Facilitated Transport of Saccharides through a Supported Liquid Membrane Containing a Neutral Lipophilic Resorcinarene Carrier

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Summary: The facilitated transport of several types of saccharidess has been studied across a supported liquid membrane containing a resorcinarene carrier. The rate-determining step is believed to be the migration of a carrier-saccharide-water ternary complex in the organic solvent. The transport kinetics obey a saturation law, that allows the calculation of the stability constants of the various complexes. The stability constants are related to the saccharide structures : important factors are chain length, configuration and substitution of key hydroxyl groups.

Introduction

The design of novel processes for the efficient utilization of renewable, natural raw materials has now become a tremendous challenge for chemists. In the current context of fear of a global economic crisis due to shortage of energy and/or chemicals derived from fossil fuels, most efforts are being devoted to the design of processes that perform the transformation of the biomass into key compounds for the chemical industry.

In this respect, natural saccharides, that are afforded in large quantities by agriculture, appear to be essential starting materials, because they are highly functionalized and contain many chiral centers for elaborate synthesis reactions. However, an important problem about the use of cheap natural sources of polysaccharides such as wood, straw, gums, corncobs, seaweed, and pectic substances, is that preliminary processing usually yields an aqueous mixture of various simple saccharides, the separation of which is not an easy task. Crystallization of pure sugars from aqueous solutions is known to be a poorly reproducible technique.

A useful method for purifying sugars is liquid-liquid extraction. For this purpose, the addition of a suitable extracting agent (the carrier) in the organic layer is necessary, since most unprotected saccharides are not soluble in organic solvents. The carrier must

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be able to form complexes with the sugars to be extracted. Ideally, if the complexes formed with various sugars had different stabilities, one could expect selective extraction of sugars from a mixture. Recovery of the purified sugar(s) is achieved through double extraction : a feed mixture is first extracted by an organic solvent, then the pure sugar is recovered from its complex by a second extraction with an aqueous receiving or strip phase.

A drawback of liquid-liquid extraction techniques is the use of large quantities of volatile organic solvents, which is not admissible in view of current regulations. However, the volume of solvent can be dramatically reduced by employing supported liquid membranes (SLMs),[1,2] in which a small volume of solvent containing the carrier is immobilized into the pores of a hydrophobic microporous membrane named the support. The other advantages of SLM processes are those of most membrane processes: they are continuous processes that require small investment and function costs, because they operate at ambient temperature and pressure. The use of SLM for removal of chromium from seawater has recently reached the industrial stage.[3]

A scheme of facilitated (or carrier-mediated) transport of a carbohydrate S by a carrier T in a SLM is represented in Figure 1, showing the "catalytic cycle" of the carrier in the organic phase.

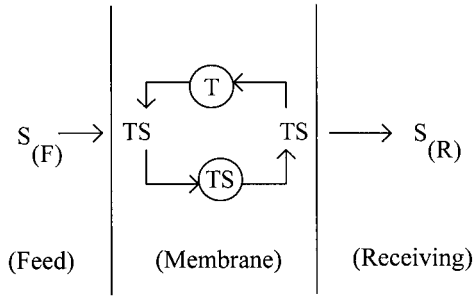

Figure 1. Scheme of the facilitated transport of a carbohydrate S by a carrier T.
TS is the complex. T and TS are soluble only in the organic phase.

Various SLM systems using boronic acid derivatives as carriers for the transport of carbohydrates have been reviewed by Smith.[4] However, carbohydrate boronate complexes are only formed in alkaline medium and require the use of aqueous solutions containing a buffer that is difficult to eliminate during the final recovery step of the separated sugars. This is why, on the basis of previous reports on the use of macrocyclic

carriers in SLMs,[5] we investigated a new concept by using as carrier the neutral, lipophilic carrier represented in Figure 2. The trivial name of this macrocycle, "resorcinarene", recalls that it is related to the calix[4]arene family. In a resorcinarene, the phenol units are replaced by resorcinol (1,3-dihydroxybenzene) units that are linked by CHR groups attached at the 4,6 positions.[6,7] Here, the R substituents are n-undecyl groups that provide the four lipophilic "tails" necessary for solubility in organic solvents. The four benzene rings form the "calix" that is "crowned" by eight hydroxyl groups. Two adjacent HO groups borne by neighboring benzene rings form a complexing site that can bind a small molecule that contains hydroxyl groups. Therefore, the carrier can bind up to four water or small carbohydrate molecules.

