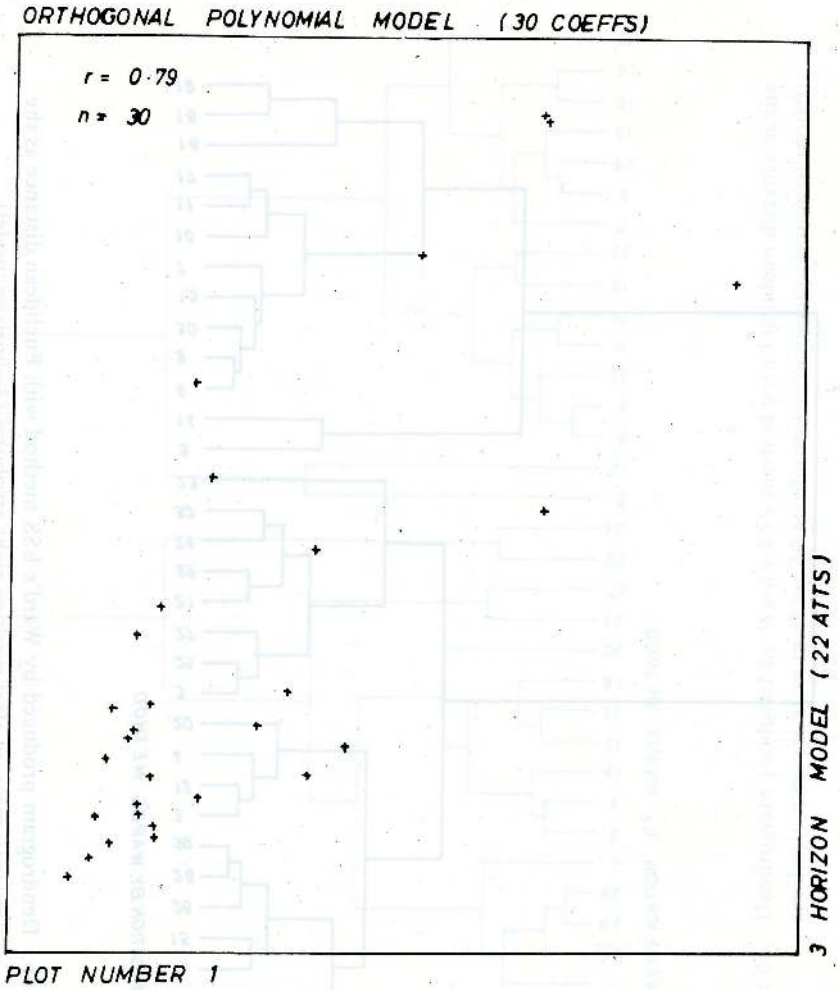

Figure 7. Relationship between two Euclidean matrices calculated for two soil profile models after masking 30 attributes of the polynomial model (Coefficient $C_3 \leftrightarrow C_5$) and 8 attributes of the 3–horizon model.

The relationship between the similarity matrices calculated using all attributes and a sub-set of attributes was examined for both soil profile models (Figure 8, a & b) assuming that it might reveal some information on the effect of inter-attribute correlation on the relative similarity between individuals. The scatter plots (Figure 8, a & b) show a curvi-linear relationship between the similarity matrices. Since the two matrices are not linearly related, the relative similarity calculated using correlated attributes tends to produce different classifications. Therefore, the selection of attributes for numerical classification is of fundamental importance. Equally, it has been shown that although goodness-of-fit of orthogonal polynomial functions depends on the value chosen for k, it is not necessary to use higher degree polynomials for numerical classification of soils. Such polynomials may be used to explain the variation of soil properties with depth.

4. Conclusions

The results reported above suggest that soils may be characterized for numerical classification by observations made at a few depth levels or horizons. Since soils are multi-level bodies, it has been the normal practice to use all observations on intrinsic soil properties made of all identified soil horizons. This has led to the collection of and the analysis of a large quantity of information despite the fact that such information may include redundant attributes. It was demonstrated in this analysis that it was necessary to eliminate redundant attributes prior to numerical classification for two reasons; (a) redundant attributes contain no additional information about the population under study and (b) they can distort the measurement space which would produce meaningless results.

The effect of inter-attribute correlation on the similarity measure seems to vary from similarity measure to similarity measure. The similarity measure (a) used in this analysis was not affected by inter-attribute correlation. Euclidean distance requires the attribute vectors chosen to be mutually orthogonal. Therefore it is required either to eliminate the correlated attributes or to transform data in order to achieve a mutually orthogonal set of vectors.

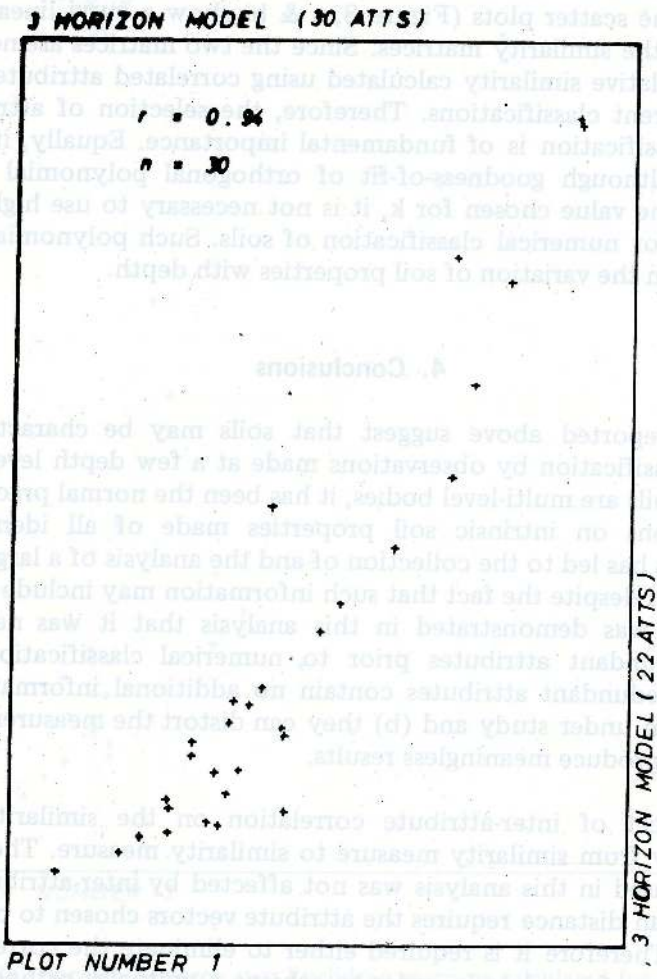

Figure 8a. Relationship between two Euclidean distance matrices calculated for the 3—horizon model before and after masking attributes.

