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The determination of sugars in beverages and medicines using on-line dialysis for sample preparation

Gillian M. Greenway & Nsanyi Kometa

School of Chemistry, University of Hull, Hull HU6 7RX, UK

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A method is described for the analysis of sugars in beverages and medicines using on-line dialysis for sample preparation. The design, construction and method development for the on-line dialysis is described. The dialysis is carried out using a novel polypropylene membrane treated for wettability (Celgard 3401) which is mechanically strong and solvent resistant when compared to traditional cellulose acetate membranes. Counter-current dialysis was used at a flow rate of 0·8 ml min^{-1}, with a recipient stream of water and the dialysate was then analysed by high-performance liquid chromatography. Fructose, glucose, lactose, maltose and sucrose were determined in a range of samples. The % RSD of the method was shown to be below 5% and the recoveries above 90% for all samples.

INTRODUCTION

The analysis of the sugar content of food involves both identification and quantification of the sugars present. Generally, because of the complex nature of the food matrix, extensive extraction and clean-up procedures have to be carried out to minimise interferences during determinations.

Extraction normally involves the use of firstly a solvent extraction step with a non-polar solvent to remove fat soluble substances, followed by the use of a polar or aqueous solution to dissolve the sugars. The old method of sugar determination also made use of clearing agents to clean-up the food extracts before measurement by polarimetry. These methods, however, suffered from some interferences because the clearing agents were not very effective at eliminating the interferences. Current methods of sugar determination make use of separation techniques such as ion-exchange and solid phase extraction to clean up the extracts (Macrae, 1991). High-performance liquid chromatography (HPLC) is the chosen method for the separation of the different sugars. Reverse-phase ODS2, ion-exchange and aminopropyl columns have been used with a range of detectors including refractive index and electrochemical detection (Folkes & Crane, 1988).

Herbreteau *et al.* (1990) and Morin-Allory *et al.* (1990) have described a method for sugar analysis with automated sample preparation in which the only preliminary sample preparation required for molasses involved passing it through a mixed Hamilton bed exchanger to eliminate ionic interferences. This was followed by chromatographic separation and light scattering detection. On-line dialysis has been shown to be a useful method for sample preparation in food analysis (Greenway *et al.*, 1992). In this work dialysis has been investigated in detail for sugar analysis. The 'in-house' system developed differs from the previously used commercial system (ASTED™, Gilson, Villiers-Le-Bel, France) in that it uses counter-current dialysis and a novel dialysis membrane. A simple HPLC method with a reverse-phase ODS2 column is used for the separation of the sugars. A range of different samples are then directly analysed with degassing and dilution being the only sample preparation step.

MATERIALS AND METHODS

Instrumentation

A Gilson HPLC System was used for separation (Gilson Medical Electronics). This consisted of two model 305 pumps, an 805 manometric module and an 811 Dynamic mixer. The detector was a differential refractometer (Waters 410) connected to an integrator (Trivector Trio MPA 7314).

The sample preparation system was designed and constructed in-house. The dialysis blocks consisted of a perspex donor and a recipient blocks each with grooves

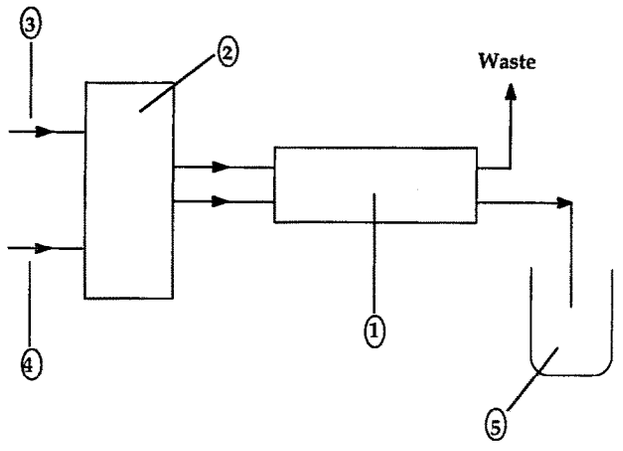

Fig. 1. Schematic arrangement of the dialysis unit 1, Dialyser; 2, two separate peristaltic pumps; 3, donor stream; 4, recipient stream; 5, dialysate.

drilled into them to provide channels (Fig. 1). The blocks were then sandwiched around the dialysis membrane and evenly clamped with screws. The dimensions of the channels were 660 mm long × 1·6 mm wide. Blocks with different channel depths were constructed so as to optimise the system for the best channel depth (0·1–1·0 mm).