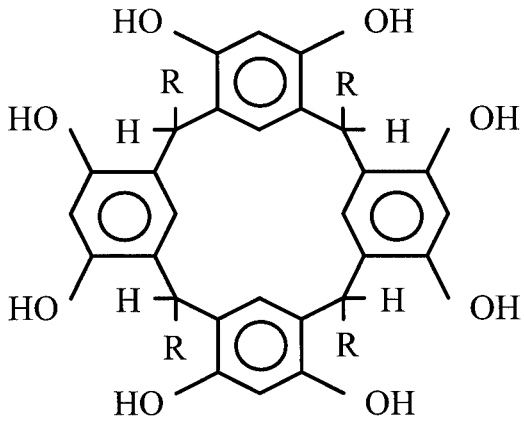

Figure 2. Structure of the resorcinarene. R = $C_{11}H_{23}$.

Aoyama et al.[8-10] reported the synthesis and use of this compound for the selective extraction of sugars into dry organic solvents. Later, we reported that extraction also occurs in solvents in contact with water, allowing the adaptation of the technique to the liquid membrane technology.[11] The aim of this work is to report some results of a research program aiming at the design of a membrane process for the separation of mixtures of carbohydrates.

Experimental Part

All chemicals were of the highest available grade. The transport cell (Figure 3) is made of two compartments of equal volumes (V = 100 mL) separated by the membrane. The cell is immersed into a thermostated bath (T, 298 K). The solutions in both compartments are stirred with magnetic bars, using a Variomag apparatus. The SLM support was a microporous PTFE film (Goodfellow) of thickness 63 µm. Characteristic values are porosity 84 % and pore size 0.45 µm. The membrane area available for diffusion was 19.6 cm^2 (diameter, 5.0 cm). The SLM was made by soaking during 15 hours a square portion of the polymer film (8 cm × 8 cm), into a 0.01 M solution of resorcinarene in pure carbon tetrachloride. After insertion in the cell, the SLM was equilibrated with pure water placed in both compartments for at least 12 hours.

Initially, the feed compartment contained the carbohydrate solution (c_0 = 0.05-1.0 M) and the receiving compartment contained pure water. Small samples (v = 0.5 mL) of the receiving phase were withdrawn at known times. These samples were analyzed using a HPLC apparatus equipped with a 30-cm Phenomenex Rezex column in calcium form, maintained in an oven at 85 °C. The flow rate of eluent (pure water) was 0.6 mL/min. The pump was a Shimadzu LC-9A model. Detection was achieved with a Varian RI-4 refractometer. For calibration of the system, standards of known concentration were injected with each run of a series of samples. The carbohydrate concentrations were determined by analyzing chromatographic data with Varian Star software. All experiments were duplicated and were reproducible within 3% accuracy. Calculations were made with the Sigmaplot 4 software.

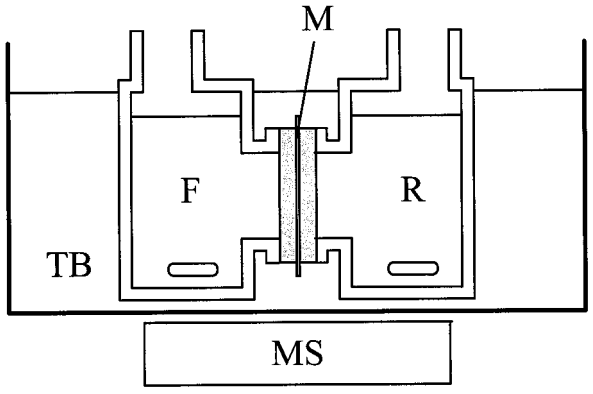

Figure 3. Scheme of the transport cell. M is the SLM, F is the feed phase, R is the receiving phase, TB is a thermostatted bath and MS is a multiple magnetic stirrer.