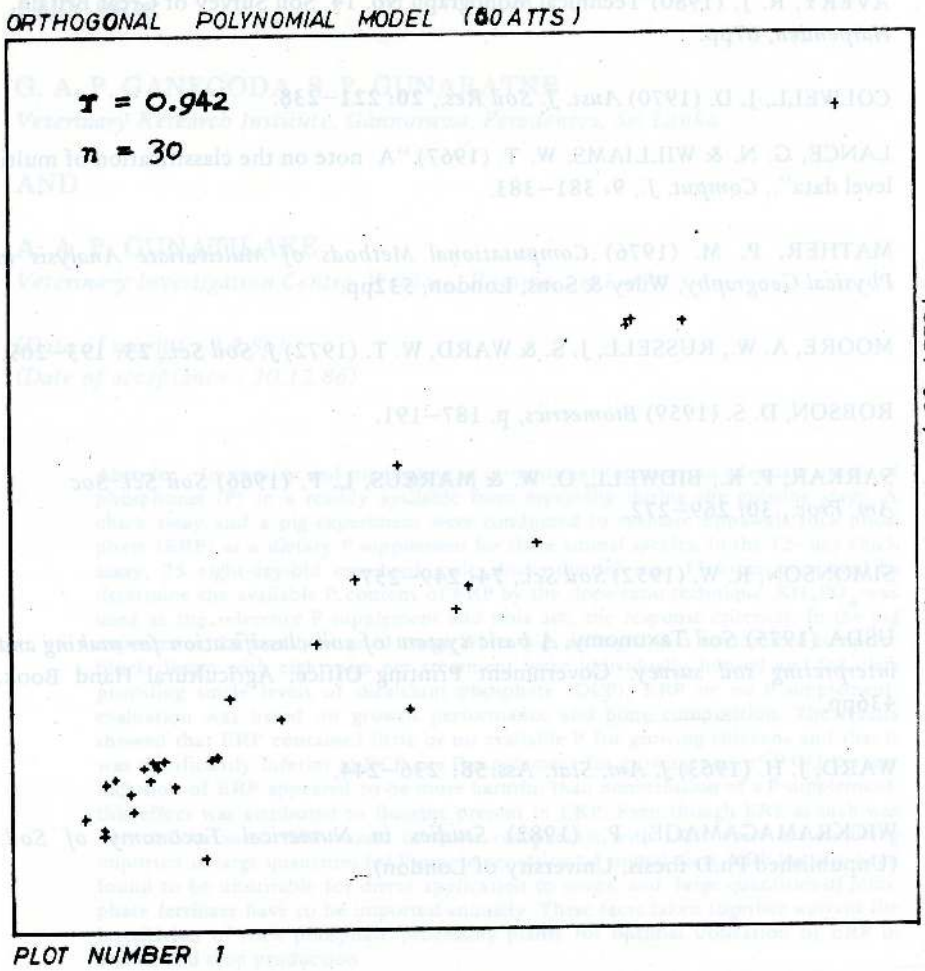

Figure 8b. Relationship between similarity matrices calculated using 80 and 30 polynomial coefficients.

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EVALUATION OF EPPAWALA ROCK PHOSPHATE AS A PHOSPHORUS SUPPLEMENT IN DIETS FOR GROWING CHICKENS AND PIGS

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Abstract : In poultry and pig feeding, it is important to ensure an adequate intake of phosphorus (P) in a readily available form especially during the growing stage. A chick assay and a pig experiment were conducted to evaluate Eppawala rock phosphate (ERP) as a dietary P supplement for these animal species. In the 12–day chick assay, 75 eight-day-old crossbred male chicks divided into 15 units were used to determine the available P content of ERP by the slope-ratio technique; KH_2PO_4 was used as the reference P supplement and tibia ash, the response criterion. In the pig experiment, 24 freshly weaned Large White pigs arranged in a randomized complete block design with eight pigs per treatment were individually housed and fed diets providing single levels of dicalcium phosphate (DCP), ERP or no P supplement; evaluation was based on growth performance and bone composition. The results showed that ERP contained little or no available P for growing chickens and that it was significantly inferior to DCP as a P supplement for growing pigs ($p<0.01$). Dietary inclusion of ERP appeared to be more harmful than noninclusion of a P supplement; this effect was attributed to fluorine present in ERP. Even though ERP as such was not, defluorinated ERP would be quite comparable with DCP which is now being imported in large quantities for P supplementation of animal diets. ERP has also been found to be unsuitable for direct application to crops, and large quantities of phosphate fertilizer have to be imported annually. These facts taken together warrant the installation of rock phosphate processing plants for optimal utilization of ERP in animal and crop production.

1. Introduction

Practical diets for poultry and pigs are based on cereals and oilseed meals. Since these feedingstuffs do not provide adequate amounts of calcium (Ca) and phosphorus (P), it is usual to include Ca and P supplements in such diets. The commonly used P supplements e.g. dicalcium phosphate (DCP) are expensive. Nevertheless, the diets for growing poultry and pigs must contain

adequate levels of P in a readily available form commensurate with their rapid growth.

Rock phosphates from the principal deposits of the world have been evaluated by many workers for P supplementation of diets for farm animals.[10] The variance of the results of those evaluations can be attributed partly to the differences in the available P and fluorine (F) contents of the various rock phosphates.

A huge deposit of light brown rock phosphate of the chlorfluorapatite type was discovered by the Geological Survey Department at Eppawala in 1971. This deposit is being exploited by the State Mining & Mineral Development Corporation to produce unprocessed phosphatic fertilizer; but none of it is being utilized in animal feeding. Defluorination improves the feeding value of rock phosphates by eliminating the hazard of F toxicity as well as by increasing the biologically available P content. However, defluorinating facilities are not available in Sri Lanka. The chick assay and the pig experiment reported herein were therefore thought to be a necessary first step for the proper utilization of Eppawala rock phosphate (ERP) in animal production.

2. Experimental

2.1 Phosphorus Supplements

ERP ground to pass through 0.15mm mesh was procured from the State Mining and Mineral Development Corporation; it contained 23.0% Ca and 14.7% P. DCP procured from the Asia Glues and Chemicals Ltd, Madurai, India for use as the control P supplement in the pig experiment contained 27.5% Ca and 17.8% P. Atomic absorptiometry and colorimetric vanadomolybdate procedure were used for the determination of Ca and P, respectively. Potassium *di*hydrogen orthophosphate (KH_2PO_4) of analytical reagent grade was used as the reference P supplement in the chick assay.

2.2 Chick Assay

A batch of 125 crossbred (Hubbard Golden Comet) males procured as day-old chicks were fed a conventional maize-soya bean meal starter diet for 7 days. They were then fasted for 12 h and after discarding the heaviest and the lightest, 75 of them were divided equally into 15 experimental units (penlots) of similar mean body weights. The penlots were housed in decks of raised wire-floor battery cages in a room with continuous lighting and a temperature of 25°C to 27°C and randomly assigned to five dietary trea-

ments. Food and distilled water were provided *ad libitum* for 12 days.

The five diets were as follows: A purified basal diet supplying 0.10% P from the reference P supplement was prepared according to the formula of Corley *et al.*[5] This minimal level of P has been shown to be adequate to prevent chick mortality. Two reference P diets to contain 0.05 and 0.10% additional P and two ERP diets to contain 0.10 and 0.15% additional P were prepared by substituting appropriate amounts of the respective P supplements for maize starch weight for weight in the basal diet.

