Dextran

A. S. AIYAR*

Introduction :

DEXTRAN is a microbiologically produced polysaccharide that has plagued the wine and sugar industries for years, retarding filtration and crystallisation processes. In recent years, it has aroused great interest and has attained a leading position as a plasma volume expander for use in cases of shock and loss of blood.

Vanquelin in 1822 found that cane juice stored in bottles changed to a thick mucilagenous mass during transport[1]. This gummy product was the subject of investigation by many workers and its true composition as consisting of glucose molecules was first described by Scheiber in 1874[2]. He named it dextran due to its similarity with dextrin.

The dextrans are an ill-defined, yet characteristic, group of water-soluble polymers of glucose with molecular weights ranging in millions. They may be roughly divided into two groups one of which consists of long and virtually unbranched chains of α 1:6 linked *d*-glucopyranose units and the other which consists of highly branched dextrans in which short chains of α 1:6 linked units are joined by α 1:4 and α 1:3 branches.

Dextran as plasma volume expander :

Hæmorrhagic shock is primarily caused by a decrease in effective blood volume and the prompt restoration of this volume, even if the composition of the blood be somewhat altered, is important for effecting a reversal of most or all of the deleterious effects[3]. This is best achieved by transfusion of blood or plasma or, lacking these, by fluids known as 'plasma volume expanders'.

Besides the very limited supply of blood and blood plasma, there are other serious limitations to their use e.g.: processing of collected blood must be rapid ; expensive and extensive facilities are required for their storage, distribution, and utilisation ; and, rigorous care must be exercised in matching blood groups. Another complication which may result from the use of plasma is the development of homologous serum jaundice in the recipients. This can be prevented only by a method of sterilisation which will destroy the virus without denaturing the plasma proteins.

Because of these difficulties in the use of blood and blood derivatives, widespread interest has centred around the development of plasma volume expanders. To be efficient in its action, the desirable properties of an ideal plasma volume expander are:

i. Colloidal solute should be retained in circulation until replaced by body proteins ; this implies that it should not be rapidly metabolised or excreted ;
ii. The solution should be similar to plasma in total osmotic pressure, oncotic pressure, and viscosity ;
iii. Composition from batch to batch must be constant within narrow and definable limits of molecular weight range ;
iv. Should be ready for use with ρH range of 6.0 – 8.0 ;
v. Should be eventually metabolised ;
vi. Should be stable during storage and sterilisation ;
vii. Should be crystal clear ;
viii. Must not be pyrogenic or toxic ; and
ix. Must not be antigenic.

*Research student, Food Technology section.

Crystalloid solutions were first tried and were found to be of very limited use as they were not retained in the vessels for any significant period. Milk infusions were next tried but discarded soon as they caused sensitisation. Gelatine-saline solutions of various compositions were in use for a short period before being displaced by gum-arabic-saline solutions which held sway for nearly twenty years and were used extensively during World War I.

Polyvinyl pyrollidine, a synthetic water-soluble polymer was developed as a plasma expander by two German workers, Hecht and Wesse, during World War II. At about the same time, Gronwall and Ingelmann in Sweden discovered the suitability of microbiologically produced dextran as a plasma expander. Since then dextran has gradually gained favour and is at present the foremost blood plasma expander.

Dextran as produced by bacterial action is known as 'native dextran' and is unsuitable for use as an expander directly. A low molecular weight fragment, known as 'Clinical dextran', is obtained by partial hydrolysis of the high molecular weight native dextran. The nature of the bonds present in the dextran molecule is an important factor determining its suitability for use as an expander.

As the α 1:4 bonds (as are present in glycogen) are attacked rapidly and the α-1:6 bonds only very slowly, by the body enzymes, it is essential that the dextran molecule must have a preponderence of α 1:6 bonds over α 1:4 bonds, so that they do not leave the blood stream too rapidly. It has been shown with C^{14} tagged dextran that 92% of the administered dextran could be accounted for in 10 days with 64% excretion in urine, 26% in the expired air as CO_2 and 2% in feces[4].

Clinical dextran has been found to satisfy the conditions for an ideal plasma expander to a degree not approached by any other substance developed and investigated so far. Accordingly, the commercial production of dextran has been stepped up enormously in recent years in many countries, notably the United States of America, where huge stocks have been built up for national emergencies.