Results and Discussion

The transport of various types of carbohydrates through the proposed SLM was investigated. We checked that transport does not occur in the absence of carrier. Moreover, a long induction period (several hours) is observed when transport experiments are carried out immediately after preparation of the membrane. However, this induction period is suppressed when the SLM has been equilibrated with water prior to use, showing that it is due to the formation of the carrier-water complex in the organic phase. In agreement with Aoyama's finding that the carrier can complex up to four water molecules; this suggests that the migrating species are indeed carrier-carbohydrate-water ternary complexes.

Kinetics of transport

The results for some aldoses and alditols have been published.[12,13] Typical experiments were carried out with four different initial concentrations of sugar c_0 in the feed phase. The transport is monitored by determining the concentration c_R of sugar in the receiving phase as a function of time t. The concentration in the feed phase is c_F.

$$c_F = c_0 - c_R. \quad (1)$$

S is the area of the SLM, l is the membrane thickness and V is the volume of the aqueous phases. P is the permeability of the SLM. J is the flux of sugar across the membrane. The flux is related to the permeability according to Fick's First Law :

$$J = P\Delta c/l = P(c_F - c_R)/l = P(c_0 - 2\,c_R)/l \quad (2)$$

The transport rate is related to the flux :

$$dc_R/dt = JS/V \quad (3)$$

The kinetic relation of c_R vs t is :

$$\text{Ln}\; c_0/(c_0 - 2\,c_R) = 2\,(P.S/l.V)\,t \quad (4)$$

The permeability P was calculated from the slope of the corresponding plot. The values of P for $c_0 = 0.10$ mol $\cdot$ L^{-1} are given in Table 1.

Since uncomplexed carbohydrates cannot cross the SLM, the overall flux of sugar S is equal to the flux of complex TS in the organic phase. The diffusion of the complex through the membrane is assumed to be the rate-determining step of the transport.

Formation of the complex TS is a fast reaction at the membrane-water interface:

$$\text{T (org)} + \text{S (aq)} \rightleftharpoons \text{TS (org)} \quad (5)$$

where (org) and (aq) refer to the organic phase and aqueous feed phase, respectively.

The concentration $[TS]_i$ of complex at the interface obeys the law of mass action, in which K is the stability constant of the complex.

$$[CS]_i = K[C]_i[S]_i \quad (6)$$

$[T]_i$ is the concentration of the carrier in the organic phase and $[S]_i$ is the concentration of the sugar in the feed phase, both near the interface.

The flux of complex TS in the organic phase is given by Fick's Law. D^* is the apparent diffusion coefficient of the complex.

$$J = (D^*/l).[TS]_i \quad (7)$$

Because the sugar S is in excess in the feed phase, $[TS]_i << [S]_i$ and $[S]_i = c_0$.

The overall $[T]_0$ concentration of the carrier, immobilized in the SLM, is constant, but the free carrier T is in equilibrium with the complex TS:

$$[T]_0 = [T]_i + [TS]_i = [T]_i(1 + K[S]_i) \quad (8)$$

The concentration of free carrier $[T]_i$ is given by:

$$[T]_i = [T]_0 /(1 + K[S]_i) = [T]_0 /(1 + K[S]_t) \quad (9)$$

Finally, the flux J is obtained as:

$$J = (D^*/l).K[T]_i[S]_t = (D^*/l).[T]_0 K[S]_t/(1 + K[S]_t) \quad (10)$$

The initial flux J_i is that observed for $[S]_t = c_0$

$$J_i = Pc_0/l \quad (11)$$

The above mechanism requires that J_i is proportional to the initial concentration of carrier $[T]_0$ and obeys a saturation law with respect to c_0. In order to test the proposed relationship, the equation was linearized as a Lineweaver-Burk plot:

$$1/J_i = (l/D^*[T]_0K)(1/c_0) + l/D^*[T]_0 \quad (12)$$

Plots of the values of $1/J_i$ vs $1/c_0$ fitted good linear relationships in every case. From the slopes and intercepts of these plots, the apparent diffusion coefficients and stability constants of the complexes were calculated (Table 1) by linear regression analysis :

K = intercept/slope and $D^* = l/[T]_0$.intercept.

