High fructose syrup production using fructose-selective liquid membranes

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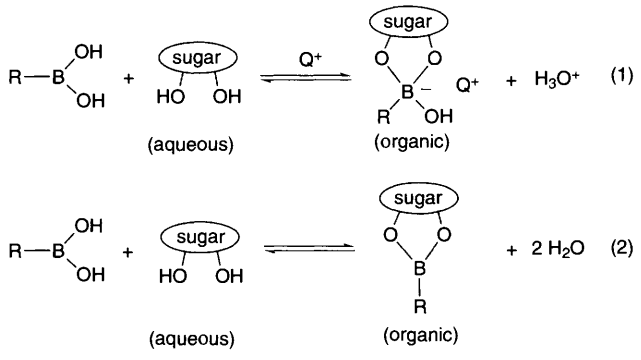
Fructose is separated from a fructose–glucose mixture using a polymer-supported liquid membrane containing a boronic acid carrier; addition of immobilized glucose isomerase to a solution of glucose in the source compartment of a liquid membrane transport cell produces a receiving phase mixture that is more than 80% fructose.

Worldwide production of high fructose syrup (HFS) is currently around 8×10^9 kilograms per year.[1] The most common process for HFS production from corn involves four major steps:[2,3] (*i*) wet milling the corn to extract the starch; (*ii*) enzymatic or acid hydrolysis of the starch to produce a feed stream that is about 94% glucose; (*iii*) enzymatic isomerization of the glucose to fructose – since the enzymatic equilibrium constant for this process is approximately one, the maximum achievable fructose concentration is around 42%;[4] (*iv*) if necessary, chromatographic enrichment of the syrup to produce a higher concentration of fructose.[5] This report deals primarily with the last step in this production sequence.

Fructose is the sweetest of all naturally occurring carbohydrates (relative sweetness ratings: fructose 128; sucrose 100; glucose 67).[2] In many applications (*e.g.* beverages) it is necessary to increase the sweetness of glucose–fructose syrup to the level of sucrose. This means that the fraction of fructose in the syrup has to be raised by chromatographic enrichment to 55% (HFS-55).[5] Here we describe a new method of producing HFS using fructose-selective liquid membranes. The method is based on our discovery that polymer-supported liquid membranes[6–8] containing boronic acid carriers are able to separate fructose from a fructose–glucose mixture.

Ten years ago, Shinbo and co-workers reported that a carrier mixture of phenylboronic acid and Aliquat 336 (which is predominantly trimethyloctylammonium chloride) was capable of transporting monosaccharides through a bulk liquid membrane (BLM).[9] Since no transport was observed in the absence of Aliquat or when the pH was below 9 (the pK_a for phenylboronic acid is 8.86), it was postulated that the equilibrium responsible for transport was as depicted in eqn. (1). Despite the precedence that boronic acids alone do not



transport monosaccharides through BLMs,[9] we find that they are effective monosaccharide carriers in supported liquid membranes (SLMs) at neutral pH.

SLM transport fluxes were determined using an apparatus that has been described before.[10] The liquid membrane was 2-nitrophenyl octyl ether supported by a thin, flat sheet of microporous polypropylene.‡ The appearance of glucose or fructose in the receiving phase was monitored by enzymatic methods.§ In the absence of carrier, the background glucose and fructose fluxes through the membrane were barely detectable (1×10^{-9} mol m^{-2} s^{-1}). When the liquid membrane contained boronic acid **1**[12] (50 mM or 2% wt) the glucose and fructose permeabilities increased substantially (entry 2 in Table 1). Raising the carrier concentration further had little effect on glucose flux, whereas fructose transport was enhanced in a linear fashion.¶ With a membrane carrier concentration of 250 mM and both aqueous phases at pH 7.3, the flux for fructose was 12 times higher than that for glucose (entry 3). On the basis of our previous work, the transport enhancement is attributed to eqn. (2).[14] Strong evidence in favour of this interpretation is the observation that facilitated transport is eliminated when the source phase pH is raised above the boronic acid pK_a (entry 4). The transport selectivity for fructose over glucose reflects the known order of sugar–boronate stability constants.[15]

When the SLM contained Aliquat alone, a significant and quite reproducible flux was observed for both glucose and fructose (entry 5). There are two possible explanations for this effect. Either the Aliquat is acting as an anion exchanger and is transporting the deprotonated monosaccharide,[16] or it is simply

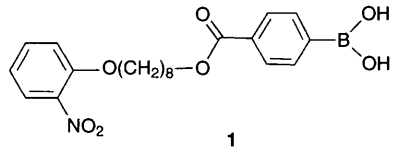


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Table 1 Non-competitive transport of fructose and glucose through SLMs containing different carriers[a]

