

REACTION OF SUGARS WITH GELATIN

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Addition of 0.5–3.0 m. mols. of aldo- and keto-sugars per 100 c. c. of 1% gelatin–water-sol gives a definite decrease in p_n values varying with the nature of the sugars in the following order : mannose > glucose = *l*-arabinose = xylose > galactose > fructose > maltose > lactose.

From the study of the electrometric titration curves of the various mixtures of gelatin sol and sugars against standard NaOH solution, it is concluded that the condensation power of each sugar varies with the p_n of the mixture.

Kostychev and Brillant (*Z. physiol. Chem.*, 1923, 127, 224) found a reaction between glucose and amino-acids at 55° in slightly alkaline solutions leading to a diminution of detectable amino group, thus showing a combination of $-NH_2$ group of amino-acid and $-CHO$ group of glucose. Gullard and Mead (*Biochem. J.*, 1935, 29, 397) titrated electrometrically with hydrogen electrode, gelatin sol, in presence of aromatic aldehydes, with NaOH and judged the chemical combination between them quantitatively by the extent of deflection from the pure gelatin curve. In the light of all these results a reaction of $-CHO$ and $-CO$ groups with $-NH_2$ group of amino-acids of aqueous sol is presumed. Further, Frankel and Katchalsky (*ibid.*, 1937, 31, 1595) showed fructose to be inert, whereas Neuberg and Kobel (*Biochem. Z.*, 1925–1928) showed its combination with amino-acids resulting in a controversy.

With a view to ascertaining whether gelatin sol behaves like amino-acids towards sugars (*a*) measurements of the changes in acidity electrometrically with hydrogen electrode and (*b*) potentiometric titrations of mixtures of gelatin and various quantities of different aldo- and keto-monosaccharides (xylose, *l*-arabinose, glucose, mannose, galactose, fructose) and -disaccharides (maltose and lactose) with standard NaOH solution were made.

EXPERIMENTAL

Throughout the investigation Jena glass apparatus and conductivity water, free from CO_2 , were used. Merk's gold-label gelatin from the same packet, extra pure sugars and electrometrically prepared calomel were used. The gelatin sol was prepared as suggested by Shephard and Houck (*J. Phys. Chem.*, 1930, 34, 273). The hydrogen electrode vessel was of Bunker type (1920); it consisted of 1 cm. length of platinised Pt wire.

Hydrogen gas, prepared electrolytically and purified with $KMnO_4$ and NaOH and hot copper filings was used for the H_2 -electrode. Calomel electrode and hydrogen electrode were both placed in a water thermostat at 37° and connected through an agar-KCl bridge. The E.M.F. set up was measured

with a slide wire potentiometer, using a sensitive ballistic mirror galvanometer. The authors found the following precautions with regard to hydrogen electrode greatly helpful:

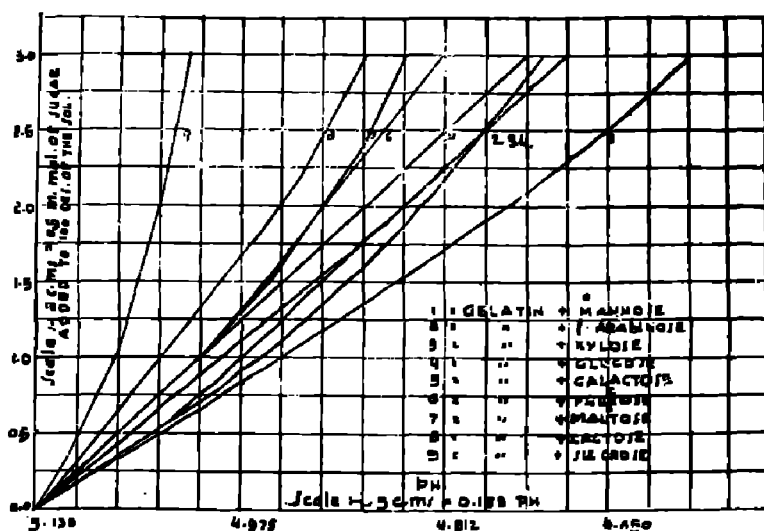
(1) Platinum wire of same length and thickness, platinised for exactly the same time, preserved under CO_2 -free water, was used.

(2) For every experiment newly platinised electrode was used, and occasionally a new electrode was inserted in the middle of the experiment to see that the E. M. F. recorded was the same, and every experiment was repeated till fairly constant results were obtained.

(3) Hydrogen was passed over the electrode for five minutes, before it was allowed to come in contact with the solution.

The influence of addition of various quantities (0.5-3.0 m.mols. per 100 c. c.) of different sugars on the p_{H} of 1% gelatin sol is shown in Fig. 1.