Stability of the SLM

The use of supported liquid membranes is often hampered by lack of long-term stability. In many cases, the organic solvent is slowly replaced, in the pores of the support, by water from the feed or receiving phases. Sometimes also, carriers that are slightly soluble in water are slowly extracted from the organic phase. In all cases, the liquid membrane is eventually destroyed and cannot transport its substrate any longer.

The origin of instability in SLMs has been discussed elsewhere.[14] The problem is so serious that solvent-free membranes have recently been designed, instead of SLMs, for the carrier-mediated transport of sugars such as sucrose, fructose and glucose. For example, plasticized cellulose triacetate membranes containing boronic acid derivatives as carriers have been patented for the separation of mixtures of carbohydrates.[15]

The SLM studied in this work represents an ideal case, since the carbohydrates are almost insoluble in the organic phase, whereas the carrier is insoluble in aqueous medium, avoiding thus its washing-out from the organic layer. The high stability of this SLM has been demonstrated for up to 20 days in the transport of sugars or alditols, without any observation of leaking. Another advantage of this system is that only facilitated transport of the sugars takes place, as passive diffusion of sugars cannot be detected in the absence of the carrier. Another interesting feature is that the SLM is permeable for oligosaccharides as well as for monosaccharides.

Finally, a specific advantage of the neutral resorcinarene carrier over arylboronic acids used in previously reported membranes[4,15] is that the complex-forming reaction does not depend on the pH. In view of application to separation processes, it is important that the receiving solution should not contain other species (such as buffers) than the transported sugar.

Relationship between permeability and carbohydrate structure

The permeabilities of the SLM to various carbohydrates are related in Table 1 to the contributions of two properties of the corresponding complex : the stability constant (a thermodynamical factor) and the apparent diffusion coefficient (a kinetic factor).

A rough correlation, which was not unexpected, is found between the size of the carbohydrate molecule and the apparent diffusion coefficient of its complex. Besides, the influence of the stability constant on the permeability seems rather complicated.

The stability constant of a carrier-carbohydrate complex is probably proportional to the number of interactions between the two molecules. Thus, since the carrier has been shown to possess four sites[8,9] that each can bind a diol group from a sugar molecule, large sugars may be expected to interact with two sites rather than with a single site.

A study of the variations of the stability constants of the complexes of alditols versus the structures of the alditols[13] suggested that the preferred site of binding is an internal diol site (i.e. a -CHOH-CHOH- system). The low reactivity of terminal CH_2OH groups is usually ascribed to an entropy effect, since such groups would lose several degrees of

freedom when engaged in bonding. Comparison of erythritol and threitol shows that the erythro complex is stronger than the threo one. Moreover, the differences found between the pentitols show that an *arabino* site forms stronger complexes than a *ribo* or *xylo* site. This finding explains why the three investigated hexitols form complexes of equal stabilities, comparable to that of the arabinitol complex, since all of them possess an *arabino* system : galactitol (HO-2,3,4), mannitol (HO-2,3,4) and glucitol (HO-3,4,5).

Table 1. Permeabilities (P), stability constants (K), and apparent diffusion coefficients (D^*) of the carrier-carbohydrate complexes at $T = 298.15$ K.

