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Pure Lac Dye: A Potential Natural Food Additive

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***Abstract*--The pure lac dye is obtained by dissolving technical grade lac dye in mild alkali for removal of insolubles, followed by acidification of the filtrate and finally keeping it for 8 days to complete the process of crystallization. The crystallized dye is filtered, washed and dried (yield approx.0.25% on the weight of sticklac, Purity 95% and above). The purified lac dye is bright reddish in colour and soluble in cold water. The pure lac dye has a great potential as a food additive for colouring food materials as it gives deep orange-red colour in aqueous solution like the colour of beverages and cold drinks available in market. Use of synthetic colours in the food products come to be looked upon with certain amount of apprehension with regard to their safety. Hence, lac dye could serve as a good substitute for the synthetic food colours. The potential of lac dye as food additive for colouring food material is further authenticated by the toxicological studies conducted at ITRC, Lucknow and AIIH&PH, Kolkata. The findings of these studies also support that the lac dye is non toxic at the limit test dose of 200 mg/kg diet *i.e.* 0.02% of the diet for acute oral toxicity as intake of lac dye 0.02% in diet for 90 days in male rats does not show any sign of toxic manifestation. Use of lac dye has been reported in China, Japan and Thailand as a colouring material in soft drinks and other products e.g., ham/ sausages, jams and chowmin noodles etc.**

***Keywords*-- Lac dye, Food additive, food colours**

I. INTRODUCTION

Colour is an important constituent of food as every food is associated with a certain type of colour. Addition of colour gives, food an attractive and appetising appearance, and enhances the acceptability. Natural colourants are extracted from natural herbs, plant parts, animals like kokam, beetroot, safflower, purple grapes and a very large source. Then natural colourants could be a good substitute for the synthetic food colours. Many countries have restricted the use of synthetic colours in food products. This has provided impetus and need for development of alternate colours and natural colours are now being considered as alternative.

Lac dye is a red coloured natural dye, which is present in the body fluid of lac insect, *Kerria lacca* (Kerr) as the alkali salt. It is obtained during washing of sticklac for preparing seedlac. Pure dye is sparingly soluble in water, is orange red in acidic medium and reddish violet in alkaline medium. In alkaline solutions, it decomposes rapidly.

It gives coloured lakes with inorganic mordant and was formerly employed for dyeing wool. Schmidt (1887) prepared pure dye from commercial cakes, in the form of a brownish-red crystalline powder decomposing without melting at 188^{o}C, and called it 'laccaic acid'. It is known as Natural Red 25 (CI Number 75450) in international trade. Dimroth and Goldschmidt (1913) suggested $C_{20}H_{14}O_{10}$ as its empirical formula. Mayer and Cook (1943) considered it to be a dicarboxylic acid of hydroxyanthraquinone. Mitrea (1936) was able to separate laccaic acid into two laccains, $C_{18}H_{12}O_9.2H_2O$ (red needles; 80%) and $C_{25}H_{18}O_{12}$ (yellow plates), through fractional crystallization from ethyl acetate. Kamath (1956) was able to separate it into two components, one containing nitrogen and other free from nitrogen. The pioneering study on the establishment of structure of laccaic acids was done by Venkataraman and Rama Rao (1972).

India, at present, produces about 20,000t of lac annually. Processing of this raw lac yields 200t of lac dye; majority of this dye is lost as by-product. Some processors use the partially pure form (technical grade) for utilization as textile dye (unspecified quantity), the rest processing industries dispose the sludge of washing as manure to villagers. Thus, an enormous potential exists for utilization of this by-product of lac factories, even if half of the potential is exploited then it will be possible to turn trade of lac dye into profitable business of specialty product, an export oriented trade with assured foreign market. An expansion of lac-based entrepreneurship is, thus, envisaged.

The study aims to highlight the potential of pure lac dye to be used as natural, non-toxic and safe food additive for coloring soft drinks and other products e.g., ham/ sausages, jams and chowmin noodles etc.

II. MATERIALS AND METHODS

Preparation of Technical and Pure grade Lac Dye

(a) Technical grade

The sticklac wash water is acidified with hydrochloric acid (0.1% on the vol. of water) and the precipitate allowed to settle in the tanks. The supernatant clear liquor is collected by filtration. The acid precipitated sludge is separately extracted thrice with for complete removal of dye and washings mixed with previous collection.

