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Chromatographic Separation of Glucose and Fructose

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The structures, mutarotation, and the physicochemical properties of glucose and fructose as well as methods for their separation are examined. Their chromatographic separation on cation exchangers in the calcium-form is discussed in detail. A theory of the formation of complexes of carbohydrates with metal cations is described and the mechanism of the separation of glucose and fructose on cation exchangers in the calcium-form is discussed in detail. Factors influencing the chromatographic separation of glucose and fructose on sulphonic acid cation-exchange resins are also considered.
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I. INTRODUCTION

The technology used previously (up to the middle 1970's) in the preparation of fructose was based on the application of a scarce and expensive raw material—inulin, which is a fructose polymer. This prevented the wide-scale use of fructose in the food and medical industry. Only in the last decade the application of chromatographic methods permitted the large-scale manufacture of fructose by its isolation from glucose-fructose syrups obtained either as a result of the inversion of sucrose or by converting glucose with the aid of glucose isomerase.

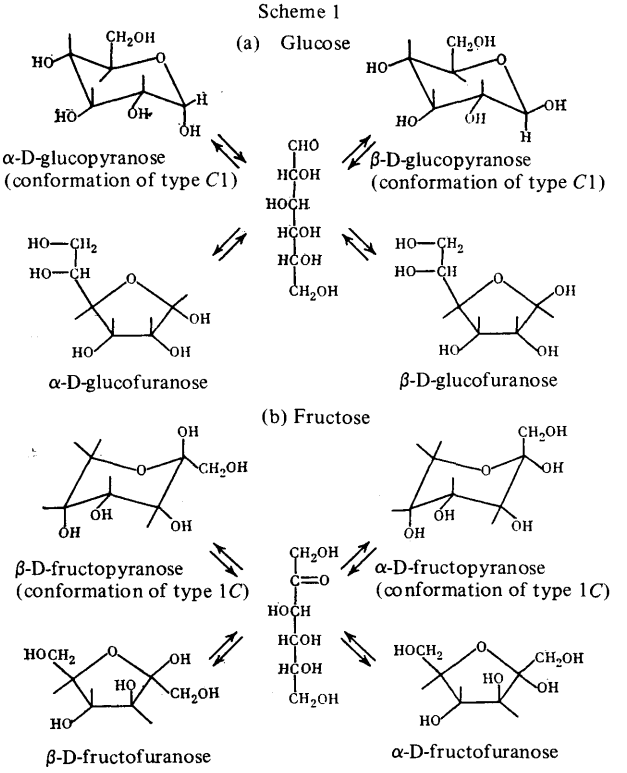
The industrial manufacture of fructose based on the chromatographic separation of glucose-fructose syrups on cation-exchange resins in the calcium-form has already been approved in some countries. The Suomen Sokeri Company [the Finnish Sugar Company] (Finland) is the largest manufacturer of fructose in the world. Its factories in Finland and the USA manufacture about two-thirds of the entire fructose produced throughout the world, i.e. 20000 tonnes annually. France occupies the second place in the manufacture of fructose and is followed by the Federal Republic of Germany and Austria.[1]

In connection with the active development of the technology for the preparation of fructose on the basis of chromatographic methods, it is important to know the properties and characteristics of the behaviour of glucose and fructose under various conditions and also the mechanism of their chromatographic separation.

II. THE STRUCTURES AND PROPERTIES OF GLUCOSE AND FRUCTOSE

Glucose and fructose mutarotate in solution, which leads to the establishment of equilibrium between five structural forms of each of these compounds, as shown in Scheme 1:

Scheme 1



The composition of the equilibrium mixture is influenced primarily by the nature of the solvent and temperature. In the case of D-glucose, the equilibrium mixture in aqueous solution at 20 °C consists of 63.8% of β-D-glucopyranose, 36.2% of α-D-glucopyranose, and trace amounts (0.0026%) of the open-chain acylic structure.[2,3]