Two types of dialysis membrane were investigated, a cellulose acetate membrane 15 kDa cut-off (Cuprophan, Gilson) and a polypropylene membrane treated for wettability with a molecular mass cut-off of 37 kDa (Celgard 3401, Hoechst, Celanese Corp., Charlotte, USA). The dialysis unit was set up as shown in Fig 2. The peristaltic pumps (Ismatec Minipuls SA 8031), fittings and silicone tubing (0·8 mm i.d.) were from Anachem (Luton, UK). One pump was used to pump the sample through the donor side of the block, whilst the other pump was used to pump the recipient stream through the other side. The outlet from the donor stream went to waste, whilst the outlet from recipient stream was collected to inject on the HPLC.

Reagents

Fructose, glucose, lactose, maltose, sucrose and raffinose were obtained from Sigma (Poole, Dorset, UK). Methanol (HPLC grade) was from Merck (Lutterworth, UK) and the purified water was prepared by reverse osmosis followed by ion-exchange. All HPLC mobile phases were degassed with helium before use.

Preliminary sample preparation

Three types of samples were analysed for their sugar content, soft drinks, medicinal syrups and powders for making hot drinks, all of which were obtained from a local supermarket. Soft drinks were diluted by a factor of 2 and the medicinal syrups were diluted by a factor of 5 in water before being degassed in an ultrasonic bath for 5 min. For the powder samples a 5 g sample was dissolved in water and made up to 25 ml, before being degassed as above.

HPLC method

The HPLC method was adopted from a method by Herbreteau *et al.* (1990) for the determination of sugars

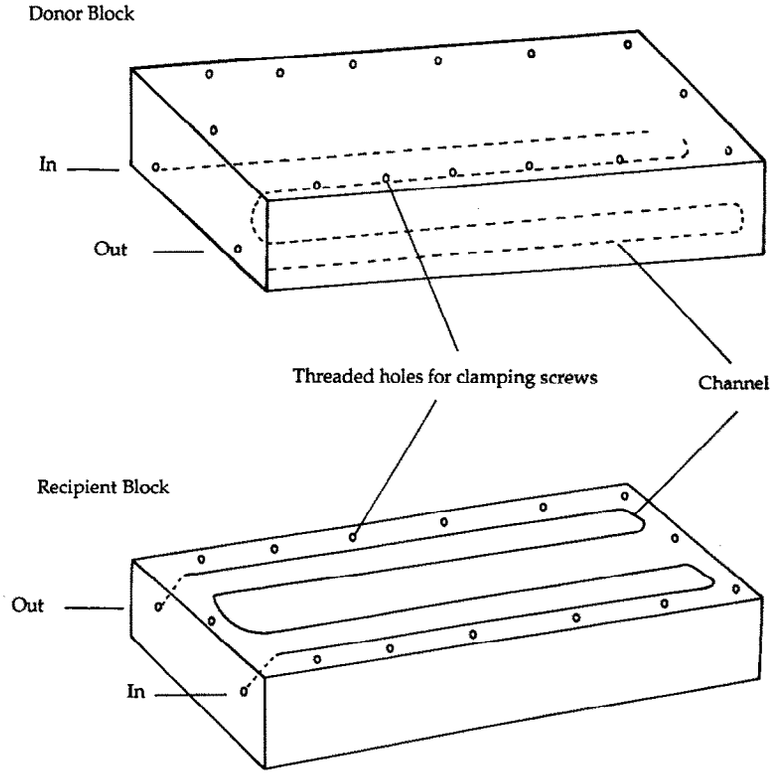

Fig. 2. Donor and recipient dialyser blocks.

