Opacity as a General Measure of Coagulation.

IN the course of a work to be published shortly in the *Indian Chemical Journal*, it has been observed that the *opacity* of manganese dioxide sol increased '*zonally*,' that is, with marked discontinuities, during coagulation, and that this feature became more pronounced the *slower* the coagulation. The curves in Fig. 1 represent but three typical cases observed during *subsequent* work on the

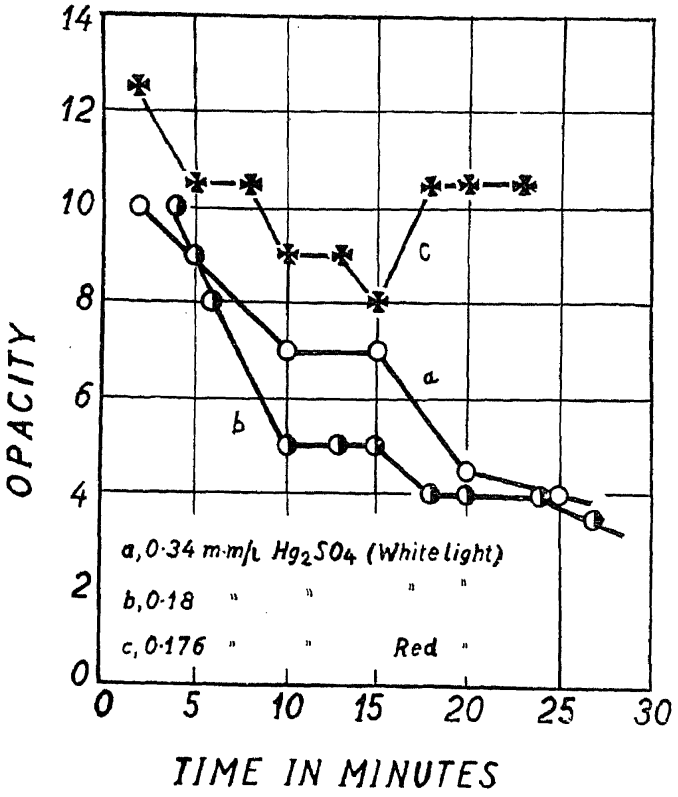

Fig. 1.

kinetics of the same sol, but using a higher colloid content and with solutions of mercurous sulphate as coagulants. These results not only confirm the '*zonal*' change of opacity during coagulation, but show, contrary to expectation from the current theories and general experience, that the opacity has *decreased* during the change. As is usual in these kinetic studies, measurement of the opacity during coagulation was discontinued, as soon as the coagulating system became heterogeneous by flocculation, that is, produced discrete particles of the coagulum sensibly subject to local variations. Curves *a* and *b* refer to experiments made with the white light from a glowing filament; these show that the opacity diminishes with coagulation. Partly different results were noticed when a narrow band, almost monochromatic near H*α*, was employed. Curve *c* is one of the results. It shows that there is an initial diminution of opacity followed by a rise, and that both the stages of the change are marked by the '*zonal effect*'. It has been almost a tacit assumption with colloid chemists that coagulation entails an increase of opacity; the adoption of the last quantity as a measure of the degree of coagulation has had a wide and long usage in the field of coagulation kinetics. It is of considerable interest, therefore, to observe, it would appear for the first time in this line, not only a limitation of the general validity of the above assumption, but of the possibly general discontinuous character of the change, independent of whether opacity and coagulation vary in the same sense or otherwise, as was also noticed previously in respect of changes of viscosity and refractive index, during coagulation.[1]

S. S. JOSHI.
A. PURUSHOTHAM.
Department of Chemistry,
Benares Hindu University,
Benares,
May 14, 1936.

[1] Joshi and Rao, *Curr. Sci.*, 1936, **4**, 481; also *J. Indian Chem. Soc.*, 1936, **13**, 141.

Fractionation of Starch.

A SURVEY of the literature on amylolysis in relation to the structure of starch reveals the existence of two contradictory views supporting either the homo- or hetero-molecular nature of starch. A study of the mutarotation phenomena accompanying the hydrolysis of starch with different amylases led Kuhn[1] to the view that the starch molecule consisted of both *α*- and *β*-glycosidic linkages which were specifically attacked by *α*- and *β*-amylases; the sense of the mutarotation was independent of the substrate but was characteristic of the enzyme. van Klinkenberg[2] from his work on the action of integrally pure *β*-amylase on starch, which hydrolysed a definite fraction (64 per cent.) of the starch substance, advanced the view that the liberation of the *α*- and *β*-maltose was not due to the alternative types of hydrolysis of a single substrate, but rather, to the specific hydrolysis of different components of starch which he designated *α*- and *β*-starches. A critical examination of the question by Hanes[3] has revealed that, while the main conclusions of van Klinkenberg can be confirmed, no

evidence exists to show that the two components pre-exist in the starch substrate, the possibility of their being fragments of a single molecule not having been disproved. Indeed, the recent work of Freeman and Hopkins[4] has led the authors to reject the hypothesis that starch is composite. Our results on the fractionation of starch furnish some evidence on this question.