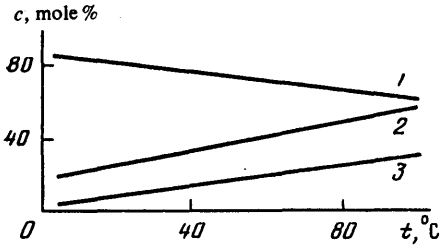
The following equilibrium composition has been established for D-fructose in aqueous solution at 20 °C: 76.4% of β-D-fructopyranose, 19.5% of β-D-fructofuranose, and 4.1% of α-D-fructofuranose.[4]

An increase of temperature leads to a linear increase of the content of fructofuranoses and a corresponding decrease of the content of β-D-fructopyranose.[5] For example, at 0 °C the equilibrium aqueous solution of D-fructose contains 84.8% of β-D-fructopyranose, 11.1% of β-D-fructofuranose, and 4.1% of α-D-fructofuranose, while at 50 °C the corresponding proportions are 55.8, 31.9, and 12.3% respectively.[6]

These data agree with the temperature dependence of the equilibrium composition of a solution of D-fructose in water obtained by Shallenberger[7] (see Figure). Equations permitting the calculation of the content of each form of D-fructose in aqueous solution as a function of temperature t are quoted in the same communication:

$$c_1 = -0.48t + 86.72 ,$$
$$c_2 = 0.26t + 14.32 ,$$
$$c_3 = 0.23t - 0.55 ,$$

where c_1, c_2, and c_3 are the contents of β-D-fructopyranose, β-D-fructofuranose, and α-D-fructofuranose respectively (in mole %).



Temperature dependence of the equilibrium composition of D-fructose in aqueous solution: 1) β-D-fructopyranose; 2) β-D-fructofuranose; 3) α-D-fructofuranose.[7]

The specific rotation of the equilibrium mixture of D-fructose at a temperature t can be calculated from the formula[7]

$$\alpha_D^t = 0.56t - 102.6 .$$

The rate of mutarotation is higher the higher the temperature. At 20 °C in aqueous solution the mutarotation process virtually terminates after 20 min.[4]

The temperature dependence of the equilibrium composition of D-glucose has a somewhat different character than for D-fructose. The contents of α-D-glucopyranose and β-D-glucopyranose are respectively 36 and 64% at temperatures ranging from 5° to 30 °C, while at 80 °C the contents are 46 and 54%.[3,8] Unfortunately there are no literature data on the ratios of glucopyranoses in the temperature range 30–80 °C.

The concentration in aqueous solutions of both glucose and fructose has almost no influence on the equilibrium composition; only a weak tendency towards an increase of the content of α-D-glucopyranose with increasing concentration of D-glucose in aqueous solution has been observed, at least in the concentration range from 5 to 65%.[8,9] Similarly, the increase of the concentration of D-fructose from 5 to 80% entails a slight decrease of the fraction of β-D-fructopyranose and the corresponding increase of the content of fructofuranoses.[9,10] The above trends lead to a difference between the equilibrium compositions of D-glucose and D-fructose by only 3–4%.

The fractions of all the forms present in solution depend on their relative free energies. The "chair" form, in which the anomeric OH group is in the axial position, i.e. the 1C conformation, is more stable for D-fructose.[11,12] β-D-Fructopyranose has this conformation also in the crystalline state. In general the furanose form in which the O(2) and O(3) atoms are in the *cis*-positions is more stable for any ketohexose. In this case the interaction between O(3) and the hdyroxymethyl group is weakened. For this reason, the β-D-fructofuranose form of D-fructose, present in aqueous solutions in greater amounts than the α-D-fructofuranose form, is more stable. The stability of furanoses increases in solution in dimethyl sulphoxide (DMSO), while pyranoses are more stable in water.[12]

The composition of the D-fructose equilibrium mixture at 20 °C is presented in the Table as a function of the nature of the solvent. It permits the conclusion that the content of fructopyranoses increases, while that of fructofuranoses diminishes with increasing solvent polarity. This is partly associated with the hydration of the hydroxy-groups, because the large solvated groups are subject to less steric hindrance when the carbohydrate exists in solution in the form of pyranose in the "chair" conformation and not in the form of the furanose ring. In non-aqueous solvents, this effect is less important, because solvation in such solvents cannot be quite so appreciable as in water, the hydroxy-group occupies a smaller volume, and the advantages of the pyranose conformations compared with the furanose conformations diminish.[14]

The influence of the nature of the solvent on the composition of the equilibrium D-fructose mixture at 20 °C.