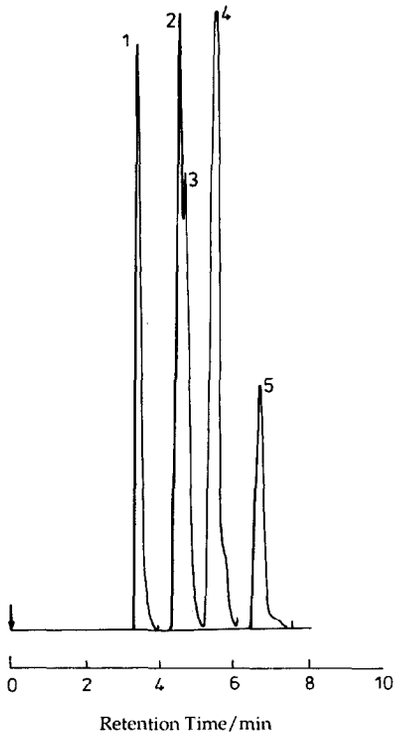

Fig. 3. Chromatogram of sugar standards 1, Fructose + glucose; 2, lactose; 3, maltose; 4, sucrose; 5, raffinose.

in the beet industry. As in the original method, the method used was unable to separate all sugars but it had the advantage of using an inexpensive ODS2 column as opposed to the more expensive aminopropyl and ion-exchange columns. The separation achieved was quite adequate for demonstrating the on-line sample preparation technique. The column used was a Spherisorb ODS2 column with 5 μm particle size (250 mm long × 4·6 mm id.). A guard column was also incorporated before the analytical column to protect it from the samples (Spherisorb ODS2 10 μm particle size, 40 mm long × 4·6 mm id.). The injection loop was 20 μl and the flow rate was 1 ml min^{-1}. The separation was isocratic with a mobile phase of 99% water, 1% methanol. Figure 3 shows a separation for fructose, glucose, lactose, maltose, sucrose and raffinose, in which fructose and glucose are seen to co-elute.

Method development for dialysis

The effect of solvent, channel depth, flow rate and flow pattern were investigated using both types of membrane. To ensure reproducibility the first 0·5 ml dialysate collected was discarded. These experiments were carried out using a 50 mg ml^{-1} standard solution of sucrose. The results were evaluated in terms of the