Industrial production of clinical dextran :

Raw materials:—Sucrose is the principal raw material used industrially in the form of refined cane sugar.

Cultures: The microorganisms found capable of synthesising dextran include, besides *Lactobacillus mesenteroides, Acetobacter viscosum*[5], *Acetobacter capsulatum*[6] and a fungus *Botrytis cinerea.* However, *L. mesenteroides* seems to be the only strain that produces dextran in sufficient yield for commercial use.

Several strains of *L. mesenteroides* have been tried for their dextran producing ability, the percentages of α 1:6 bonds varying from about 100% to about 60%. The Northern Regional Research Laboratories (NRRL) isolated from a bottle of unpasteurised root beer, which had become viscous, a strain which yield a product with 95% α 1:6 bonds. This strain, given the number NRRL B-512 has since proved to be the standard one used in commercial production procedures.

Mechanism of formation: Hehre[7] isolated an enzyme, that was capable of synthesising dextran from sucrose, from the cell-free extract of *L. mesenteroides.* He termed this enzyme 'Dextran-Sucrase'. 'Dextran-Sucrase' is probably a mixture of enzymes, one of which synthesises the main α 1:6 chain, and the others (transglycolases) which synthesise the α 1:4 and α 1:3 links.

The end products of dextran fermentation were found to be dextran and fructose along with minute amounts of

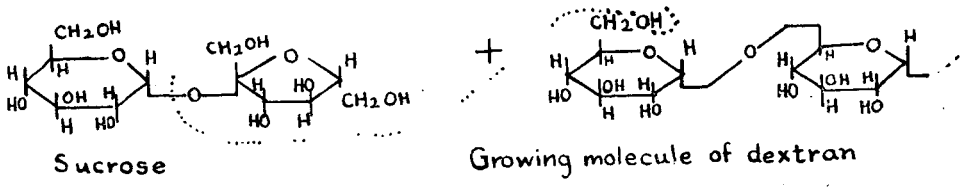
glucose. On the basis of this, Hehre postulated that the formation of dextran from sucrose proceeded according to the equation.

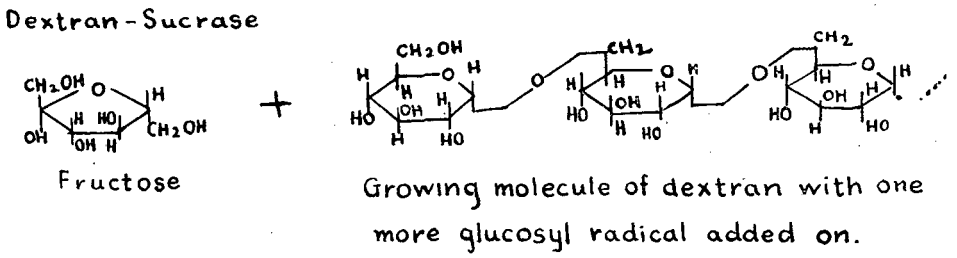
$$n(\text{Sucrose}) \rightarrow \underset{\text{Dextran}}{(\text{Glucose})n} + n(\text{Fructose})$$

The presence of the small amounts of glucose may be accounted for by the hydrolysis of some sucrose into glucose and fructose molecules due to the slight acidic ρH of the fermentation.

For sometime it was believed that phosphorylated sugars like glucose-l-PO_4 played a part in the synthesis of dextran, on the same lines as their participation in the formation of starch and glycogen. This belief was dispelled by Hehre who showed that the dextran-sucrase system does not require any phosphorylated sugar for its function[8].

The growth of the dextran polymer, catalysed by dextransucrase, takes place in a series of steps, each step consisting of the cleavage of the glucosyl radical from a molecule of sucrose and its linking up with the C_6 hydroxyl of the non-reducing, terminal glucose unit of the growing polymer. This process of chain growth continues to virtually complete conversion.





Fermentation: Bixler has described in detail the production of clinical dextran on an industrial scale[9]. The plant consists mainly of the fermentation and the recovery sections. The medium consists of a solution of refined sucrose (about 10%) in water along with small amounts of minerals and nitrogen and vitamin sources.