A 10 per cent. solution of soluble starch (Riedel-E de Haën A-G., A.R., according to Zulkowski) in 30 per cent. solution of calcium chloride was employed for the fractionation of starch. Absolute alcohol was gradually added to the solution under vigorous stirring to a concentration of 20 per cent.; the precipitate obtained was centrifuged and washed three times with a calcium chloride-alcohol mixture of the same composition as that of the mother liquor. The alcohol concentration of the centrifugate was then raised to 50 per cent. when a further quantity of precipitate was obtained. This was separated and treated in the same way as the first precipitate. A further amount of alcohol was added to the centrifugate to raise the proportion of salt solution to alcohol to 1 : 10. The precipitate was separated and washed. The three fractions were repeatedly washed with alcohol and finally extracted in a Soxhlet with absolute alcohol to remove the last traces of the salt.

The specific rotations of the fractions are tabulated below:—

TABLE I.

	Fraction I	Fraction II	Fraction III
$[\alpha]_D$	169·1	170·5	176·7

The three fractions give different colourations with iodine: thus, Fraction I gives a blue colour and Fraction III a reddish colour with hardly any blue in it; the colour given by Fraction II lies between the two. The three fractions are hydrolysed at different rates by Taka diastase and β-amylase prepared from ungerminated barley. The results obtained with β-amylase employing the same quantities of enzyme and starch are graphically represented (Fig. 1).

The difference between the rates of hydrolysis of Fractions I and III is striking. The quantity of starch hydrolysed in 30 minutes from the three fractions which may be taken

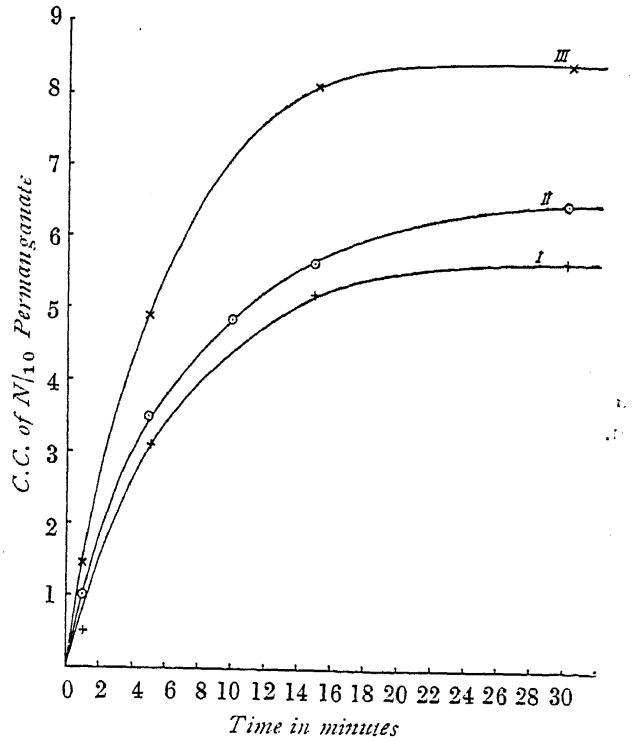

Fig. 1.

Starch 1·0 per cent.; β-amylase 0·5 per cent.

50 c.c. of starch + 10 c.c. of McIlvin's Buffer (pH, 4·8) + 10 c.c. of β-amylase.

10 c.c. used for the estimation of Maltose (Bertrand).

as an indication of their β-amylose contents, are respectively

0·5897; 0·6414; 0·8713 grams.

The results clearly show that by a process of solution and precipitation it is possible to fractionate soluble starch into two components differing with respect to their colour reactions with iodine and β-amylose contents. It can be reasonably assumed that the procedure adopted to effect the fractionation does not bring about any chemical change in the starch, and the conclusion that starch is composite and that the components pre-exist in the starch substance, therefore, appears justified.

Further work on the other characteristics of the fractions is in progress.

B. N. SASTRI.
A. KRISHNAMURTI.

Department of Biochemistry,
Indian Institute of Science,
Bangalore,
June 10, 1936.

1 Kuhn, *Liebigs' Ann.*, 1925, **443**, 1.
2 van Klinkenberg, *Ergebnisse Enzyme forsch*, 1934, **3**, 73.
3 Hanes, *Can. J. Res.*, 1935, **13B**, 185.
4 Freeman and Hopkins, *Biochem. J.*, 1936, **30**, 451.