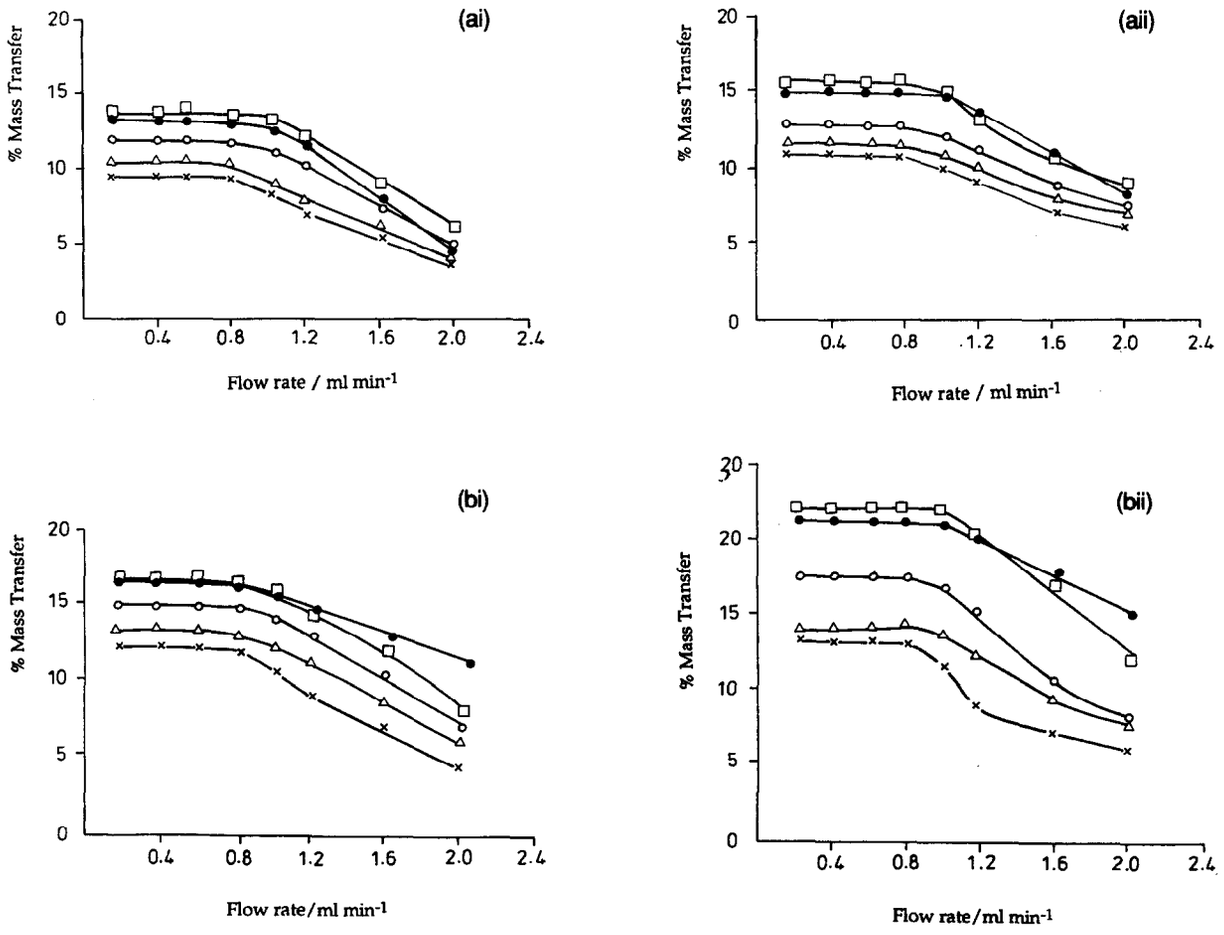

Fig. 4. The effect of flow rate on the dialysis of sucrose at different channel depths: × 0·1, Δ 0·2, ○ 0·6, □ 0·8 and ● 1·0. (a) Flow pattern: co-flow; (i) Cuprophan membrane, and (ii) Celgard membrane. (b) Flow pattern: counter-flow; (i) Cuprophan membrane, and (ii) Celgard membrane.

percentage mass transfer of sucrose through the membrane ((peak area for dialysate/peak area for original standard solution) × 100).

Water and methanol/water (99:1, i.e. the HPLC mobile phase) were investigated as recipient solvents. The methanol could only be used with the Celgard 3401 as the Cuprophan membrane has no resistant to solvents. No difference was seen however for the Celgard 3401 membrane when the mass transfer for the two different recipient solutions was compared and water was used for all subsequent tests. Figure 4(a) shows the effect of flow rate on the dialysis of sucrose in water at different channel depths and with co-flow (i.e. both donor and recipient streams flowing in the same direction) for (i) the Cuprophan membrane, and (ii) the Celgard 3401 membrane. Figure 4(b) shows the same but this time dialysis is by counter-flow (i.e. donor and recipient stream flowing in opposite directions). For all conditions the highest % mass transfer was obtained for flow rates between 0·2 and 0·8 ml min^{-1} and after 0·8 ml min^{-1} the % mass transfer decreased significantly. This was because at higher flow rates the contact time between the particular portions of donor and recipient stream decreases so reducing the number of sucrose molecules diffusing through the membrane. As would be expected, counter-current flow gives higher mass transfer than co-flow for equivalent conditions. This is because the sample stream is continuously being exposed to a new section of recipient stream which contains no dialysate. Therefore, in terms of analyte molecules there is a 'zero osmotic pressure' in each new section of the recipient stream resulting in diffusion of analyte molecules through the membrane into the recipient stream in an attempt to establish equi-osmotic pressure on both sides of the membrane. Equi-osmotic pressure is not easily attained however because the recipient stream encounters an increasing concentration of analyte molecules in the sample stream as it flows through the dialyser. For both Figs 4(a) and (b) the Celgard 3401 membrane (ii) gives the greater % mass transfer. This is probably due to the larger pore size of the Celgard 3401 membrane. Although this would give it less size selectivity it had several advantages over the Cuprophan membrane in that it had greater strength and did not easily tear or break. It was also not affected by water, unlike the Cuprophan membrane that swelled in water and had to be kept moist at all times after initial use if it was to operate reliably. The Celgard 3401 membrane could be reused even if it dried and was resistant to solvents such as methanol.