Solvent	Content, mole %		
	pyranoses	furanoses	refs.
Dimethyl sulphoxide	18.5	81.5	[12]
Ethanol (90%) + water (10%)	43	57	[2]
Ethanol (80%) + water (20%)	49	51	[2]
Pyridine*	53	42	[13]
Water	76	24	[4,12]

*The solution in pyridine contains 5% of the keto-form.

The majority of aldopyranoses, including glucopyranose, exist in aqueous solution in a conformation of type $C1$, because it is energetically more favourable. For example, the free energy of the $C1$ conformation of α-D-glucopyranose is 2.4 kcal mol^{-1}, while the energy of the 1C conformation is

6.55 kcal mol^{-1}; in the case of β-D-glucopyranose, the free energies of these conformations are 2.05 and 8.0 kcal mol^{-1} respectively.[15] Thus the type *C*1 conformation predominates for D-glucose (see Scheme 1).

The free energy of α-D-glucopyranose is somewhat higher than that of β-D-glucopyranose owing to the axial disposition of the anomeric OH group. For this reason, a smaller amount of α-D-glucopyranose (36%) is observed in aqueous solution compared with β-D-glucopyranose (64%), in which all the OH groups are equatorial.[2] In aqueous solutions D-fructose exists in a type 1*C* conformation[12] (Scheme 1).

The equilibrium aqueous solution of D-glucose has $\alpha_D^{20} = +52.7°$, while for the D-fructose solution under the same conditions we have $\alpha_D^{20} = -92.4°$.[16]

D-Glucose and D-fructose are monosaccharides, which readily undergo chemical changes. Under the influence of bases, they undergo isomeric interconversion via the intermediate enol, accompanied by the formation of mannose. Thus a mixture of D-glucose, D-fructose, and D-mannose is obtained from D-glucose in a very dilute solution of alkali.[14] In aqueous alkaline solutions at higher pH (pH ≥ 9), the sugar forms a complex with the alkali, which is followed by alkaline transformation and decomposition.[17] In a study[18] of the kinetics of the decomposition of fructose and glucose in an alkaline medium on heating, it was noted that fructose decomposes appreciably faster than glucose.

In general, fructose is chemically much less stable than glucose. Thus heating of an alkaline solution of D-fructose at pH 8 and 80 °C for 20 min leads to the development of an appreciable yellow colour in solution and, as the pH is raised, the intensity of the colour rises rapidly.[19]

The study of the transformation of sugars in solution in pyridine showed that the double bond can migrate via the enediol mechanism along the entire carbon chain. The same mechanism constituted the basis of a wide variety of reactions of monosaccharides, in particular their cleavage by strong acids and bases.[20] Glucose and fructose, especially glucose, are very resistant to the action of acid. Thus, under mild conditions, the interconversion of the sugars is possible, while under severe conditions dehydration, leading to the formation of furfural and related compounds, takes place.

Heating of D-glucose in dilute sulphuric acid solution at 100 °C leads to the formation of D-fructose, D-arabinose, and small amounts of D-mannose and D-xylose. Under the same conditions, fructose undergoes the aldose–ketose isomerisation.[21]

The products of the conversion of D-glucose by various dilute acids are D-fructose, D-mannose, isomaltose, gentiobiose, etc. The mixture composition depends on the nature of the catalyst employed.[22] Analogous results have been obtained in another study,[23] where, however, it was emphasised that oligomers are formed only in 10% solution of glucose when a 0.01–0.2 *N* solution of hydrochloric acid is used.