At the conclusion of the 12—day assay period, each chick was weighed and killed by bleeding. The left tibiae of the chicks from each penlot were freed from adherent tissues after steaming for 5 min, defatted with chloroform-methanol (2:1 v/v) and ashed at 600°C for 36 h.

Data on P intake and tibia ash of the penlots that were fed the basal and reference P diets were subjected to linear regression. The reference P response equation so obtained was applied to the penlots that were fed the ERP diets to calculate the fraction of tibia ash due to P intake from the reference P supplement; the remaining tibia ash was attributed to P intake from ERP. Data on ERP intake and tibia ash due to ERP could then be used to obtain the ERP response equation. The available P content of ERP could be estimated by dividing the regression coefficient of the ERP response equation by that of the reference P response equation (slope-ratio technique).

2.3 Pig Experiment

Twenty-four freshly weaned Large White pigs were arranged in four blocks of three littermate males and four blocks of three littermate females, and assigned to three dietary regimes in a randomized complete block design. The pigs were individually housed on concrete floors.

Table 1 gives the ingredient composition and nutrient contents of the diets used for two-stage feeding. The positive control and test diets contained supplemental P as DCP and ERP, respectively, while the negative control diet did not contain any supplemental P. Daily feed allowance for each pig, adjusted at weekly intervals according to body weight, was computed from the equation

$$Y = 0.44 + 0.385\ X$$

where X was the body weight and Y the daily feed allowance, both in kg. The daily allowance was fed in two meals after moistening with water at the rate of 2.51 per kg of feed.

Table 1. Ingredient composition and nutrient contents of the diets in the pig experiment *(expressed as g/kg of air-dry diet unless stated otherwise)*

Feeding stage	Up to 20 kg body weight			From 20 to 50 kg body weight		
Diet designation	Positive control	Negative control	Test	Positive control	Negative control	Test
Ingredients:						
Ground yellow maize	320	320	320	300	300	300
Wheat flour	265	265	265	255	255	255
Rice polishings	100	100	100	100	100	100
Soya bean meal, 46–%	271	265.8	273	200	196.3	201
Coconut meal, expeller	8.8	21	5	112	120.6	109.7
Dicalcium phosphate	22.1	–	–	15.5	–	–
Eppawala rock phosphate	–	–	25.5	–	–	18
Powdered oyster shell	5.1	20.2	3.5	9.5	20.1	8.3
Powdered common salt	5	5	5	5	5	5
Vitamin–trace mineral premix[a]	2.5	2.5	2.5	2.5	2.5	2.5
Zinc bacitracin, 10–%	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient contents:						
Digestible energy, MJ/kg	13.73	13.80	13.71	13.68	13.74	13.66
Crude protein	200	200	200	185	185	185
Lysine	10.6	10.5	10.6	9	9	9
Methionine + cystine	6.6	6.6	6.6	6.1	6.1	6.1
Calcium	8	8	8	8	8	8
Total phosphorus	8	4.3	8	7	4.4	7

[a]Zoodry VM 1 (F. Hoffmann–La Roche & Co. AG, Basel, Switzerland)

Each pig was slaughtered when it reached 50 kg in body weight and its left fourth metacarpal bone was dissected out. The individual bones were defatted and ashed as in the chick assay; their dry fat-free weights were recorded before ashing. Bone Ca and P were determined as in the case of the P supplements. Response to dietary P supplementation was judged by time taken to reach 50 kg in body weight, mean daily feed intake, mean daily weight gain, feed:gain ratio and bone composition. Observations on each criterion were subjected to analysis of variance; differences between treatment means were assessed by Duncan's new multiple range test at 1—% significance level.

3. Results

3.1 Chick Assay

The results of the chick assay for the estimation of available P in ERP is summarised in Table 2. There was virtually no accumulation of tibia ash attributable to P intake from ERP, indicating that the available P content of ERP for growing chickens was virtually nil.

3.2 Pig Experiment

Table 3 gives a summary of the results of the pig experiment. The pigs on the ERP-supplemented dietary regime took a longer time to attain the final weight and consumed more feed per unit weight gained than those on the DCP-supplemented dietary regime ($p<0.01$). Inclusion of ERP as a dietary P supplement appeared to be worse than noninclusion of a P supplement. However, retention of Ca and P in bone was not affected by the dietary treatments.

4. Discussion

The principal rock phosphate deposits of the world can be broadly typed as continental and island deposits. Continental deposits such as those occurring in North Africa and North America contain 3 to 4% F, whereas deposits found in the Pacific and Indian Ocean islands generally contain only half that or even less.[15] Island deposits had been generally regarded as safe for P supplementation of cattle, pig and poultry diets.[13]

However, it is evident from the results that ERP is virtually ineffective for P supplementation of diets for growing poultry, Since ERP contains about 2% F,[2] the ERP diets fed during the starter and grower stages of the pigs (Table 1) must have contained at least 510 and 360 ppm F, respectively. That these levels are deleterious to pig performance is indicated by the fact

Table 2. Summary of the chick assay for estimation of available phosphorus (P) in Eppawala rock phosphate (ERP)
(mean values of three replicates of five male chicks per treatment)

Diet designation:	Basal[a]	Basal + 0.05% reference P	Basal + 0.10% reference P	Basal + 0.10% ERP P	Basal + 0.15% ERP P
Initial weight at 8 days of age, g	67	67	67	67	67
Weight after 12–day feeding, g	129	130	133	130	127
Food intake, g	115	117	122	117	114
Total P intake, mg	115.5	176.0	244.3	234.2	285.5
P intake from ref. P supplement[b], mg	115.5	176.0	244.3	117.1	114.2
ERP[c] intake, mg	—	—	—	796.6	1165.3
Total tibia ash, mg	100.6	118.2	142.8	100.8	99.8
Tibia ash due to reference P, mg	100.6	118.2	142.8	100.2[d]	99.3[d]
Tibia ash due to ERP, mg	—	—	—	0.6[e]	0.6[e]

[a]Contained 0.10 % P from reference P supplement.

[b]KH_2PO_4

[c]Contained 14.7 % P

[d]Computed from the reference P response equation $Y = 61.6003 + 0.33\ X$ where X is the P intake from the reference P supplement in mg and Y the tibia ash content due to reference P, also in mg ($r = 0.995$).

[e]No linear correlation with ERP intake ($r = 0.015$).