A channel depth of 0·8 mm gave the highest % mass transfer for the experimental conditions investigated. At smaller channel depths the donor volume is reduced effecting transfer, but at 1·0 mm the % mass transfer again decreases, probably due to lack of accessibility to the membrane for molecules furthest away.

The conditions finally selected for dialysis were a channel depth of 0·8 mm with a counter-current flow of 0·8 ml min^{-1} flow rate for each stream and a recipient stream of water.

RESULTS AND DISCUSSION

Calibration curves were prepared for fructose, glucose, lactose, maltose and sucrose in the range 0–800 mg ml^{-1}. The equations of the lines with errors and correlation coefficients are given in Table 1. Good linear correlation was obtained for all the calibration curves in the range investigated.

Table 2 shows the values obtained for simple sugars in the samples analysed. It was not possible to make direct comparisons of the amount of sugars obtained with the manufacturers labels as they only expressed the sugar content as part of the total carbohydrates, but the value agreed well with those quoted in the literature (Hurst *et al.*, 1979; Hurst & Martin, 1980). The values for fructose/glucose and lactose/maltose have been expressed as total values for the pairs because the

Table 1. Calibration equations of sugars analysed (where x is concentration in mg ml^{-1} and y is area in arbitrary units)

Sugars	Slope of best straight line[a]	Intercept[a]	Correlation coefficient, r
Fructose	15·4 ± 0·29	46·1 ± 108·6	0·9998
Glucose	17·7 ± 0·46	56·5 ± 170·8	0·9996
Lactose	13·3 ± 0·41	−34·7 ± 150·9	0·9994
Maltose	12·2 ± 0·25	−25·1 ± 91·8	0·9997
Sucrose	14·7 ± 0·34	−53·3 ± 127·1	0·9996

[a]Confidence limits at 95%, $n = 9$.

Table 2. The concentration of sugars in some common beverages and medicinal liquids

Sample	Sugar found	Concentration (mg ml^{-1}) (mean value of five measurements)
Simple linctus BP	Sucrose	324·2
Codeine linctus BP	Sucrose	332·8
Syrup BP	Sucrose	664·3
Pholcodine linctus	Sucrose	330·0
Sudafed elixir	Sucrose	209·6
	Glucose/fructose	118·1
Zaditen syrup	Fructose/glucose	248·1
	Sucrose	69·6
Malt extract drink	Maltose/lactose	130·7
	Fructose/glucose	22·2
Sunkist orange drink	Sucrose	49·4
	Glucose/fructose	18·7
Tango orange drink	Sucrose	53·6
	Glucose/fructose	14·9
Citra lemonade drink	Sucrose	38·1
	Glucose/fructose	23·4
Ovaltine	Lactose/maltose	640·4 mg g^{-1}
Fresh milk, whole	Lactose	38·4
Horlicks	Lactose/maltose	571·3 mg g^{-1}
	Sucrose	38·4 mg g^{-1}
Chocolate (brown)	Maltose/lactose	77·4 mg g^{-1}
	Sucrose	527·5 mg g^{-1}
Grandways lemonade	Sucrose	35·8
	Glucose/fructose	21·6
Pepsi Cola	Sucrose	48·7
Coca Cola	Sucrose	44·5