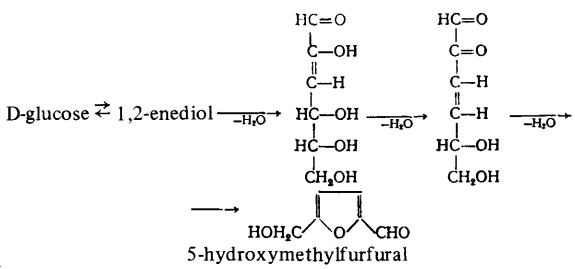
Under more severe conditions, the dehydration becomes the dominant process. The dehydration of hexoses in an acid aqueous medium leads to the formation of not only 5-hydroxymethylfurfural but also of 2-hydroxyacetylfuran.[24] In addition difuryl ether may be formed.[25]

Fructose undergoes condensation in an acid medium mainly at moderate temperatures (~60 °C) and pH 1.3–3.5 with formation of levanbiose, inulobiose, and other products.[26] At pH ≥ 3.9, D-fructose is dehydrated without the formation of 5-hydroxymethylfurfural, while at pH > 4.5 it isomerises to D-glucose.[27]

Thus the conversion of glucose and fructose in alkaline and acid media can result in the formation of a wide variety of products whose composition depends on the pH of the medium, the reaction time, temperature, the nature of the catalyst, and the concentrations of the monosaccharide and catalyst.

The dehydration of D-glucose on heating in aqueous solution can be described by the scheme[28]

Scheme 2

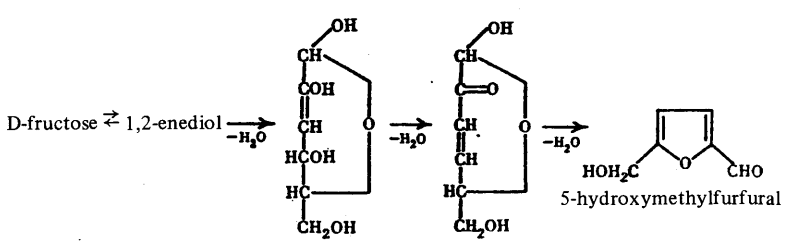


5-hydroxymethylfurfural

A 2,3-enediol, which is ultimately converted into 2-hydroxyacetylfuran, can also be formed from D-glucose.

Dehydration of D-fructose on heating in aqueous solution can be described by the scheme[29]

Scheme 3



5-hydroxymethylfurfural

D-Fructose reacts chemically extremely readily, which has been demonstrated by Shamgar and Leibowitz.[30] After D-fructose has been kept in absolute ethanol at room temperature for some time, methylglyoxal, difructosoanhydrides, diheterolevulosans, β-methyl fructopyranoside, and two glucose derivatives are detected in solution after a time. Thus, under these conditions, D-fructose undergoes anhydridisation, isomerisation, dimerisation, and decomposition into fragments, each containing three carbon atoms. D-Glucose undergoes no changes whatever under the same conditions.[30]

In a cold aqueous solution, fructose is 1.6 times sweeter than sucrose; however, in hot aqueous solution the sweetness of fructose diminishes appreciably. It has been shown[2,4] that the sweetness of fructose depends on the ratio of the tautomeric forms. Fructofuranoses have almost no sweet taste, the carrier of which is in fact β-D-fructopyranose. If the sweetness of sucrose is assumed to be 100%, then that of β-D-fructopyranose is 180–200% and that of fructofuranoses is 0%. The sweet taste of β-D-fructopyranose is determined by the presence of three-component groups incorporating the anomeric hydroxy-group, the oxygen atom in a primary alcoholic group, and the ring methylene carbon atom.[31] Since it has been shown[7] that the amount of β-D-fructopyranose diminishes with increase of temperature and is accompanied by the corresponding increase of the amount of furanoses, the weakening of the sweet taste of fructose in a hot aqueous solution becomes understandable.

III. METHODS OF SEPARATION

There exist many methods for the separation of glucose–fructose mixtures. It is possible to distinguish five main groups of methods.

1. Methods based on dialysis using ion-exchange membranes.[32,33] These methods require the application of very complex equipment and also the expenditure of much electrical energy.[33] For this reason, they are extremely expensive.

2. Methods based on chemical reactions with one of the mixture components (glucose or fructose). Here use is made of the oxidation of glucose to gluconic acid with subsequent precipitation of the latter by calcium salts and its removal from fructose solution,[34,35] the formation of hydrazones from one of the sugars and their subsequent hydrolysis,[36,37] the interaction of the sugars with metal carbonyls and subsequent hydrolysis of one of the resulting products,[38] the formation of chemical compounds of phenol-containing resins with glucose,[39] etc. The fructose obtained by these methods requires careful purification and glucose, which is a valuable sugar, is converted into waste.