The medium is sterilised and a part of it goes to the seed tank while the main bulk flows into the fermentor. Contents of the seed tank is used to inoculate the main batch and the fermentation is carried out at about 25°C with good agitation. As fermentation proceeds ρH drops and viscosity increases. Fermentation is stopped when ρH drops to approximately 4.5 (from 24 to 48 hours) and the native dextran is agitated with an equal volume of methanol. Essentially all the dextran is thrown out of solution, while the impurities remain in the supernatant. The precipitated dextran is allowed to settle and the supernatant treated for methanol recovery.

Native dextran is redissolved at 60-70°C in pyrogen-free water and the precipitation procedure repeated. The precipitated dextran is once more dissolved in pyrogen-free water and hydrolysed with hydrochloric acid at

100-105°C in a glass-lined vessel. Viscosity is determined at frequent intervals of time and the hydrolysis is stopped such that after cooling and neutralising, the viscosity will be less than 5.0 cs. The solution is cooled, neutralised with sodium hydroxide, filtered and sent to fractionation vessels. A calculated amout of methanol is added with good agitation such that high molecular weight dextran precipitates and is removed. An additional amount of methanol is added and material with molecular weight in clinical range is precipitated. The fractionation is repeated and clinical dextran from the final fractionating tank is dissolved in pyrogen-free water and deionised. The deionised solution is evaporated to a concentration suitable for spray-drying and then spray dried to get uniform particles of 40 microns size. The powder is dissolved in pyrogen-free water to give 6% W/V and NaCl is added to give 0.9% concentration. The solution is filtered, filled in bottles vacuumised, sealed and then sterilised.

A new process that is likely to revolutionise production methods is one in which *L. mesenteroides* is grown in a manner conducive to good secretion of the extra-cellular dextransucrase[10]. By proper control, it is possible to get dextran with molecular weight predominantly in the clinical range.

Recent work has also shown that by addition either large numbers of small molecules of dextran of approximately 8,000 molecular weight, or high concentrations of maltose as primers, the synthesis can be controlled to give a dextran that requires no hydrolysis and could be used with only minimum fractionation as a plasma expander[11].

In addition to the acid hydrolysis method, enzymatic[12], ultrasonic[13], and thermal[14] hydrolytic methods have also been indicated.

Miscellaneous uses of dextran:

Several ethers, esters and mixed ether-esters of dextran are described as suitable for use in the production of lacquers. The sulfuric acid ester of dextran has been reported to possess an anticoagulant potency comparable to that of heparin. Dextran itself is a good stabiliser for sugar sirups, ice cream and other confections. Its use as a stabiliser for drilling muds for oil and gas wells has also been suggested.

REFERENCES

1. Vanquelin (quoted), **J.Am.Chem.Soc.**, 28, 453 (1906).
2. Scheibler, C. (quoted), **ibid.**, 28, 460 (1906).
3. Martin, L. E., **Chemistry and Industry**, 8, 184 (1955).
4. Scully, N. J., Stavely, H. E., Skok, J., Stanley, A. R., Dale, J.K., Craig, J.T., Hodge, E. B., Chorney, W., Watanabe R., and Baldwin, R., **Science**, 116, 87 (1952).
5. Hehre, E. J., and Hamilton D.M., **Proc.Soc.Exp.Biol.Med.**, 71, 336 (1949).
6. Hehre, E. J., and Neill J. M., **J.Exp.Med.**, 83, 147. (1946).
7. Hehre, E. J., **Science**, 93, 237 (1941).
8. Hehre, E. J., **Proc.Soc.Exp.Biol.Med.**, 54, 240 (1943).
9. Bixler, G. H., Hines G. E., McGhee R. M., and Shurter R. A., **Ind.Eng. Chem.**, 45, 692 (1953).
10. Tsuchiya, H. M., Hellman, N. N., and Koepsell, H. J., **J.Am.Chem.Soc.**, 75, 758 (1953).
11. Koepsell, H. J., Tsuchiya, H. M., Hellman N. N. Kazenko A., Hoffman, C. A., Sharpe, E. S. and Jackson, R. W., **J.BiolChem.**, 200, 793 (1953).
12. Tsuchiya, H. M., Jeanes, A., Brucker, H. M., (eriol.) and Wilham, C.A., **J.Bact.**, 64, 513 (1952).
13. **Chem.Eng.News**, 29, 650 (1951).
14. Wolff, I. A., Watson, P. R., Sloan, J. W., and Rist, C. E., **Ind.Eng.Chem.**, 45, 755 (1953).