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The mixed filtrate is then treated with calcium carbonate or quicklime till the liquor becomes almost colorless. The calcium salt of dye, which separates out, is collected by filtration and washed with water. The cold suspension of calcium salt of lac dye in water is acidified with 10% aqueous solutions, and kept for 7-10 days at room temperature when the dye crystallizes out (Ghosh et al 1964; Ghosh and Sengupta 1977). It is filtered, washed and dried (yield approx.0.5% on the weight of sticklac, Dye content 80-90% depending on the quality and age of sticklac)

(b) Pure (food) grade

The technical grade dye is further purified by dissolving it in mild alkali, filtering the insolubles, acidifying the filtrate and finally keeping it for 8 days for crystallization (Prasad 2005). The crystallized dye is filtered, washed and dried (yield approx.0.25% on the weight of sticklac, Purity 95% and above). The purified lac dye is bright reddish in colour. It is sparingly soluble in cold water (0.13-0.14%) but completely soluble in dimethyl formamide. It can be used for colouring food materials as it gives deep orange-red colour in aqueous solution like the colour of beverages and cold drinks available in market.

(c) Properties

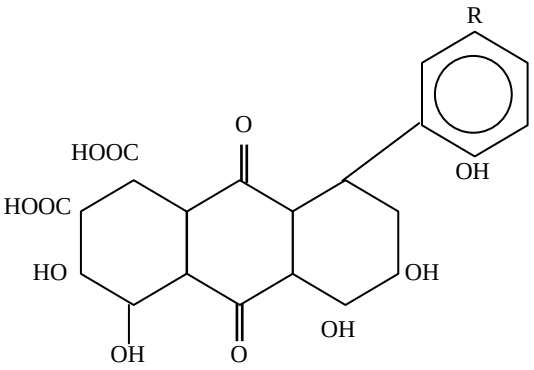
Various physico-chemical properties of lac dye such as colour, solubility, ash content, melting point, acid value, dye content, estimation of heavy metals were studied. Melting profiles of lac dye isolated from the wash water of lac factory effluents, and lac dye crystallized from simple water extract of sticklac, without addition of any additive were investigated by a Perkin-Elmer Differential Scanning Calorimeter (DSC 7).

(d) Toxicity tests

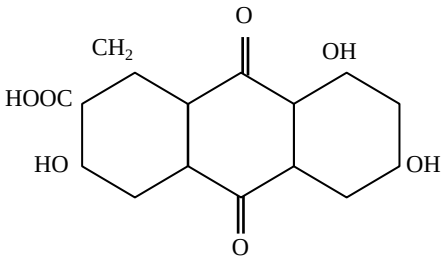
In view of promoting lac dye to be used as a food colouring agent, the toxicological studies were undertaken at Industrial Toxicological Research Centre (ITRC), Lucknow and All India Institute of Hygiene and Public Health (AIIH&PH), Kolkata (published by SEPC, Kolkata)

III. Results and Discussion

Lac dye is a red colored natural dye produced by the insect *Kerria lacca*. It is recovered by processing the washed water obtained during seedlac and shellac purification. Five compounds are found in lac dye, designated as laccaic acids A, B, C, D and E, laccaic acid A being the major fraction.



Laccaic acid



Laccaic acid D

A : R = $CH_2CH_2NHCOCH_2$
B : R = CH_2CH_2 0H
C : R = $CH_2CH(NH_2)$ COOH
D : = Structure shown separately.
E : R = CH_2CH_2 NH_2

The lac dye, has anthraquinone-type structure resembling Alizarin, Indathrona Golden yellow, is a mixture of at least five closely related pure compounds, all being the anthraquinone derivatives.

They have been assigned the names as laccaic acid A, B, C, D and E (Pandhare et al.1966, 67,69; Rama Rao et al 1968, 69; Bhide et al 1967and Mehandole et al 1968).

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For food sanitation and safe manufacturing practice, the development of a simple and precise method for identification of each component is required. High-speed counter –current chromatography (HSCCC), an advanced liquid-liquid partition method that does not require a solid support has been used as a powerful technique for the purification of lac dye (Oka et al. 1997).