3. Methods based on the differential crystallisation of glucose and fructose. Many such methods exist. For example, the following procedures are used: cooling of the invert syrup with subsequent introduction of glucose seed crystals, the crystallisation of glucose, and its separation from the fructose solution;[40] the formation of a complex of glucose and sodium chloride, which crystallises from the solution;[41] preliminary differential crystallisation of glucose and fructose with subsequent separation on a cation exchanger;[42] differential crystallisation in methanol using the difference between the crystallisation temperatures of glucose and fructose.[43,44] However, completely pure glucose and fructose cannot be obtained by these methods.

4. Methods based on the isolation of fructose in the form of complexes with a calcium compound, for example $Ca(OH)_2$ [45–47] or $CaCl_2$.[48–52] These methods are relatively ineffective owing to the low yield of the final product (10–20%).

5. The group of ion-exchange chromatographic methods, which can be in its turn subdivided into three main subgroups:

(a) Chromatographic separation of glucose and fructose on zeolites, including zeolite X, containing K^+ cations and capable of selective adsorption of glucose;[53–56] on a zeolite containing NH_3^+, K^+, Na^+, Ca^{2+}, and Ba^{2+} cations and capable of the selective adsorption of fructose;[57] on zeolites X, Y, or L containing the Li^+, Na^+, K^+, Cs^+, Be^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, etc. cations[58–60] and also on a mineral adsorbent of the "Sorbex" type.[61]

(b) Separation of glucose and fructose on anion-exchange resins. The separation is usually carried out at 40–60 °C on anion-exchange resins, usually of the Dowex I type, in the bisulphite- or sulphite-form, using water as the eluent.[62–72] However, the employment of an anion exchanger in the above forms results in the presence of SO_3H^- and SO_3^{2-} ions in the glucose and fructose obtained, because these are readily eluted by water.[65] In order to avoid the presence of these impurities, it is necessary to employ additional purification columns with the anion exchanger in the OH-form, which entails additional dilution of the sugar solutions. A mixture of an anion exchanger in the bisulphite-form with 5% of a cation exchanger in the sodium- and hydrogen-forms has been used,[77] which made it possible to improve the separation of the glucose–fructose mixture.

(c) The separation of glucose and fructose on cation-exchange resins. Sulphonated polystyrene resins cross-linked by divinylbenzene (DVB) and containing alkali metal or alkaline earth metal cations are usually employed. Other forms are also used. When glucose and fructose were separated on the Dowex 50 × 4 cation-exchange resin in the Ba-form, the glucose and fructose peaks were found to be markedly diffuse, on the Ag-form a weak separation was observed, while on the Sr-form a fairly effective separation was achieved.[78] The use of the sodium-form of the cation exchanger[79] does not allow the separation of the glucose-fructose mixtures, but partial separation of mono-, di-, and tri-saccharides was noted. Comparison of the efficiencies of the various forms of cation-exchangers leads to the conclusions that the Ca^{2+} cation is most suitable for the separation of glucose and fructose. The same conclusion follows from the study of Mountfort.[80]

Cation-exchange resins in the Ca-form with a degree of cross-linking equivalent to 2–8% of DVB are used in most studies.[81–97] The eluent is water, the separation is carried out at 50–70 °C, and the concentration of the syrup introduced amounts to 50% and above in terms of the dry substances. The resin is normally employed in the form of 0.2–0.5 mm beads and an attempt is made to employ as narrow a fraction as possible. An important factor, which influences the efficiency of the chromatographic separation, is the density and uniformity of the packing of the resin bed in the column. A study[98] has been devoted to this question and a method has been proposed whereby a dense packing of the resin in the Ca-form with a bead size of 0.3–0.6 mm can be achieved. It is based on the ability of sulphonated polystyrene resins to swell in a concentrated $CaCl_2$ solution (20%) to a lesser extent than in water. A suspension of the resin in calcium chloride solution is supplied into the column and the resin is then washed with water.

