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POLAR BRILLIANT CRIMSON AS A STAINING DYE FOR ELECTROPHORETICALLY SEPARATED PROTEINS

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ABSTRACT

A new staining procedure, using polar brilliant crimson for electrophoretically separated protein fractions, is described which possesses the advantages of simplicity, sensitivity, stability of dye-protein complex and adaptability to elution and densitometric measurements.

INTRODUCTION

MANY procedures have been described for protein stains that colour protein components after electrophoresis. The stains used include bromophenol blue[1], amidoschwarz 10B[2], azocarmine B[3], lissamine green SF[4], bromocresol green[5], ponceau 2R[6], neococeine-acilan scarlet[7], nigrosine[8], ponceau S (fast ponceau 2B)[9] and procion brilliant M-RS[10]. The present report describes a new technique using the dye, polar brilliant crimson (Suhrid Geigy), for staining the protein components after electrophoresis with applicability of densitometric measurements and elution analysis.

MATERIALS AND METHODS

Paper electrophoresis.—Fractionation of serum proteins was accomplished by using barbitone-sodium barbitone buffer of pH 8·6 with 0·05 ionic strength.

Agar gel electrophoresis.—Agar shreds (50 mg) were added to 7·5 ml of barbitone-sodium barbitone buffer and heated. The molten agar (1 ml) was layered on 2·5 × 7·5 cm microscope slide (or polyester film) and allowed to set for 40 to 60 min. After application of serum, a constant current at 200 volts was applied for 30 min and at the end of the run the slide (or polyester film) was placed in methanol for the fixation of the protein bands. The slide (or film) was finally dried at room temperature.

Dye solution.—0·5 gm of polar brilliant crimson (PBC) in 20 ml ethanol was made up to 100 ml with 3% sulphosalicylic acid.

Clearing solution—95% methanol containing 10% acetic acid V/V.

Eluting solution.—1% sodium carbonate in 50% aqueous ethanol.

The protein components separated on the paper and on the agar gel were stained with the dye for 10 minutes at room temperature, washed for 4 min in two changes of clearing solution, then rinsed with methanol and allowed to dry at room temperature. The quantitative evaluation of the stained protein components was made by scanning in Photovolt Densitometer Model 525 and by photometric determination in Spectronic 20, of the dye eluted from the segregated fractions, at 535 nm. The electrophoretically separated protein fractions were cut out and eluted with 6 ml of eluting solution for 30 minutes, mixing at intervals

RESULTS AND DISCUSSION

By the use of the dye, polar brilliant crimson, the protein components in electropherogram show as red bands against clear background without any free dye adsorbed on the media. The dye is sensitive to stain all protein fractions and the patterns do not show any deterioration even after 7 years indicating that the dye-protein complex is quite stable. The

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polar brilliant crimson dye solution is stable at room temperature and the eluate is constant showing only 1% change in transmission in 48 hours. The characteristic maximum of absorption is at 535 nm and is recorded in Spectronic-20.

Tables I and II show mean, standard deviation and P values of protein components of normal and pathological sera obtained by staining with polar brilliant crimson and bromophenol blue. There is no appreciable difference between the results of the two dyes. The 't' test has been applied for the differences in the mean and they are not statistically significant. In all cases $P > 0 \cdot 05$ and the difference between the two mean values of the two different dyes are not statistically established.

The dye-binding properties of both the dyes (polar brilliant crimson and bromophenol blue) with the serum protein components may be the same since little variation is observed in the values. Though Rees and Lawrence[11] observed the obedience of Beer's law by different stains, Wurm and Epstein[12] discredited the applicability of Beer's law. The dye-binding properties of different fractions are different leading to the introduction of correction factors[13]. Correction factors that have been recommended can be criticized since they are based on normal sera and are not necessarily valid for abnormal sera[14]. It has been observed that the best solution to this probelm would seem to establish a normal range for a particular dye and other electrophoretic conditions employed and to avoid

TABLE I

Serum protein fractions as stained by polar brilliant crimson and bromophenol blue
(PER CENT OF TOTAL PROTEIN)

Serum protein fractions	Normal n=50					Nephrotic syndrome n=20				
	Polar brilliant crimson		Bromophenol blue		P value	Polar brilliant crimson		Bromophenol blue		P value
	Mean	±S.D.	Mean	±S.D.		Mean	±S.D.	Mean	±S.D.	
Albumin	51·5	3·5	51·0	3·9	>0·05	23·9	4·1	25·0	3·5	>0·05
Globulins :										
Alpha$_1$	2·9	0·7	3·1	0·9	>0·05	2·3	0·6	2·7	0·7	>0·05
Alpha$_2$	10·7	1·5	11·0	1·2	>0·05	36·4	2·2	37·3	2·5	>0·05
Beta	11·4	1·5	11·9	1·6	>0·05	12·5	1·5	12·0	0·9	>0·05
Gamma	23·5	3·5	23·0	3·2	>0·05	25·0	3·2	23·0	3·5	>0·05

n = number.

TABLE II

Serum protein fractions as stained by polar brilliant crimson and bromophenol blue
(PER CENT OF TOTAL PROTEIN)

Serum protein fractions	Cirrhosis of liver n = 30					Infective hepatitis n = 30				
	Polar brilliant crimson		Bromophenol blue		P value	Polar brilliant crimson		Bromophenol blue		P value
	Mean	±S.D.	Mean	±S.D.		Mean	±S.D.	Mean	±S.D.	
Albumin	35·1	3·0	34·8	4·1	>0·05	36·3	3·2	36·2	3·6	>0·05
Globulins :										
Alpha$_1$	4·1	0·5	4·3	0·6	>0·05	3·8	0·6	4·0	0·7	>0·05
Aplha$_2$	5·1	1·5	5·8	1·8	>0·05	8·8	0·7	8·8	0·8	>0·05
Beta	6·4	3·8	4·3	3·5	>0·05	10·0	1·2	10·0	0·8	>0·05
Gamma	49·3	7·0	50·8	6·5	>0·05	41·1	5·0	41·0	6·2	>0·05

n = number.

affinity correction factors[15]. The new staining procedure affords a convenient, simple and stable staining method for widespread use in electrophoresis.

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STUDIES ON TOBACCO RING SPOT VIRUS FROM BRINJAL (*SOLANUM MELONGENA* L) WITH PARTICULAR REFERENCE TO PURIFICATION AND ASSESSMENT OF LOSSES

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ABSTRACT

Virus causing ring spot symptoms on brinjal is studied and identified to be due to Tobacco ring spot virus (TRSV). The virus is spherical with 26 mμ in diameter and is related to TRSV in other physical properties also. The loss in yield due to this virus infection is 55·2% to 70·3%.

RING spot symptoms of brinjal were found to be of common occurrence in the fields around Tirupati (Andhra Pradesh) and Bangalore (Karnataka). The disease incidence ranged from 60% to 80%. The characteristic symptoms of the disease were chlorotic concentric rings as well as mosaic mottling of leaves (Fig. 1). The infected plants produced less fruits which were small, disfigured and with concentric rings. Sastry and Nayudu[6] reported that the virus producing concentric rings on brinjal induced only necrotic local lesions without becoming systemic on forty-five plant species belonging to the families like Solanaceae, Chenopodiaceae, Cucurbitaceae and Leguminosae. The virus had thermal inactivation point between 65–70° C, the dilution end point between 1/1000 to 1/3000 and longevity *in vitro* for 7 days (28–32° C). Further investigations pertained to the purification of the virus and the losses in yield caused by this virus are presented in this paper.

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FIG. 1. A diseased brinjal plant showing chlorotic rings.