Entry	Carrier[c]	Aqueous phase pH[d]	Flux (10^{-8} mol m^{-2} s^{-1})[b] Glucose	Fractose
1	None	7.3	0.1	0.1
2	**1** (50 mM)	7.3	3.8	6.2
3	**1**	7.3	7.2	83
4	**1**	Gradient[e,f]	0.4	0.4
5	Aliquat	7.3	14	11
6	Aliquat	Gradient[e]	19	14
7	**1** + Aliquat	7.3	7.0	84
8	**1** + Aliquat	10	9.0	17
9	**1** + Aliquat	Gradient[e]	35	110

[a] Source phase: sodium phosphate (100 mM), fructose or glucose (300 mM); liquid membrane: carrier(s) dissolved in 2-nitrophenyl octyl ether supported by a flat sheet of Accurel (16 cm^2); receiving phase: sodium phosphate (100 mM); room temperature. [b] Initial flux extrapolated to $t = 0 \pm 10\%$. [c] Unless stated otherwise, each carrier component was 250 mM in 2-nitrophenyl octyl ether. [d] pH in source and receiving phases. [e] Source phase pH 10, receiving phase pH 6. [f] There was no evidence of carrier leakage into the aqueous phases as determined by UV spectroscopy.

extracting the neutral sugar into the SLM. Evidence against the first explanation is the observation that the transport fluxes did not increase much when a basic source phase was used (entry 6). On the other hand, there is literature precedent in favour of the second explanation. It is known from phase transfer catalysis chemistry that Aliquat can extract neutral hydrophilic compounds (including associated ion pairs) into organic phases.[17] In addition, Coterón *et al.* recently reported that tetrabutylammonium chloride associates with glycosides in chloroform solution to form hydrogen bonded complexes (K_{assn} ~ 500 dm^3 mol^{-1}).[18] Thus, it appears that Aliquat transports the monosaccharides through the SLM by forming lipophilic hydrogen bonded aggregates.

When the membrane contained a carrier mixture of boronic acid **1** and Aliquat, the observed fluxes at pH 7.3 were similar to those obtained with **1** alone, *i.e.* a twelve-fold enhancement of fructose over glucose (entry 7). Increasing the pH of the source and receiving phases to 10 produced little change in glucose flux and greatly decreased fructose flux (entry 8). Our previous experience suggested there was a problem with slow fructose release from the membrane into the receiving phase.[19] This was verified when a pH gradient was used, which produced the highest fructose and glucose fluxes recorded (entry 9; note however that the fructose–glucose ratio has dropped to three). Overall, it appears that the carrier mixture of **1** and Aliquat operates by a combination of boronate transport pathways, eqn. (1) and eqn. (2), as well as the Aliquat extraction pathway; the fraction that each pathway participates depends on the experimental conditions.

Competitive transport experiments were conducted using a source phase containing equal amounts (150 mM) of glucose and fructose. Under these competitive transport conditions, carrier **1** at pH 7.3 exhibited an eighteen-fold transport selectivity in favour of fructose (entry 1 in Table 2). A carrier mixture of **1** and aliquat at pH 7.3 transported fructose 12 times faster than glucose (entry 2). When this carrier mixture was tested using a pH gradient, both fluxes increased as expected; however, the transport selectivity decreased to five-fold in favour of fructose (entry 3).

With a fructose-permeable membrane in hand, it was a logical progression to combine this separation step with the enzymatic glucose isomerization. Immobilized glucose isomerase (Taka-Sweet™, 1 g, 150 units)[4] was added to a solution of glucose in the source compartment (300 mM, 50 ml, pH 7.3) of a transport cell operating at 50 °C, and the fructose levels in both aqueous phases were monitored over time. After 5 h with an SLM containing carrier **1** (250 mM), the source phase had reached its equilibrium position of 50% fructose, whereas the fraction of fructose in the receiving phase was greater that 80%. Thus, glucose isomerization and fructose enrichment have been converted from two discrete steps into a single operation.

Table 2 Competitive transport of fructose and glucose through SLMs containing different carriers[a]

Entry	Carrier[c]	Aqueous phase pH[d]	Flux (10^{-8} mol m^{-2} s^{-1})[b]	
			Glucose	Fructose
1	**1**	7.3	2.9 (2.6)[e]	55 (32)[e]
2	**1** + Aliquat	7.3	4.6	57
3	**1** + Aliquat	Gradient[f]	17 (2.4)[e]	91 (31)[e]

[a] Source phase: sodium phosphate (100 mM), fructose (150 mM), glucose (150 mM); liquid membrane: carrier(s) dissolved in 2-nitrophenyl octyl ether supported by a flat sheet of Accurel (16 cm^2); receiving phase: sodium phosphate (100 mM); room temperature. [b] Initial flux extrapolated to $t = 0$ ± 10%. [c] Unless stated otherwise, each carrier component was 250 mM in 2-nitrophenyl octyl ether. [d] pH in source and receiving phases. [e] Repeated run with the same membrane after it had been washed for 20 h. [f] Source phase pH 10; receiving phase pH 6.