Physico-chemical Properties of Lac dye

Lac Dye is known as Laccaic acid or Natural Red 25 (CI Number 75450) in international trade.

It is red to purple powder. Its colour in aqueous solution varies with its pH. Variation in colour with pH is as follows: pH 3-5 : orange-red; pH 5-7: red-purple;

pH>7 : red-purple. The resistance to light and heat is good in acid medium. Some important characteristics of pure lac dye are highlighted in Table1.

Table.1
Important Characteristics of Pure grade lac Dye

Sl. No.	**Characteristics**	**Pure grade dye**	***Method of Test***
1.	Colour	Bright red	-
2.	Solubility		
	a. water	0.13-0.14%	-
	b. Dimethyl formamide	Completely soluble	-
3	Ash content	0.7%	*A-3 of IS 12921:1990*
4	Melting point	Above 230^{o} (melting followed by charring)	*IS 5762:1970*
5	Volatile matter at $135^{o}C$	0.1%	*A-2 of IS 12921:1990*
6	Acid value	234-245	*A-6 of IS 12921:1990*
8	Dye content (purity)	95%	**A-7 of IS 12921:1990**
9	*Lead ppm max	Nil	**9 of IS 1699:1974**
10	*Arsenic, ppm max	Nil	**10 of IS 1699:1974**

- *The maximum permissible values for lead and arsenic in food colour is 10 and 3 ppm respectively as per IS 1699:1974 standard.*

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Melting profiles of lac dye by differential scanning calorimeter

Melting profiles of lac dye isolated from the wash water of lac factory effluents, and lac dye crystallized from simple water extract of sticklac, without addition of any additive were investigated by a Perkin-Elmer (DSC 7) Differential Scanning Calorimeter.

Prasad *et al.*, (2005):isolated lac dye from the wash water of lac factory effluents, through preparation of calcium salt and treatment with acid. The crystallized dye exhibited three melting peaks around 99.3, 195.1 and 274.5°C (Fig.3). The peaks, in general, were not sharp, multiple crystallization although resulted in some degree of sharpness, yet presence of multiple peaks indicate presence of different fractions of lac dye, presence of which was indicated by Venkataraman *et al.*, (1972). Srivastava et al (2007) crystallized lac dye from simple water extract of sticklac, without addition of any additive unlike observed by Prasad. Melting profile of the dye obtained after multiple crystallization by DSC revealed interesting information. The spectrum revealed only one melting peak around 112.4°C (Fig.4), which was sharper than any of the peaks obtained for laccaic acid isolated by earlier workers. The single peak indicates more homogeneity, absence of different fractions unlike that obtained by Prasad *et al.*, (2005).

