RUBIN NUMBERS

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Introduction

The protective action of a hydrophilic colloid is generally measured by the method of Zsigmondy[2] and is expressed as its "Gold Number". Considerable difficulty is however experienced in the precise determination of Gold numbers. The method of preparation of the Zsigmondy red gold sol is elaborate. Red gold sols prepared by other methods[3] have to be standardised and the methods available are tedious.[1] Closely allied to the gold number is the 'Rubin Number' defined by Ostwald[4] as the amount of the protective colloid present in 100 c.c. of a standard congorubin solution which prevents the red dye turning bluish red when the rubin solution is made 0·16 N with respect to potassium chloride. Lüers[7] has shown that the change in colour can also be brought about by acidifying the solution to pH 5·2. A detailed knowledge of the effect of pH on the colour change in the dye is essential before the rubin number technique can be employed to determine the protective action of colloids. The following investigation was undertaken with a view to find out the optimum conditions for obtaining reproducible rubin numbers.

Experimental

1. *Materials used*

(i) *Congo rubin.*—The Kahlbaum product was purified by the method employed by Doss[5] for the purification of benzopurpurin 10 B. The purity of the dye, as determined by the ignition method, was found to be 99·5%. 0·02% solution was employed.

(ii) Sorensen's acetate buffers were employed for regulating the pH. The pH was determined by using the quinhydrone electrode.

(iii) *Gelatin.*—12–15 g. of "Gold label" gelatine sheets were soaked in 1 M-ammonium sulphate solution for 10–15 mins. This helped to set

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free the chloride normally present in gelatin. Then, the supernatant solu tion was drained off. The gelatin was washed several times with distilled water. It was then dispersed in water and was subjected to dialysis (at the laboratory temperature) for about three weeks until the dialysate gave no test for sulphate. Bacterial growth was prevented by using toluene. The dialysed sol was then filtered through a clean Pasteur-Chamberland filter. The last operation helped to remove the opacity.

(*iv*) *Albumin.*—Merck's pure product was dispersed in water and used.

(*v*) *Casein.*—0·1 g. of casein (Hammersten) was dissolved in about 3 c.c. of N/2 sodium hydroxide and diluted to about 80 c.c. Acetic acid was added to reduce the pH to 5·23 and the solution filtered.

(vi) *Starch.*—Kahlbaum's C.P. quality was used.

2. *Optimum pH for the determination of the rubin numbers*

For the determination of rubin numbers 10% sodium chloride solution was employed, in order to bring the procedure in line with that adopted for gold numbers. The colour change of the dye due to addition of the electrolyte was found to be greatly influenced by the pH of the solution. At high pH values the electrolyte had hardly any effect on the red congo-rubin sol. At very low pH, the congorubin changed to blue without the addition of any electrolyte, and did not show any further change on the addition of the lectrolyte. It was clear from this behaviour, that a certain pH should exist, at which the colour change would be most vivid. Quantitative investigations were carried out to determine this optimum pH.

The intensity of the colour developed by the dye was determined by the method of Gillespie[6] using the Hellige colorimeter. In this technique, the proportion of the blue to the red colour was measured on the assumption that an aqueous solution of pure congorubin was 100% red while the dye in 0·025 N acetic acid was 100% blue. The two solutions (containing 0·0018% dye) could conveniently be used as the red and the blue standards and were found to keep well for several weeks.

3. *Preparation of the test solution*

1 c.c. of the acetate buffer (0·2 M electrolyte concentration) was diluted to 9 c.c. with water and was mixed with 1 c.c. of the stock solution of congorubin. It was noticed that this mixture developed a slight blue colour which attained an equilibrium value within 12 hours. On keeping for 15 hours, 1 c.c. of 10% sodium chloride solution was added to the mixture and the blue colour developed was measured at intervals of (*a*) 3 and (*b*) 30 minutes. Half an hour was found to be

quite adequate for attainment of equilibrium. The results obtained are given in Table I and indicate that a pH of the order of 5·3 is most suitable for the determination of congorubin numbers since at this pH the change of colour due to the addition of sodium chloride is very large and reproducible and the time necessary for the full development of colour is only 3 minutes.

TABLE I

Equilibrium pH of the test solution	% Blue before addition of NaCl	% Blue after the addition of NaCl		Increase in the percentage of blue due to the addition of NaCl
		3 min. value	30 min. value	
4·37	66·7	68·0	68·0	1·3
4·73	43·3	68·3	68·3	25·0
5·09	22·0	60·3	60·3	38·3
5·33	6·0	46·7	46·7	40·7
5·47	3·7	36·7	46·7	33·0
6·10	3·3	26·0	33·3	22·7
Rubin in pure water pH = 6·8	0·0	5·3	17·7	5·3

4. *Optimum concentration of congorubin*

It was found that concentrations of the dye lower than 0·0015% led to large errors in the measurement of colour. When the concentration was of the order 0·008%, the sol coagulated even without the addition of sodium chloride, while with solutions of a concentration higher than 0·003% flocculation occurred on the addition of sodium chloride. There was no such complication in 0·002% solutions, and this concentration of the dye was therefore considered to be most suitable.