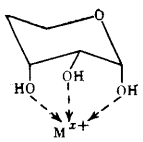
Cation-exchange resins of the Dowex 50 type have been used,[81–98] but other cation exchangers, for example Duolite C20 × 4,[99] Amberlite IR-120B,[100] Permutit Q,[111] and Duolite C25[102] have also been employed in the calcium-form under conditions analogous to those indicated above.

Semicontinuous processes have been used in a number of investigations[103-107] for the separation of glucose and fructose: the "modelled moving bed" method[103] on a sulphonic acid cation-exchange resin in the Ca-form; the "fluidised bed" method[104] on a strongly acid cation-exchange resin in the Ca-form; the "cyclic zonal separation" method[105] on an aminoethylcellulose derivative; the "moving inlet" method[106] on a cation-exchange resin in the Ca-form. A procedure has also been developed in which use is made of a column containing alternating reaction and adsorption zones.[107] Glucose then isomerises in the reaction zone to fructose, which is adsorbed in the adsorption zone.

IV. PHYSICOCHEMICAL ASPECTS OF THE CHROMATOGRAPHIC SEPARATION OF GLUCOSE AND FRUCTOSE

Fructose, glucose, sucrose, and other carbohydrates form 1:1 molecular complexes with bases. Reducing sugars, which include glucose and fructose, give rise to more stable complexes than non-reducing sugars, for example, sucrose.[108-110] Metal cations have different complex-forming capacities, which depend on their valence and size. Thus, according to Dangre,[111] univalent metal cations can be arranged in the following sequences in terms of their complex-forming capacities: $Na^+ > K^+ > Li^+$ for glucose and $K^+ > Na^+ > Li^+$ for fructose. The sequences of bivalent cations are as follows: $Sr^{2+} > Mg^{2+} > Ca^{2+} > Ba^{2+}$ for glucose and $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$ for fructose. However, not all carbohydrates form complexes with cations in aqueous solution and the complexes themselves have different stabilities. It has been possible to demonstrate by NMR and electrophoretic methods that complex formation with metal cations is possible in the presence of three OH groups in favourable steric positions. This situation arises when three neighbouring OH groups are arranged in the sequence axial–equatorial–axial (*a–e–a*).[112-115]

On the basis of NMR and electrophoretic data, Goulding proposed in 1975[116] his theory of the formation of complexes of carbohydrates with metal cations. The most stable complexes are formed in the presence of the *a–e–a* sequence of three neighbouring OH groups in the pyranose ring or the *cis,cis*-sequence of three OH groups in the furanose ring:



a–e–a

The stability constants K_S of such complexes are in the range 1–5 $mole^{-1}$. $K_S = 0.1$ mol^{-1} for the *a–e*(*cis*) sequence. The *e–a–e* sequence gives rise to a less stable complex. Finally the *e–e* sequence is even less favourable than the *a–e* sequence and complexes are not formed at all for the *a–a* sequence. Hence follows an important conclusion: the greater the number of favourably oriented pairs of OH groups in the carbohydrate molecule, the more stable the complexes. For example, α-D-glucopyranose and α-D-fructopyranose each have one pair of OH groups in the *a–e* sequence, β-D-fructopyranose has two pairs, while β-D-glucopyranose has no such pairs.

In relation to allose, it has been demonstrated[112] that the complexes of furanoses are always less stable than those of pyranoses.

The optimum radius of the cation for complex formation is ~1.0 Å. The greater the charge of the cation, the easier the formation of the complex: the sizes of Na^+, Ca^{2+}, and La^{3+} are 0.97, 0.99, and 1.02 Å respectively.[112]

It follows from the data of Goulding,[116] who determined the capacity coefficients of sugars (k') on the Aminex A-5 cation-exchange resin with a particle size of 11 ± 2 μm using different cations, that fructose forms fairly stable complexes with K^+, Ag^+, Tl^+, and Ca^{2+} and slightly less stable complexes with Sr^{2+}. On the other hand, on the cation exchanger containing the K^+, Ag^+, and Tl^+ cations, the values of k' for glucose are also fairly high, which means that, when these cations are employed, it is not possible to achieve a satisfactory separation of glucose–fructose mixtures. Since strontium is toxic, one may conclude that the most suitable cation for the selective complex formation with fructose in the presence of glucose is that of calcium.