Table 3. Growth performance and bone composition of pigs fed diets with dicalcium phosphate (DCP), Eppawala rock phosphate (ERP) or no phosphorus supplement from weaning to 50–kg body weight
(mean values of four males and four females per treatment in randomised complete block design)

Phosphorus supplement:	Positive control DCP	Negative control None	Test ERP	SE of diff.	CV (%)
Initial wt., kg	10.4	10.5	10.4		
Time taken to reach 50–kg wt, days	83.1[a]	89.3[a]	96.3[b]	2.1	4.9
Mean daily feed intake, kg	1.48[a]	1.45	1.48[a]	0.02	2.3
Mean daily wt. gain, kg	0.49[a]	0.45[ab]	0.42[b]	0.01	5.6
Feed:gain ratio	3.02[a]	3.28[ab]	3.49[b]	0.12	7.4
Left fourth metacarpal:					
Fat-free dry wt., g	6.395	6.231	6.340		
Ash, g/kg	609.4[a]	608.0[a]	611.0[a]	7.2	0.3
Calcium, g/kg	234.0[a]	232.2[a]	232.9[a]	5.3	0.6
Phosphorus, g/kg	111.0[a]	108.6[a]	106.7[a]	2.9	0.7

In a given row, values with different superscripts are significantly different ($p < 0.01$).

Table 4. Maximum safe dietary fluorine levels for different farm animals according to form of fluorine *(expressed as mg/kg dry matter)*

	Sodium fluoride	Rock phosphate
Young cattle	30–50	30
Dairy cow		65–100
Beef cattle	40–50	65–100
Young sheep and goats	70–100	60
Adult sheep and goats		100–200
Pigs	150–300	150
Chickens		300–400
Laying hens	300–400	300
Turkeys		300

that the pigs given ERP fared marginally worse than those given no P supplement. Kick *et al*[10] have found that rock phosphate levels giving more than 175 and 700 ppm F in the diet were not safe for pigs and chickens, respectively, even for short-term feeding.

The etiology of F toxicity (fluorosis) in livestock has been comprehensively discussed by Underwood.[15] As in the case of the other toxic mineral elements, the maximum safe level of dietary F varies according to the age and species of the animal, the physical and chemical form of the element, the duration and continuity of the intake and the nature and proportions of the other dietary ingredients. Highly soluble F sources like sodium fluoride are much more toxic than highly insoluble sources like calcium fluoride; rock phosphates are intermediate. Table 4 gives the maximum safe dietary levels of F for various farm animals and F sources.

Highly effective defluorinating procedures have been developed to reduce the F content of rock phosphates to safe levels. The degree of heat treatment required to defluorinate rock phosphate is also adequate to convert its P to the readily available *ortho*-phosphate form and may sometimes be severe enough to convert some of the *ortho*-phosphate form to less available *pyro*- and *meta*-phosphate forms.[15] However, there is ample evidence to show that defluorinated rock phosphates are as good as DCP for supplementation of diets for growing chickens,[6] laying hens,[8] growing pigs[3,4,7,11] and growing sheep.[9] The maximum level of residual F in defluorinated rock phosphates for pig feeding is considered to be 0.2 %.[14]

5. Conclusion

To sum up, ERP as such is unsuitable as a P supplement for farm animals due to its low P availability and high F toxicity. It is possible to reduce its F content to a perfectly safe level and simultaneously increase the available P content to that of DCP by an appropriate defluorinating procedure. Defluorinated ERP would be quite comparable with DCP which is now being imported in large quantities for P supplementation of diets for livestock and poultry. In crop production too, ERP as such is unsuitable for direct application, especially to quick-growing crops[16] and huge quantities of phosphatic fertilizer have to be imported annually. Technological and agronomic studies have clearly shown that ERP is quite suitable for production of single and triple superphosphates.[2] These facts taken together warrant the installation of appropriate processing plants for the optimum utilization of ERP in animal and crop production.

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THE CATALYSIS OF WATER PHOTO–OXIDATION BY HEAVY METAL HEXACYANIDES

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Abstract : A number of heavy metal hexacyanides are tested for their ability to catalyse photo-oxidation of water with $bipy_3Ru^{2+}$ as the sensitizer and $K_2S_2O_8$ as the sacrificial agent. Strongest catalytic activity is seen in $Zn_3(Fe(CN)_6)_2$, $Cd(Fe(CN)_6)_2$ and $Fe_3(Fe(CN)_6)_3$. Semiconducting properties of heavy metal hexacyanides and their relevance to catalytic activity are discussed.

1. Introduction

Photo-oxidation of water in the presence of sacrificial agents has attracted much attention as models of photosystem II in natural photosynthesis.[1,2,3,4,5,6] Heterogeneous sensitizers (semiconductors) generally photo-oxidise water under sacrificial conditions even in the absence of other catalysts.[2,3,6] However, in the case of homogeneous sensitizers the presence of catalysts become essential for oxygen generation.[1,3,4,8] It is known that one electron oxidant tris (2 2' — bipyridyl ruthenium (III) ($bipy_3Ru^{3+}$) could bring 4 — electron transfer leading to photo-oxidation of water in the presence of catalysts such as RuO_2, IrO_2 and MnO_2.[9–11] Recently it has also been noted that prussian blue (PB) could also catalyse the same reaction with persulphate as the electron acceptor.[12] A suspension of PB in a solution containing $bipy_3Ru^{2+}$ and $K_2S_2O_8$ photogenerate O_2 via following reaction scheme.[12]

$$bipy_3Ru^{2+} + S_2O_8^{-} \xrightarrow{h\nu} bipy_3Ru^{3+} + SO_4^{-} + SO_4^{2-}$$

$$bipy_3Ru^{2+} + SO_4^{-} \rightarrow bipy_3Ru^{3+} + SO_4^{2-} \qquad (1)$$

$$4bipy_3Ru^{3+} + 2H_2O \xrightarrow{PB} 4bipy_3Ru^{2+} + 4H^{+} + O_2$$

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We have tested a number of other water insoluble heavy metal ferro— and ferri— cyanides (PB type structure) for the above catalytic activity and found that $Zn_3(Fe(CN)_6)_2$, $Cd_3(Fe(CN)_6)_2$ and $Fe_3(Fe(CN)_6)_3$ are superior to PB. It was also noted that $Cu_4(Fe(CN)_6)_2$ behave differently, this material does not catalyse water oxidation reaction with persulphate in the presence of $bipy_3Ru^{2+}$. However, in the absence of $bipy_3Ru^{2+}$, $Cu_4(Fe(CN)_6)_2$ catalyses photo-oxidation of water with sacrifice of persulphate. Again $Cu_4(Fe(CN)_6)_2$ is found to catalyse sacrificial photoreduction of water. These observations can be attributed to semiconducting properties of $Cu_4(Fe(CN)_6)_2$.

2. Experimental

Ferro— and ferri— cyanides of several heavy metals (Zn, Cd, Cu, Fe, Mn, Cr) were prepared by double decomposition of the solutions of their salts (Chloride or Sulphate, Analar) with a solution of $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$. The metal salt was kept in large excess to prevent the formation of double salts containing K.[13] Precipitates were washed and dried at 90°C. The photochemical reactions were carried out in a thermostated (26°C) quartz cell (35 ml) equipped with a polarographic detector (Applied Photophysics). The solution contained 9.2×10^{-3} mol dm^{-3} of $K_2S_2O_8$ and various concentration of different catalysts. All solutions were purged with argon and the cell was sealed before irradiation. The light source used was a 90 W medium pressure mercury lamp (UV and IR filtered off). Light intensities were measured with an International Light IL 700 Radiometer.