For the determination of congorubin numbers, the stock solution of the dye was prepared by mixing 10 c.c. of an acetate buffer of pH 5·2 (0·2 M electrolyte concentration) with 80 c.c. of water and 10 c.c. of 0·04% congorubin solution.

5 c.c. of this stock solution when diluted with an equal volume of the acetate buffer of 5·3 pH (0·02 M electrolyte concentration) used to give a colour which was only 6% blue but which rose to 47% blue, 3 minutes after the addition of 1 c.c. of 10% sodium chloride solution. In determining rubin numbers of protective colloids, the same quantities of the stock solution of the dye and of the buffer were employed and the effect of varying amounts of the protective colloid was studied.

Results obtained with different hydrophilic colloids are given below and are graphically represented in Figs. 1–4.

TABLE II. *Gelatin*

Mg. of geletin used	% Blue colour
0·00	47
1·45	40
2·90	34
3·77	23
5·33	14
7·25	6·0
No colloid and no salt	6·0

TABLE III. *Casein*

Mg. of casein	% Blue colour
0·00	47
0·17	42
0·33	37
0·55	33
0·66	26
0·77	18
0·88	14
1·32	6·0
No colloid and no salt	6·0

TABLE IV. *Albumin*

Mg. of albumin	% Blue colour
0·00	47
0·67	40
1·18	37
1·68	33
2·28	29
2·86	24
3·36	19
3·86	16
4·20	11
4·54	7·0
5·04	6·0
No colloid and no salt	6·0

TABLE V. *Starch*

Mg. of starch	% Blue colour
0·00	47
10·55	45
21·10	42
27·43	37
31·65	30
35·87	23
42·20	18
54·86	6·0
63·30	6·0
No colloid and no salt	6·0