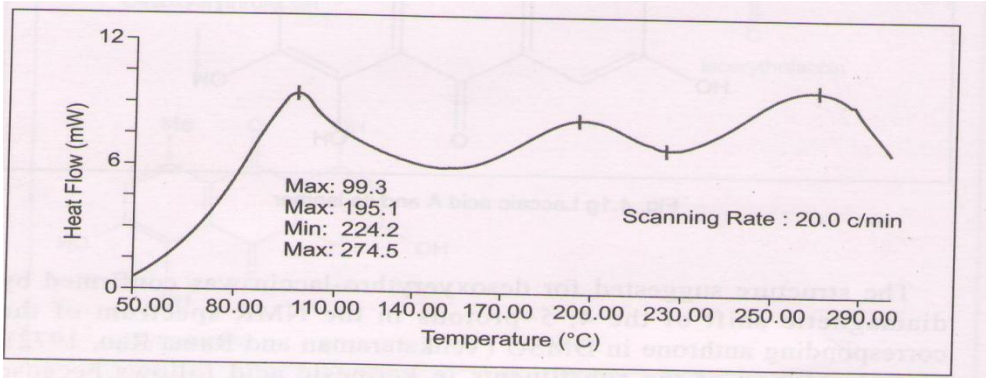

Fig. 2.Melting profile of lac dye extracted from effluents of lac factory

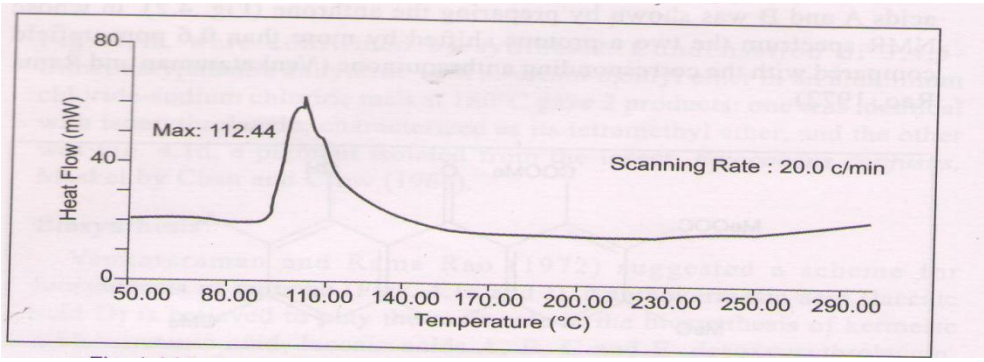

Fig. 3. Melting profile of lac dye crystallized from water extract of stick-lac

IV. Toxicological Studies

As part of a series of tests of the suitability of lac dye for use in foods as a colouring agent its detailed toxicological evaluations were performed.

Lac dye was non-mutagenic in Ames tests using five strains of *Salmonella typhimurium* with or without metabolic activation.

No cytotoxicity or mutagenicity was observed in Chinese hamster lung (V79) cells exposed to lac dye *in vitro* (Banerjee **et al.,** 1984).

The short term –term dietary intake of lac dye (0.2, 1 and 5% in diet) for 90 days in male rats did not show any significant changes in the body weight, relative organ weight and values obtained in treated samples were comparable to controls.

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Biochemical and hematological parameters in lac dye (up to 5% in diet) treated animals showed no significant changes over to controls. Histo-pathological examination of vital organs did not reveal any changes when compared to control group of animals (Table 2).

According to Food Adulteration Act. No. 30 (1955) of the Govt. Of India, the maximum permitted dose of any dye is 200 mg per kg diet (*i.e.* 0.02% of diet).

Keeping this in view, 7 days feeding study of lac dye in rats was undertaken and the results of which indicated that within the maximum permitted dose limit of treatment, the dye had no apparent toxic effect on acid and alkaline phosphate activities of liver. Lac dye showed some effect on a few parameters of hepatic biochemistry (RNA, DNA, total lipid, total sterol and bound sterol contents of the liver) only at higher dose but safe on lower doses (Table 3).

Table No. 2:
Toxicological Studies on lac dye at ITRC, Lucknow

Sl. No.	***Parameter***	***Characteristic / Inference***
1.	Nature of test	Acute and short term oral toxicity in Albino Rats
2.	Time periods of treatment	90 days
3.	Doses	A limit test dose of 2000 mg/kg body weight
4.	Tests undertaken	
	Morbidity and mortality, Food consumption, Water intake, Body weight, Relative organ weight, Haematology, Histo-pathological studies, Bio chemical studies.	

Table No. 3:
Toxicological Studies on lac dye at AIIH&PH, Kolkata

Sl. No.	***Particular***	***Characteristic / Inference***
1.	Nature of test	Acute, sub-acute and chronic toxicity
2.	Time periods of treatment	a. Acute – 6 hrs, 24 hrs.
		b. Sub-acute – 7 days, 1 month
		c. Chronic – 3 months, six months
3.	Doses	a. 0.2 mg per rat/day
		b. 1.0 mg -do-
		c. 2.0 mg -do-
		d. 10.0 mg -do-
		e. 20.0 mg -do-
4.	Tests undertaken	
.	***Histo-pathological studies, Bio chemical studies (In the LIVER, In the SERUM, In the BRAIN), Multi-generation studies, Mutagenicity & Carcinogenicity study.***	

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V. CONCLUSION

Lac dye is non toxic at the limit test dose of 200 mg/kg diet i.e. 0.02% of the diet for acute oral toxicity as intake of lac dye 0.02% in diet for 90 days in male rats does not show any sign of toxic manifestation. Toxicological evaluation lac dye shows a great potential to be used as natural food additive (food grade, purity 95% and above) for colouring ham/ sausages, jams and chowmin noodles beverages and cold drinks etc.

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