Carbohydrate	$P \cdot 10^{7}$ a)	K	$D^* \cdot 10^{4}$
	$cm^2 \cdot s^{-1}$	$mol^{-1} \cdot L$	$cm^2 \cdot s^{-1}$
Erythritol	13.75	1.58	1.00
DL-Threitol	18.89	0.81	2.53
D-Arabinitol	13.53	1.58	0.99
Ribitol	7.09	0.68	1.11
Xylitol	8.38	0.46	1.91
Galactitol	5.22	1.53	0.39
D-Glucitol	7.11	1.48	0.550
D-Mannitol	10.22	1.46	0.89
D-Arabinose	10.5	0.18	5.95
D-Glucose	13.53	0.20	6.97
Methyl β-D-glucopyranose	13.70	0.37	3.79
Methyl α-D-glucopyranose	11.74	0.36	3.33
Sucrose	9.91	0.58	1.85
Melezitose	11.40	2.84	0.52
Cellobiose	7.86	3.00	0.36
Maltose	14.11	4.02	0.45
Maltitol	6.87	1.80	0.45

a) Initial concentration in the feed phase: $c_0 = 0.10$ mol $\cdot$ L^{-1}. Sucrose is β-D-fructofuranosyl-α-D-glucopyranoside. Melezitose is *O*-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl-α-D-glucopyranoside. Cellobiose is 4-*O*-β-D-glucopyranosyl-D-glucose. Maltose is 4-*O*-α-D-glucopyranosyl-D-glucose. Maltitol is 4-α-D-glucopyranosyl-D-glucitol.

Finally, if the intuitive idea that a good complexing diol site should possess two syn hydroxyl groups is accepted, then it follows that alditols would be preferably complexed in the sickle conformation rather than in zigzag conformation.

The complexes of aldoses (e.g. arabinose and glucose) are weaker than those of alditols. The reason may be that aldoses are present in solution mainly as pyranoses, and that in such stiff cyclic forms, the hydroxyl groups suitable for complexation are not as free as in the acyclic alditols. Accordingly, both cyclic methyl glucopyranosides form complexes with small stability constants. Since the pyranosides cannot exist in acyclic form, it rules out that the glucose complex could involve the acyclic aldehydo form. Moreover, the α-and β-glucopyranosides have similar stability constants, showing that the configuration of the anomeric MeO group is not essential for binding with the carrier. Nevertheless, the pyranoside complexes are stronger than the glucose complex, which may be due to the lipophilic character of the methoxy group.

The transport of oligosaccharides, namely melezitose (a trisaccharide) and various disaccharides containing glucose units, was studied and the results were compared to those for the parent monosaccharide, e.g. glucose. First, it should be noted that permeabilities to large sugars are not much smaller than those of glucose or glucopyranosides, whereas the apparent diffusion coefficients are clearly smaller for oligosaccharides. On the contrary, the stability constants of the oligosaccharide complexes are larger than those for monosaccharides. This difference strongly supports the hypothesis that because of their extended size, large sugars can interact with several sites of the carrier, whereas small sugars only interact with a single site. An exception to this rule is the case of saccharide, a disaccharide that forms a weak complex, suggesting poor interaction with the carrier. In saccharide, the glucopyranosyl unit is probably bonded in the same way as other glucopyranosides, and the fructofuranosyl unit is expected to interact with low intensity by the trans-3,4-diol system. A comparison of sucrose and melezitose is interesting, since the trisaccharide melezitose contains a β-D-fructofuranose unit substituted at O-2,3 by two α-D-glucopyranosyl moieties (it can be represented as a O-3-substituted saccharide). In melezitose, the disubstituted fructose ring does not possess enough free adjacent HO groups for bonding with the carrier (only HO-1,4 are unsubstituted). In this case, the strong interaction with the carrier would only take place through the two glucopyranosyl units separated by the deactivated fructose unit.

The melezitose complex is weaker than those of disaccharides such as maltose and cellobiose. This may be due to the fact that both disaccharides are bonded to two sites of the carrier through their two adjacent glucopyranose units. Here, contrary to the case of methyl glucopyranosides, the different orientations of the anomeric substituent (α for maltose and β for cellobiose) result in stability differences.