FIG. 1



The electrometric titration curves of 20 c. c. of 1% gelatin sol containing 0.5 m.mol. of various sugars per 100 c. c. against 0.04*N*-NaOH are shown in Fig. 2; the quantities of 0.04*N*-NaOH required to reach the neutral point (p_{H} 7) in case of various sugars are found to be nearly the same.

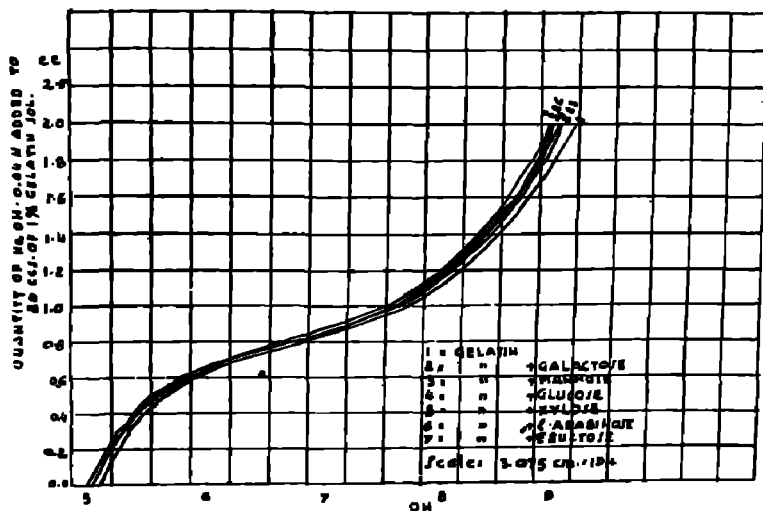
DISCUSSION

Additions of aldo- and keto-monosaccharides to gelatin sol result in a definite decrease of p_{H} of the sol, and with an increase in the concentration of sugar, the p_{H} goes on decreasing as shown in Fig. 1, indicating condensation

of sugar with NH_2 group. Pentoses, xylose and *l*-arabinose, follow practically the same course showing that the condensation power of pentoses with gelatin is nearly the same; the hexo-aldoses condense to different extents, following the order: mannose > glucose > galactose. Condensation power of fructose is less than that of aldoses. Combining both pentoses and hexoses we get the order: mannose > glucose = *l*-arabinose - xylose > galactose > fructose > maltose > lactose.

Potentiometric titration curves for the mixtures of gelatin and monosaccharoses (Fig. 2) are nearly similar to those obtained by Gulland and Mead

FIG. 2



(*loc. cit.*) in case of the mixtures of gelatin with formaldehyde or aromatic aldehydes, where maximum convergence takes place at $6.5 p_H$. The reaction as judged from the shifts in titration plots (not shown here) of various concentrations (0.5-3.0 m. mols) of each sugar from pure gelatin curve, is maximum in the case of first few concentrations and then goes on decreasing; the maximum convergence in case of xylose, *l*-arabinose, mannose, glucose and galactose takes place at 6.69, 6.76, 6.53, 6.78 and $6.7 p_H$ values respectively; plots of fructose are slightly different from those of aldoses, maximum convergence in this case taking place at $6.0 p_H$; the reaction with first 0.5 m. mol. % is much more than any other concentrations.

Addition of aldo-disaccharides of gelatin sol decreases the p_H value of the sol as shown in Fig. 1, though to a much lesser extent than monosaccharides. In the case of non-reducing sugar, saccharose, there is a slight decrease in p_H , perhaps due to decrease in free solvent owing to hydration of sugars, but much less

than that of aldo-sugars, showing that there is practically no condensation of this sugar with NH_2 group of gelatin.

Alkaline potentiometric titration curves (not shown here) for mixtures of gelatin and aldo-disaccharides resemble those in the case of monosaccharides though the shifts in the case of the former are much less than those of the latter. The reaction as judged from the shifts in titration plots (not shown here) of various concentrations (0.5-3.0 m. mols %) from pure gelatin curve, is maximum in the case of first few concentrations and then goes on decreasing; the maximum convergence in the case of lactose and maltose occurs at 6.2 and 6.59 pH values respectively.

Generalising the results, it is concluded that aldo- and keto-sugars condense with NH_2 group of gelatin sol, resulting in an increase in the acidity of the sol, though the condensation power of each sugar varies to a different extent, and that the shifts in the titration curves are true measures of the reaction, since Urban and Shaffers (*J. Biol. Chem.*, 1932, 94, 697) have shown that negligible quantity of alkali is required for neutralisation of sugars. From the increase in the divergence of the titration curves with alkalinity or acidity of the medium, it is concluded that the condensation power of the sugars increases with alkalinity and acidity.

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