We have described a novel method of preparing HFS using a semi-permeable liquid membrane to separate the fructose produced by enzymatic glucose isomerization. In principle, it should be possible to continuously remove fructose from a glucose–fructose isomerization reaction and drive the isomerization equilibrium towards more complete fructose production.

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Footnotes

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‡ The transport cell consisted of two identical, water-jacketed cylindrical halves (half-cell volumes 50 ml) that were stirred by turbines, driven by externally situated magnets. The membrane was a flat sheet of Accurel™ (thickness 0.1 mm, area 16 cm^2) that supported a solution of carrier (30 μmol) dissolved in 2-nitrophenyl octyl ether (0.12 ml).

§ Glucose concentrations were determined using a coupled hexokinase–glucose-6-phosphate dehydrogenase assay which produces an NADPH absorption at 340 nm.[11a] The concentrations of fructose, or fructose plus glucose, were obtained by including phosphoglucose isomerase in the glucose assay.[11b] Initial fluxes were calculated after extrapolating to $t = 0$. All runs were repeated at least once.

¶ One explanation why fructose flux has a stronger dependence on carrier concentration than glucose involves a difference in sugar–boronate stoichiometries. It is known that glucose forms a 1 : 2 bisboronate, whereas there is evidence that fructose can form a 1 : 4 tetrakisboronate.[13]

References

1 S. Vuilleumier, *Am. J. Clin. Nutr.*, 1993, **58**, 733S.
2 L. M. Hanover and J. S. White, *Am. J. Clin. Nutr.*, 1993, **58**, 724S.
3 *Starch Hydrolysis Products: Worldwide Technology, Production and Applications*, ed. F. W. Schenck and R. E. Hebeda, VCH, New York, 1992.
4 V. J. Jensen and S. Rugh, *Methods Enzymol.*, 1987, **136**, 356.
5 J. R. Teague and E. C. Arnold, *Sugar Azucar*, 1983, 18.
6 M. Igawa, T. Sekimoto and H. Okochi, *J. Membr. Sci.*, 1995, **98**, 177.
7 H. C. Visser, D. N. Reinhoudt and F. De Jong, *Chem. Soc. Rev.*, 1994, **23**, 75.
8 E. L. Cussler, in *Membrane Separation Systems. Recent Developments and Future Directions*, ed. R. W. Baker, E. L. Cussler, W. Eykamp, W. J. Koros, R. L. Riley and H. Strathmann, Noyes Data Corp., Park Ridge, 1991, ch. 4.
9 T. Shinbo, K. Nishimura, T. Yamaguchi and M. Sugiura, *J. Chem. Soc., Chem. Commun.*, 1986, 349.
10 T. B. Stolwijk, E. J. R. Sudhölter and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1987, **109**, 7042; W. Van Straaten-Nijenhuis, F. De Jong and D. N. Reinhoudt, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 317.
11 (*a*) S. C. Kinsky, *Methods Enzymol.*, 1974, **32**, 501; (*b*) H.-O. Beutler, in *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, Verlag, Weinheim, 3rd edn., 1983, vol. 6, 321.
12 M.-F. Paugam, J. T. Bien, B. D. Smith, L. A. J. Chrisstoffels, F. De Jong and D. N. Reinhoudt, *J. Am. Chem. Soc.*, in the press.
13 C. D'Silva and D. Green, *J. Chem. Soc., Chem. Commun.*, 1991, 227; J. C. Norrild and H. Eggert, *J. Am. Chem. Soc.*, 1995, **117**, 1479.
14 B. D. Smith, *Supramol. Chem.*, 1996, **7**, 55 and references cited therein; P. R. Westmark and B. D. Smith, *J. Pharm. Sci.*, 1996, **75**, 266; P. R. Westmark, S. J. Gardiner and B. D. Smith, *J. Am. Chem. Soc.*, in the press.
15 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769; T. D. James, K. R. A. Samankumara Sandanayake and S. Shinkai, *Supramol. Chem.*, 1995, **6**, 141.
16 Y. Shigemasa, S. Okamoto, H. Sashiwa and H. Saimoto, *Chem. Lett.*, 1990, 433.
17 E. V. Dehmlow and S. S. Dehmlow, *Phase Transfer Catalysis*, Verlag Chemie, Weinheim, 2nd edn., 1983, 17.
18 J. M. Coterón, F. Hacket and H.-J. Schneider, *J. Org. Chem.*, 1996, **61**, 1429.
19 G. T. Morin, M. P. Hughes, M.-F. Paugam and B. D. Smith, *J. Am. Chem. Soc.*, 1994, **116**, 8895.

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