The complex of maltitol is weaker than that of maltose. This result is surprising at first sight because maltitol possesses a glucitol moiety that should complex with the carrier more readily than the glucose moiety of maltose. However, it must be considered that the postulated binding site of glucitol is HO-3,4,5. In maltitol, O-4 of the glucitol moiety is no longer available for bonding since it is attached to a glucopyranosyl entity. Therefore, we believe that maltitol binds with the carrier through the glucopyranosyl unit and the threo 2,3-diol system of the glucitol moiety, resulting in a weaker complex than with maltose.

Separation of mixtures of carbohydrates

The separation of mixtures of small alditols has been reported elsewhere.[13] Moderate selectivity was noticed, as expected because the permeabilities to the components were very close. Thus, new experiments were made with several mixtures of methyl α-D-glucopyranoside (MGP) and alditols of different chain lengths that present more differences in their permeabilities (Table 2). When the feed phase contains a single carbohydrate, the SLM is more permeable to MGP than to alditols.

Table 2. Transport of mixtures of methyl α-D-glucopyranoside (MGP) and alditols.

Carbohydrate[a)]	c_0 (feed phase)	$P \cdot 10^7$	s[b)]
	mmol · cm^{-3}	cm^2 · s^{-1}	
MGP (s)	0.10	11.74	
Galactitol (s)	0.10	5.22	
Ribitol (s)	0.10	7.09	
MGP / Galactitol (m)	0.10 / 0.10	10.09 / 8.97	1.13
MGP / Ribitol (m)	0.10 / 0.10	14.33 / 15.76	0.91
Ribitol / Galactitol (m)	0.10 / 0.10	11.69 / 9.06	1.29
MGP/Ribitol/Galactitol (m)	0.10 / 0.10 / 0.10	11.56 / 12.41 / 11.70	

[a)] Conditions are the same as in Table 1. T = 298.15 K. (s) :single solute. (m) : mixture. c_0 is the initial concentration of carbohydrate(s). P is the permeability.
[b)] s (selectivity factor) is the ratio of permeabilities, i.e. $s = P(\text{MGP})/P(\text{alditol})$.

In a MGP-galactitol mixture, the permeability to MGP decreased while that to galactitol increased. In addition, in a MGP-ribitol mixture, the permeability of ribitol was increased and this pentitol was transported faster than MGP. In a mixture of ribitol and galactitol, both permeabilities of ribitol and galactitol increased, whereas the order of permeabilities was not reversed. However, in all these mixtures, a reasonable selectivity was observed. The most disappointing result was obtained for a ternary mixture MGP-ribitol-galactitol, in which the three compounds displayed similar permeabilities.

Obviously, such differences in the transport behavior of carbohydrates in their mixtures may reflect important mechanistic changes and warrant further studies. At present, we suspect that two main mechanisms operate :

- competition for the carrier (present in small concentration) may take place between two carbohydrates, depending on their relative affinities to the carrier. In this case, the permeability to one constituent (or both) is expected to *decrease* in the mixture.
- formation of mixed complexes containing a carrier molecule and several molecules of different carbohydrates may occur. This is possible because the carrier may bind with up to four sugars simultaneously. In this case, the permeabilities to these carbohydrates are expected to become *equal*. Moreover, if the mixed complex has a high mobility, the permeabilities of one or several sugars may be *enhanced* in the mixture.

If the latter hypothesis was confirmed by experiments that are currently carried out in the laboratory, it would suggest that the design of a carrier containing a smaller number of complexing sites might improve the transport selectivity.

Conclusion

A SLM containing a neutral resorcinarene carrier transports mono- and oligo-saccharides at different rates. The transport is due to the migration of a lipophilic carbohydrate-carrier-water complex through the organic phase. The stability constants and apparent diffusion coefficients of the various complexes have been determined and related to the structures of carbohydrates (aldoses, alditols, and glucopyranosides). The remarkable stabilities and the large permeabilities of the proposed SLM open a new perspective on membrane processes for the separation of aqueous mixtures of sugars.

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