The mechanism of the separation of glucose and fructose is most probably based on the presence in β-D-fructofuranose of two *cis*-OH groups attached to the five-membered ring.[117] The two pairs of OH groups in the *a–e* sequence in β-D-fructopyranose have hardly any significant influence on the separation, because they can ensure the formation of only very weak complexes with the cation. Glucose, for which only α-D-glucopyranose among all the possible isomers has one pair of OH groups in the *a–e* sequence, forms an even weaker complex. In the course of separation on the column, the sugars mutarotate. The binding of β-D-fructofuranose to calcium shifts the mutarotation equilibrium in the liquid phase. The faster this process, the narrower the peak of the fructose eluted.

An analogous phenomenon takes place also with glucose. Under favourable conditions, where the mutarotation is slow, the glucose and especially fructose peaks can be appreciably diffuse, which can be explained by the fact that β-D-glucopyranose is eluted initially, is followed by α-D-glucopyranose, then β-D-fructopyranose, and finally β-D-fructofuranose. The available data, demonstrating an appreciable narrowing of the carbohydrate peaks with increase of temperature, may serve as confirmation of this finding.[83,84,118] In the case of fructose, an increase of temperature accelerates the mutarotation process itself in the first place and shifts the equilibrium towards furanose in the second place.[7] However, another factor then begins to operate—the stability of the complex of fructose with the cation; an increase of temperature diminishes the stability of the complex. In order to achieve the optimum separation, it is essential to select a temperature at which the optimum combination of the stability of the complex, the rate of mutarotation, and the composition of the initial equilibrium mixture is achieved. Furthermore, there are other factors, which will be described below.

The presence of H^+ and also OH^- ions sharply accelerates the mutarotation and also facilitates the separation of glucose and fructose.[92] It is of interest to note that, when the cation exchanger is converted into the calcium-form by a standard method with a 10% solution of calcium chloride, part of the resin is bound to remain in the H^+-form (3–5%). If the resin is fully converted into the Ca-form (and this is possible at an elevated temperature or when an alkaline solution of calcium chloride is used), then the separation of glucose and fructose is impaired. This demonstrates the possibility of mutarotation processes in chromatographic columns. Apart from the H^+ and OH^- ions, triethyl- and trimethyl-amines cause a sharp acceleration of the mutarotation. The addition of very small amounts of triethylamine to the eluent (water) narrows the fructose and especially glucose peaks.[119] The effect of triethylamine can apparently be explained by the fact that it makes the solution weakly alkaline and this accelerates sharply the mutarotation.

It has also been noted that the stability of the complexes of sugars with metal cations is influenced by the nature of the eluent. The stability constants of the complexes in ethanol are known to be appreciably higher than in water;[120,121] however, owing to the similarity of the partition coefficients of glucose and fructose, it is usually not possible to achieve their satisfactory separation in ethanol.

The employment of anion-exchange resins for the separation of glucose–fructose mixtures leads to undesirable transformations in the individual sugars.[122,123] The decomposition of glucose and fructose with formation of glycolic and citric acids is actually observed on strongly basic anion-exchange resins, for example on Amberlite IRA-400.[124]

The decisive process in chromatography on granular materials, including cation-exchange resins, is diffusion of the sugar within the resin particles,[125] which depends in its turn on the flow rate of the mobile phase, temperature, the resin grain size, the degree of cross-linking of the resin, and the nature of the solvent and the sugar.

An increase of temperature accelerates the diffusion,[126] but, since it entails the narrowing of the peaks of the components separated, it does not always improve the separation of the mixture, since k' diminishes at the same time.[127] On the other hand, at low temperatures sugars are adsorbed more strongly and the resolution can therefore be improved, provided that the influence of the decrease of the rate of mass transfer is small and the influence of mutarotation is suppressed.[119] It has been noted[117,128] that a decrease of temperature improved the separation of glucose and fructose on a cation-exchange resin in the Ca-form.