To measure the electrical conductivity of $Cu_4(Fe(CN)_6)_2$, the material in the powder form was compacted between stainless steel electrodes to a pressure $\sim$ 80 K bar, in a glass tube (diameter $\sim$ 0.6 cm, pellet length $\sim$ 0.4 cm), the ends of the tube were sealed with epoxy resin and the resistance at different temperature was measured with an ohm-meter. The diffuse reflectance spectra of the catalyst in the dry powder form or the absorption spectra of the suspensions were determined using an Unicamp SP 500 Series II spectrophotometer.

3. Results and Discussion

As the catalyst and $bipy_3Ru^{2+}$ absorb light in the same spectral region, the reaction rates vary with the concentration of the catalyst and in each case an optimum concentration is found to exist.[12] Figure 1 indicates the variation of the initial reaction rate with the concentration of $Zn_3(Fe(CN)_6)_2$. Figure 2 shows O_2 photogeneration with different catalysts at their optimum concentrations (pH 7, as in PB[12] the reaction rates are

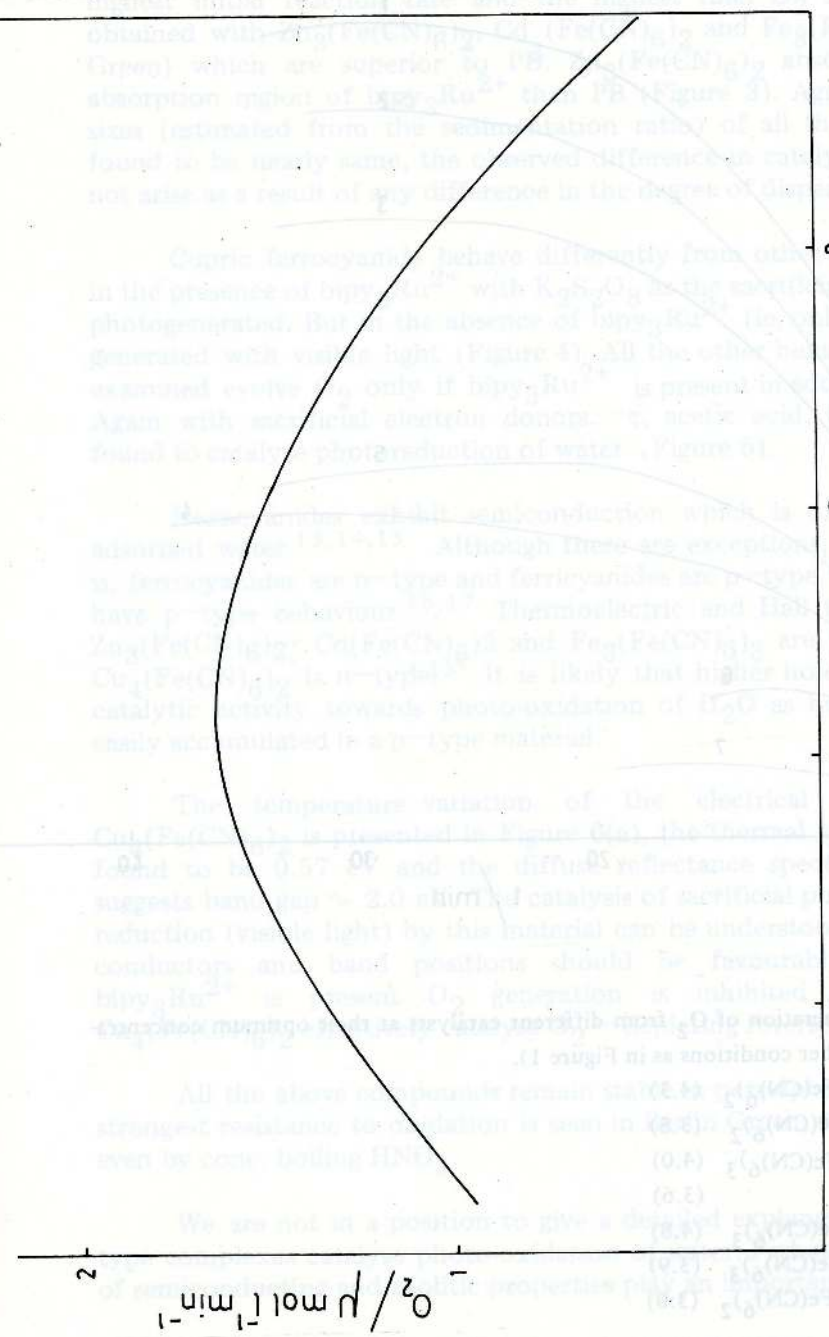

Figure 1. Variation of the initial reaction rate with concentration of $Zn_3(Fe(CN)_6)_2$. Reaction conditions ($bipy_3Ru^{2+}$) = 9.2×10^{-3} mol dm^{-3} ($K_2S_2O_8$) = 0.03 mol dm^{-3}. Illumination = 400W/m^2 at the window of the reaction cell.