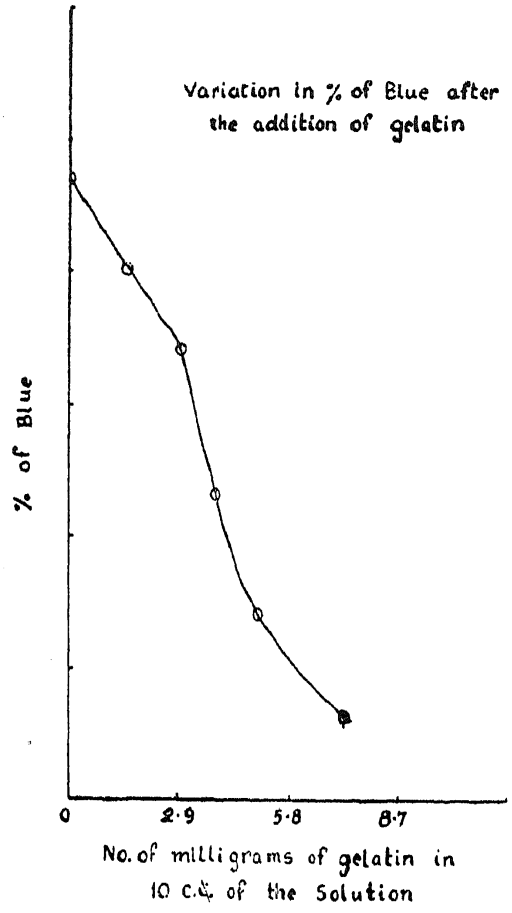

FIG. 1

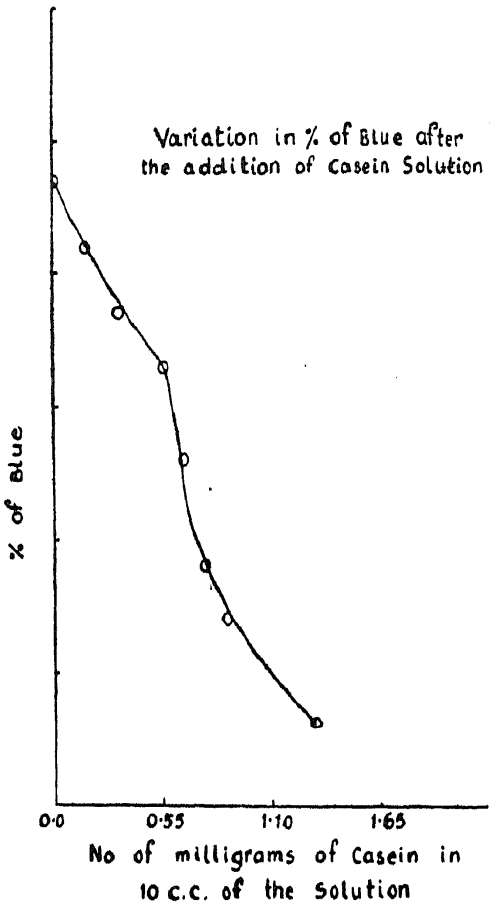

FIG. 2

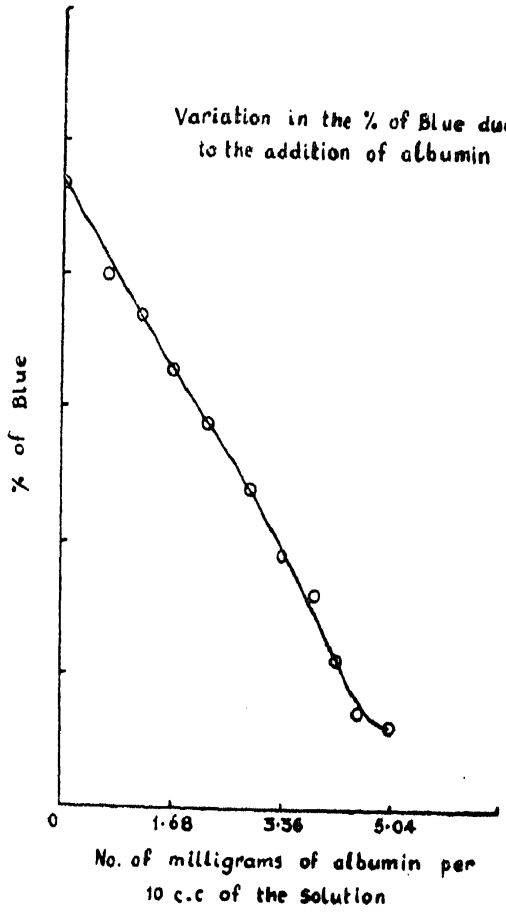

FIG. 3

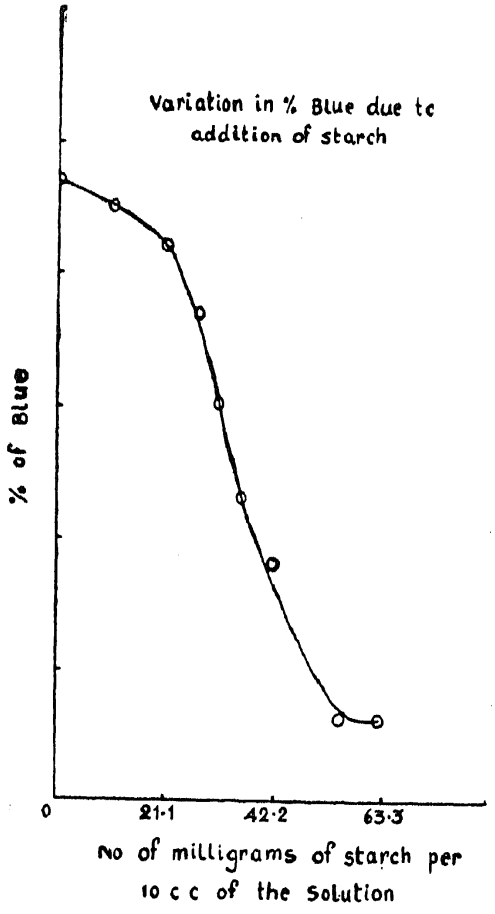

FIG. 4

Discussion

A study of the curves in Figs. 1–4 shows that in the middle portion the slopes of all the curves happen to be maximum indicating a maximum colour change with concentration in this region. As already pointed out, the dye is 47% blue in colour in absence of the protective colloid but the colour drops to 6% blue when an excess of the protective colloid is present. It follows therefore that the region of maximum colour change which is the middle point of the curve corresponds to $\frac{47+6}{2}$ or 26·5% blue. From the above considerations, the rubin number of a protective colloid can best be defined as the number of milligrams of the colloid which develops in the dye solution 26·5% blue under the experimental conditions. The rubin number is thus the weight in mg. of the colloid which when present in 10 c.c. of 0·002% rubin solution of pH 5·3 (electrolyte concn. 0·02 M) causes a colour 26·5% blue, on addition of 1 c.c. of 10% sodium chloride.

The following table gives the rubin numbers of the protective colloids studied.

Table VI. *Rubin Numbers*

Colloid	Rubin No.
Gelatin ..	3·5
Casein ..	0·66
Albumin ..	2·5
Starch ..	34·0

As has already been pointed out, the preparation of reproducible red gold sols is difficult. Preparation of rubin sols however is very easy and the technique employed in determining rubin numbers is comparatively simple. Rubin numbers therefore provide a convenient measure of protective action.

Summary

The best conditions for determining rubin numbers have been worked out. The rubin number is defined as the number of milligrams of the protective colloid which when present in 10 c.c. of a 0·002% congorubin solution of pH 5·2 and electrolyte concentration of 0·02 N produces 26.5% blue colour on addition of 1 c.c. of 10% solution sodium chloride. The rubin number of a protective colloid can be accurately determined with the help of an Hellige colorimeter employing Gillespie's technique.

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