One of the important factors in the chromatographic separation of sugars is the flow rate of the mobile phase. It is limited by the diffusion of sugars within the resin particles.[129–131] At a high flow rate of the mobile phase, the peaks of the substances being separated become asymmetric, and long "tails" appear, their presence being explained by the fact that a proportion of the sugars continue to diffuse from the resin particles into the mobile phase.

A decrease in the resin particle size increases the rate of mass transfer. For example, the change in the bead size of the Dowex 21(K) resin from 45–75 to 15–40 μm improved the separation of glucose, sucrose, and raffinose by a factor greater than 3, causing a sharp narrowing of the peaks of the test substances.[129] The greater efficiency of the Dowex 50 × 8 cation-exchange resin in Ba-form[132] compared with the Ca-form[133] can be explained by the smaller size of the resin particles employed in the former case (0.038–0.075 mm compared with 0.15–0.30 mm). A relation between the cation exchanger bead size and the yield of pure fructose has been observed:[134] the smaller the bead size, the greater the yield. Furthermore, the employment of narrower resin bead fractions causes an additional improvement in the separation of the test substances, which is apparently largely related to a more uniform packing of the beads in the bed. The use of a resin with a large bead size necessitates the employment of very low flow rates of the mobile phase, owing to the low rate of mass transfer in the large resin beads, and precludes the attainment of a satisfactory separation of the glucose–fructose mixture.[135]

Since sugar molecules have a complex steric configuration and in order to achieve complex formation with the active centre of the cation exchanger they must assume a definite position which ensures the formation of bonds of approximately equal length between the metal and the complex-forming OH groups of the carbohydrate,[114,136] it is clear that the steric structure of the cation-exchange resin must influence the chromatographic separation. The steric structure of the cation exchanger is in its turn determined by the degree of cross-linking. The degree of cross-linking of the cation exchanger must not exceed a definite limit.[79,84,88,90,92,101,137] On the other hand, analysis of the above studies leads to the conclusion that the degree of cross-linking should not be below a definite limit. The most favourable conditions are apparently ensured by a degree of cross-linking by DVB of 4–8%. The use of cation-exchange resins with a lower degree of cross-linking, for example of the KU-2-2(Ca) cation-exchange resin, in which the degree of cross-linking is 2%,[138] has been shown to[137] lower the efficiency of the separation of glucose and fructose. The causes of this are not altogether clear, but the main deficiency of cation-exchange resins with a low degree of cross linking (2% of DVB and below) is their low mechanical strength.

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Thus, in devising various technologies for the manufacture of fructose, based on chromatographic methods for the separation of glucose–fructose mixtures on cation-exchange resins, account must be taken of several most important factors.

Firstly, glucose and especially fructose are chemically extremely unstable carbohydrates and any operations with these sugars require mild conditions (pH 5–7, temperature $\leq$60 °C). Secondly, calcium cations are the most suitable for the selective complex formation with fructose in the presence of glucose and the mechanism of their separation on cation-exchange resins in the Ca-form is most likely to be based on the formation of a relatively stable complex of β-D-fructofuranose with calcium. Thirdly, the degree of cross-linking of the cation-exchange resin, equivalent to 4–8% of DVB, apparently ensures the optimum separation of glucose and fructose. Finally, when the cation-exchange resin is treated with a solution of calcium chloride, an attempt to achieve its conversion into the 100% of the Ca-form should not be made; not less than 3–5% of the sulpho-groups of the cation-exchange resin should remain in the H-form.

In optimising the conditions for the chromatographic separation of glucose and fructose, it is essential to take into account the fact that an increase of process temperature lead in this instance not only to the acceleration of the mass exchange of the carbohydrates within the cation exchanger beads but also to a shift of the mutarotation equilibrium of the sugars in the liquid phase and to a decrease of the strength of the complexes of the monosaccharides with calcium. A significant increase of the efficiency of the separation of glucose and fructose can be attained by employing cation exchanger beads with the smallest possible size. The latter makes it possible to improve the resolution of the glucose and fructose peaks, to increase the flow rate of the mobile phase, and to increase the amount of charge applied to the column.

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