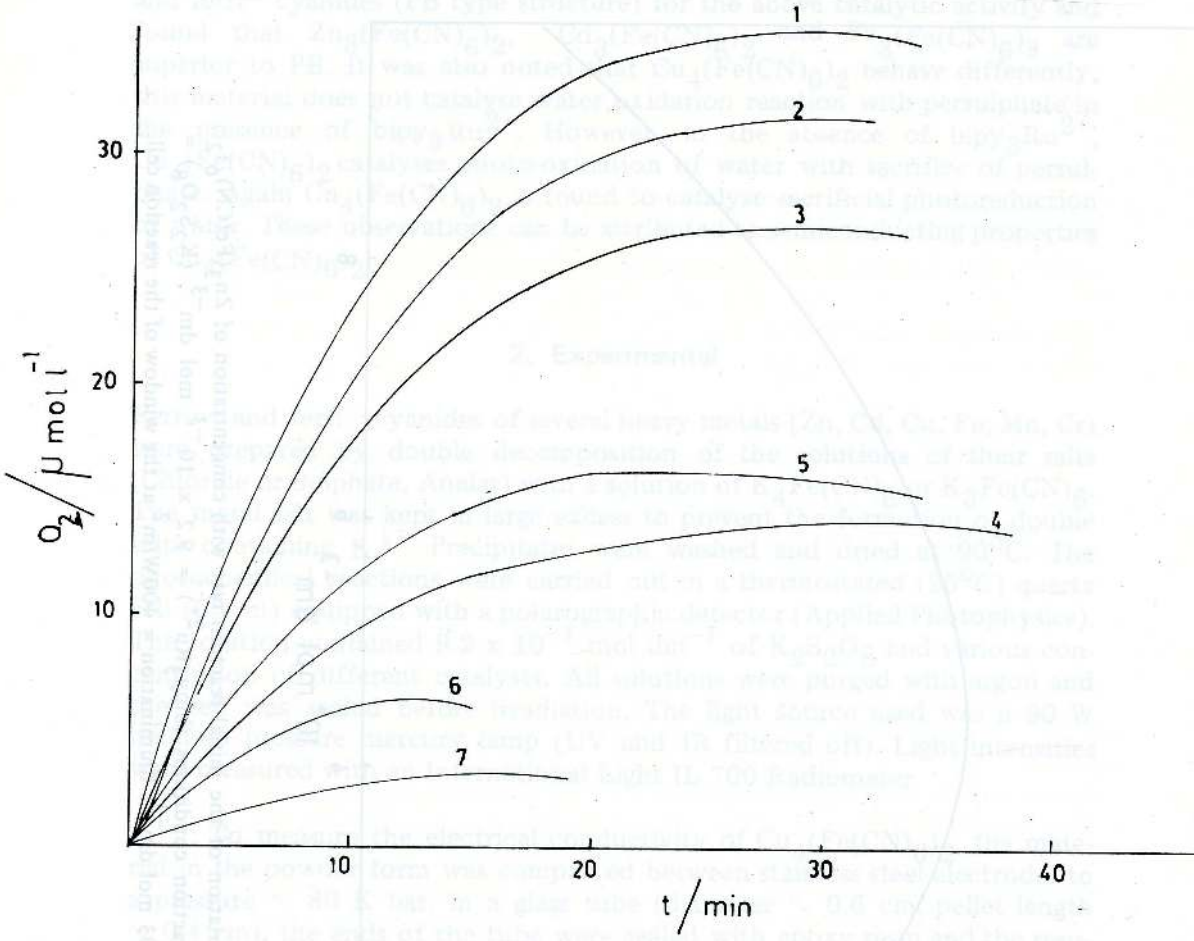

Figure 2. Photogeneration of O_2 from different catalysts at their optimum concentrations (other conditions as in Figure 1).

1. $Zn_3(Fe(CN)_6)_2$ (4.3)
2. $Cd_3(Fe(CN)_6)_2$ (3.8)
3. $Fe_3(Fe(CN)_6)_3$ (4.0)
4. PB (3.6)
5. $Cu_3(Fe(CN)_6)_3$ (4.8)
6. $Cr_4(Fe(CN)_6)_3$ (3.9)
7. $Mn_4(Fe(CN)_6)_2$ (3.0)

Numbers given inside the brackets are catalyst concentrations in 10^4 mol dm^{-3}.

maximum when the pH is ∼ 7), the other conditions kept constant. The highest initial reaction rate and the highest final O_2 concentration are obtained with $Zn_3(Fe(CN)_6)_2$, $Cd_3(Fe(CN)_6)_2$ and $Fe_3(Fe(CN)_6)_3$ (Berlin Green) which are superior to PB. $Zn_3(Fe(CN)_6)_2$ absorbs more in the absorption region of $bipy_3Ru^{2+}$ than PB (Figure 3). Again as the particle sizes (estimated from the sedimentation ratio) of all the compounds are found to be nearly same, the observed difference in catalytic activity could not arise as a result of any difference in the degree of dispersion.

Cupric ferrocyanide behave differently from other compounds. Here in the presence of $bipy_3Ru^{2+}$ with $K_2S_2O_8$ as the sacrificial agent, O_2 is not photogenerated. But in the absence of $bipy_3Ru^{2+}$ (ie, only $K_2S_2O_8$), O_2 is generated with visible light (Figure 4). All the other hexacyanides we have examined evolve O_2 only if $bipy_3Ru^{2+}$ is present in addition to $K_2S_2O_8$. Again with sacrificial electron donors. e.g, acetic acid, $Cu_4(Fe(CN)_6)_2$ is found to catalyse photoreduction of water (Figure 5).

Hexacyanides exhibit semiconduction which is often enhanced by adsorbed water.[13,14,15] Although there are exceptions, the general trend is, ferrocyanides are n—type and ferricyanides are p—type.[15] PB is known to have p—type behaviour.[16,17] Thermoelectric and Hall tests indicate that $Zn_3(Fe(CN)_6)_2$, $Cd(Fe(CN)_6)2$ and $Fe_3(Fe(CN)_6)_3$ are p—type, where as $Cu_4(Fe(CN)_6)_2$ is n—type.[15] It is likely that higher hole mobility favours catalytic activity towards photo-oxidation of H_2O as holes can get more easily accumulated in a p—type material.

The temperature variation of the electrical conductivity of $Cu_4(Fe(CN)_6)_2$ is presented in Figure 6(a), the thermal activation energy is found to be 0.57 eV and the diffuse reflectance spectrum (Figure 6 b) suggests band gap ∼ 2.0 eV. The catalysis of sacrificial photo-oxidation and reduction (visible light) by this material can be understood as in other semiconductors and band positions should be favourably located. When $bipy_3Ru^{2+}$ is present O_2 generation is inhibited possibly because $Cu_4(Fe(CN)_6)_2$ effectively catalyse O_2 — depleting reverse reaction.

All the above compounds remain stable in persulphate(9pH≲10). The strongest resistance to oxidation is seen in Berlin Green, which is unaffected even by conc. boiling HNO_3.

We are not in a position to give a detailed explanation as to why PB type complexes catalyse photo-oxidation of water. Perhaps the combination of semiconducting and zeolitic properties play an important role.