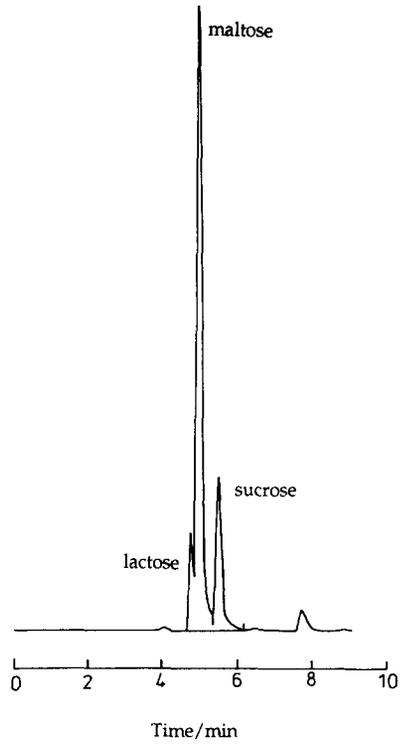

Fig. 5. Chromatogram of Horlicks drink.

HPLC method did not fully separate them. Figure 5 shows the chromatogram for Horlicks with a poorly resolved lactose/maltose peak. Poor reproducibility was obtained if the peak areas were measured individually but good reproducibility was obtained if the sum of the area under the two peaks was taken.

To investigate the reproducibility experiments were carried out on both samples and standards (Table 3). Two different concentrations of standards were investigated for all sugars (20 and 160 mg ml $^{-1}$). Sunkist orange drink and Ovaltine were selected as the samples for investigation. All % RSD values were below 5% with the reproducibility for lactose/maltose being the worst. This is not surprising since the lactose/maltose peaks are not resolved.

Table 3. Precision of dialysis HPLC method

	% RSD (n = 10)	
	At 20 mg ml^{-1}	At 160 mg ml^{-1}
For standards		
Fructose	2·1	1·5
Glucose	2·4	2·2
Lactose	3·3	3·4
Maltose	2·9	2·5
Sucrose	1·6	1·2
For samples		
Sunkist orange drink		
Fructose/glucose	2·7	
Sucrose	1·8	
Ovaltine		
Lactose/maltose	4·1	
Sucrose	3·2	

Table 4. Percentage recovery values[a]

	% Recovery		
Sample	Sucrose	Fructose	Glucose
Sunkist orange drink	94·4	94·8	95·9
Grandways lemonade	95·7	96·2	97·4
Tango orange drink	94·7	96·8	92·5
Simple linctus BP	92·7	91·1	—
Codeine linctus BP	95·2	94·6	—
Sudafed elixir	100·0	95·3	96·4
Temazepam elixir	—	96·1	93·3
Zaditen elixir	98·9	93·4	95·2
Ovaltine	96·1 (Maltose)	—	94·8 (Lactose)

[a]Recovery of sugars from pure water was 100%.

Recovery studies were carried out to determine the effectiveness of the method in (Table 4). Nine samples were selected and four sets of sample solutions were prepared for each, of which three sets were spiked, with either fructose, glucose or sucrose. All four sets of solutions were then analysed and the difference between the spiked and unspiked solutions were used to calculate the % recovery. Three sets of Ovaltine samples were also prepared and two of the sets were spiked, one with lactose and the other with maltose. Recoveries were good all being over 90% for both samples and sugars. This suggests that Ovaltine can be successfully analysed with this sample preparation although better results would be obtained with a different HPLC column.

CONCLUSIONS

The on-line dialysis system for sample preparation was shown to be effective for the analysis of sugars in beverages and medicines. The system could easily be fully automated to operate in a similar manner to the Gilson commercial system (ASTED™) but the in-house system allowed greater flexibility for method development. The Celgard 3410 membrane was found to be more robust than the Cuprophan membrane having resistance to solvents and good mechanical properties. It also had more efficient mass transfer, but the larger pore size would give less size selectivity.

As well as providing a very simple separation step for sample preparation, the dialysis step also diluted the sample which was particularly useful for the type of samples analysed which had high concentrations of analyte and matrix salts which could affect the analytical column. If the analyte had been present at low concentrations preconcentration would have been required before separation.

The concentration of sugars found in the samples agreed with the values quoted in the literature for similar samples and the recoveries for all samples were above 91%. A typical analysis took 15 min, 8 min being for the chromatography with most samples introduced directly and the only sample preparation being dissolution of powders and dilution.

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