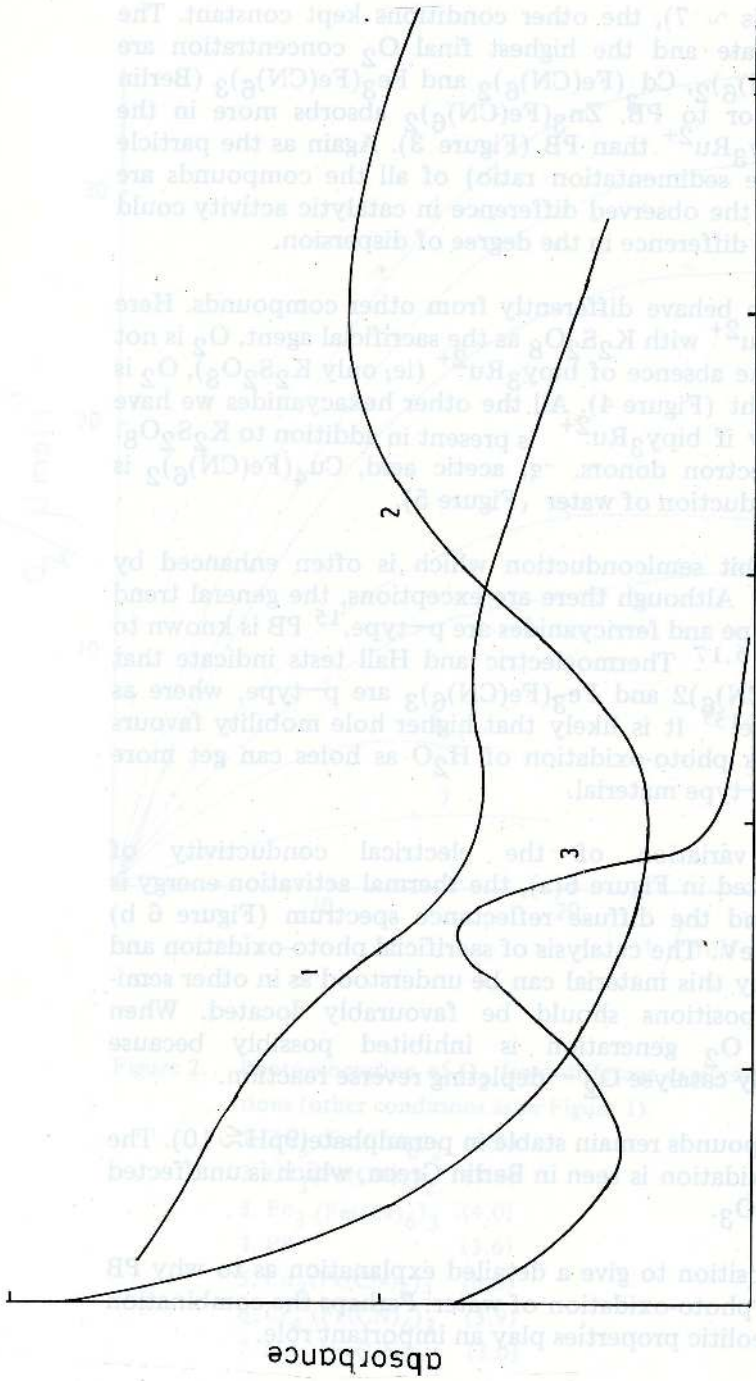

Figure 3. Absorption spectra of aqueous suspensions of
1. $Zn_3(Fe(CN)_6)_2$ (4.3)
2. PB (3.6)
3. $bipy_3Ru^{2+}$ (9.2)
The numbers given inside the brackets are concentration in 10^{-4} mol dm^{-3}.

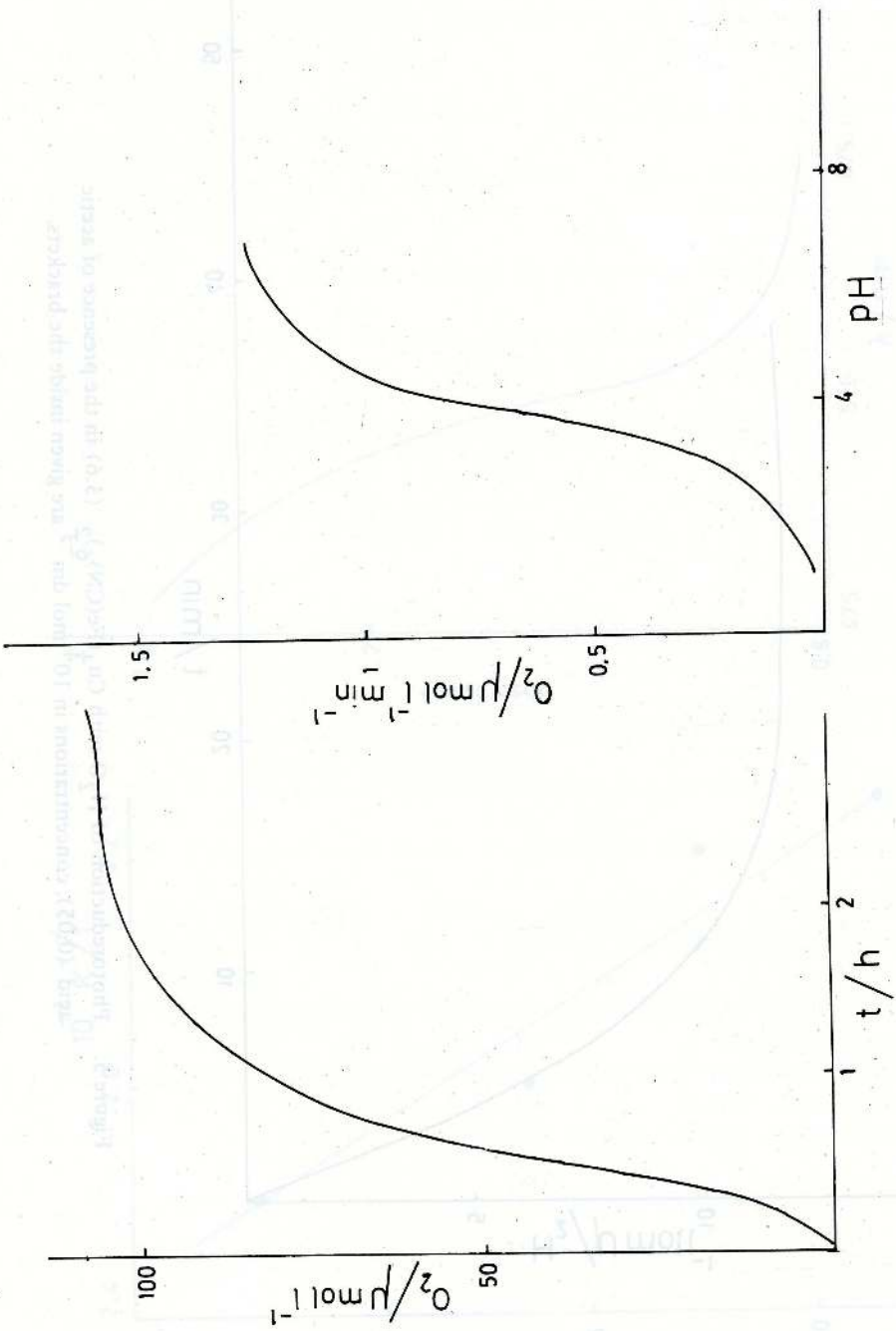

Figure 4. Photogeneration of O_2 with $Cu_4(Fe(CN)_6)_2$ (3.6) in the presence of $K_2S_2O_8$ (0.03). The numbers given inside the brackets are concentrations in 10^4 mol dm^{-3}.

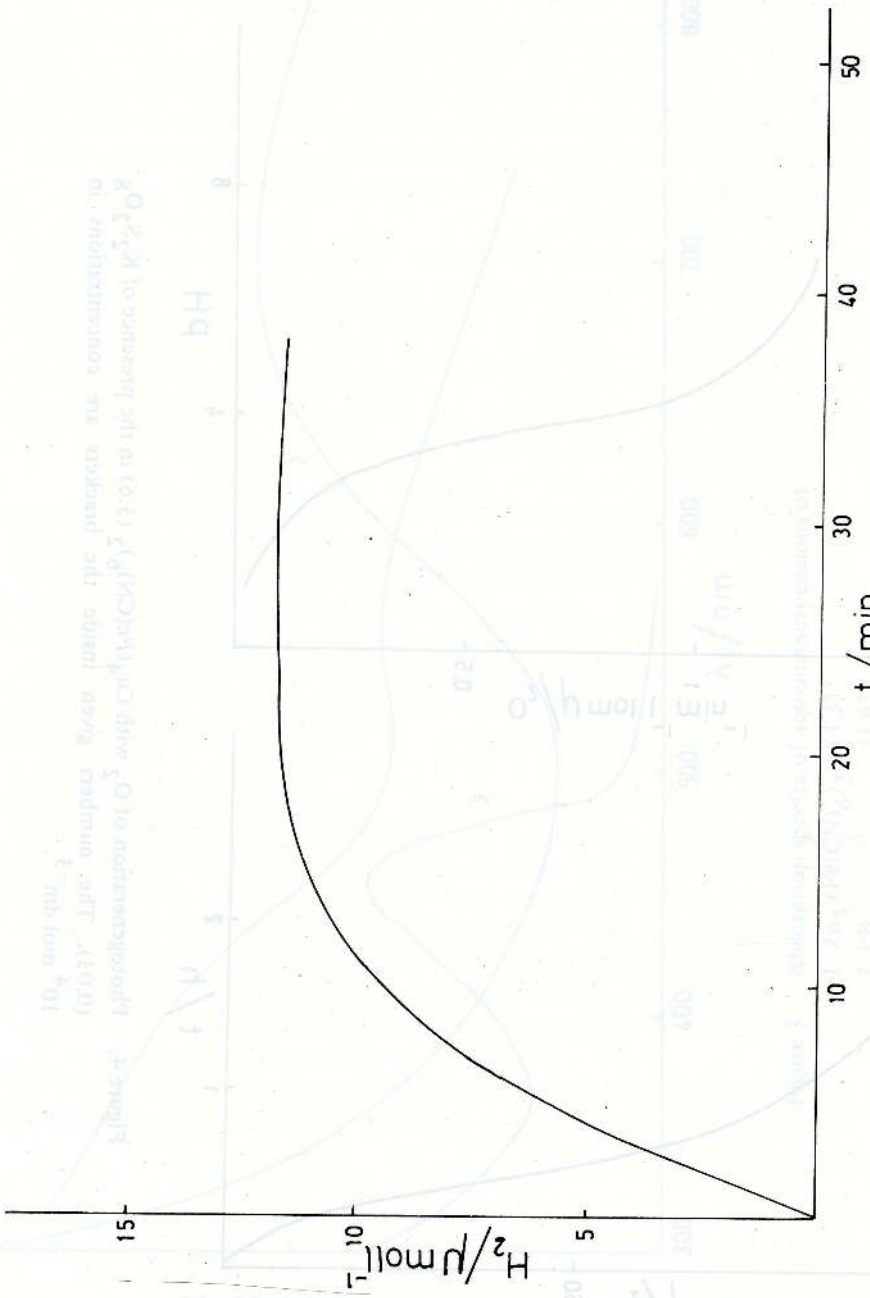

Figure 5. Photoreduction of H_2O with $Cu_4[Fe(CN)_6]_2$ (3.6) in the presence of acetic acid (0.05), concentrations in 10^4 mol dm^{-3} are given inside the brackets.

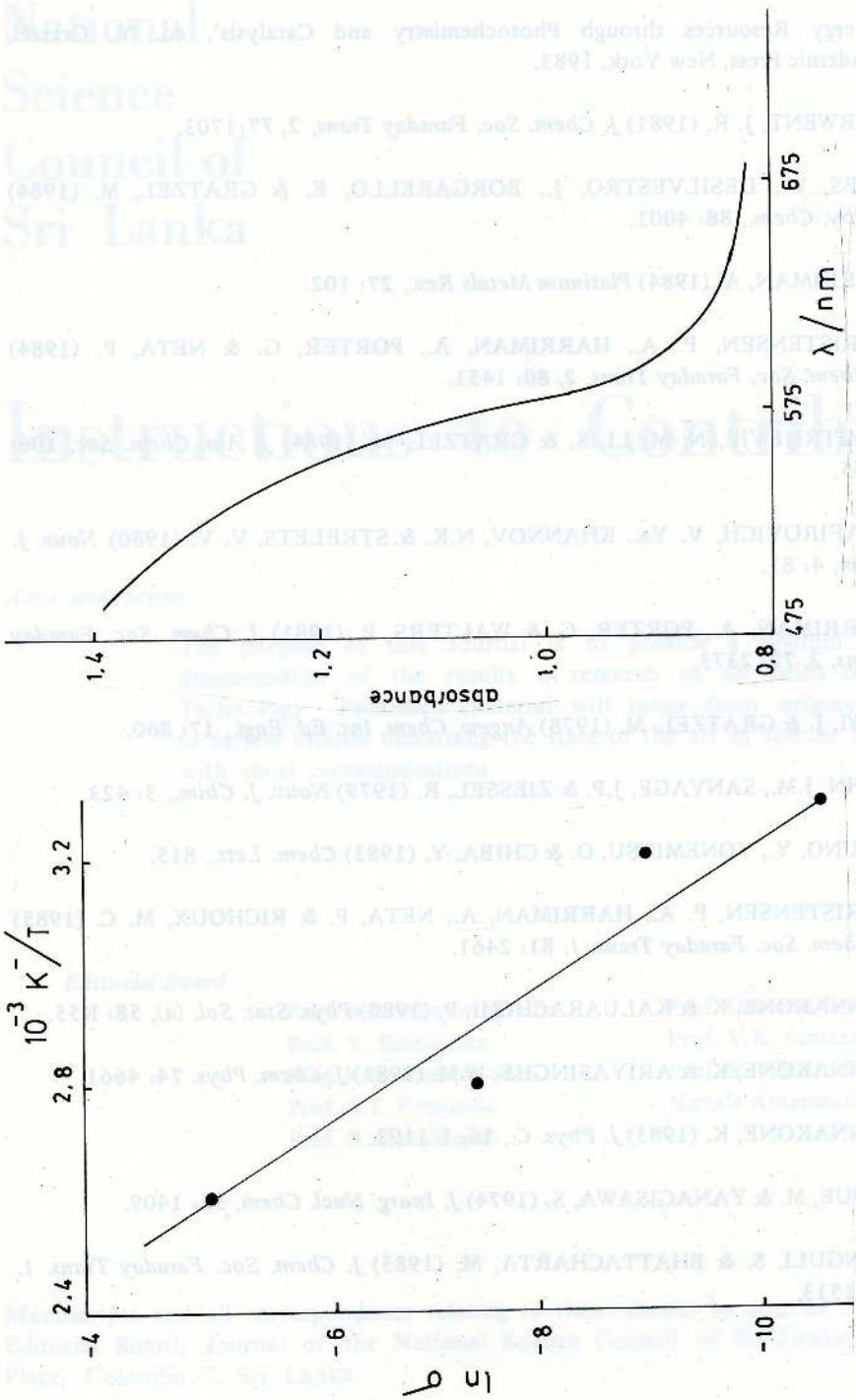

Figure 6. (a) Plot of log σ (σ in Ω^{-1} m^{-1}) *vs* T^{-1} for $Cu_4(Fe(CN)_6)_2$.

(b) Diffuse reflectance spectrum of $Cu_4(Fe(CN)_6)_2$.

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