Centrifugal partition chromatography in a biorefinery context: Separation of monosaccharides from hydrolysed sugar beet pulp☆

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ABSTRACT

A critical step in the bioprocessing of sustainable biomass feedstocks, such as sugar beet pulp (SBP), is the isolation of the component sugars from the hydrolysed polysaccharides. This facilitates their subsequent conversion into higher value chemicals and pharmaceutical intermediates. Separation methodologies such as centrifugal partition chromatography (CPC) offer an alternative to traditional resin-based chromatographic techniques for multicomponent sugar separations. Highly polar two-phase systems containing ethanol and aqueous ammonium sulphate are examined here for the separation of monosaccharides present in hydrolysed SBP pectin: L-rhamnose, L-arabinose, D-galactose and D-galacturonic acid. Dimethyl sulfoxide (DMSO) was selected as an effective phase system modifier improving monosaccharide separation. The best phase system identified was ethanol:DMSO:aqueous ammonium sulphate $(300\,g\,L^{-1})\,(0.8{:}0.1{:}1.8, v{:}v{:}v)$ which enabled separation of the SBP monosaccharides by CPC (200 mL column) in ascending mode (upper phase as mobile phase) with a mobile phase flow rate of $8\,mL\,min^{-1}$. A mixture containing all four monosaccharides (1.08 g total sugars) in the proportions found in hydrolysed SBP was separated into three main fractions; a pure L-rhamnose fraction (>90%), a mixed L-arabinose/D-galactose fraction and a pure D-galacturonic acid fraction (>90%). The separation took less than 2 h demonstrating that CPC is a promising technique for the separation of these sugars with potential for application within an integrated, whole crop biorefinery.

1. Introduction

Over 8 million tonnes of sugar beet are grown annually in the UK and sugar beet pulp (SBP) is a significant waste product from processing. Currently the SBP is pressed, dried and pelleted in an energy intensive process before being sold as low value animal feed [1]. SBP is a rich source of carbohydrates that consists mainly of cellulose (a polymer of D-glucose) and pectin (a co-polymer of various hexose and pentose sugars) as shown in Fig. 1. Its abundance and low cost suggests that SBP could provide a sustainable feedstock for the production of chemical and pharmaceutical intermediates within the context of a whole crop biorefinery [2].

The pectin fraction of SBP consists primarily of L-arabinose (Ara) and D-galacturonic acid (GA), with smaller amounts of D-galactose (Gal) and L-rhamnose (Rha) as shown in Fig. 2. The structure and composition of pectin depends on its source. Sugar beet pectin is constructed from a polymeric GA backbone with intermittent blocks of alternating Rha–GA monomers. Unbranched galactan (polymeric Gal) and highly branched arabinan (polymeric Ara) side chains complete the pectin structure [3–5].

The extraction of pectin from SBP has been widely studied [5–9] while more selective release of the pectin, with minimal cellulose degradation, is a promising new development for biorefinery applications [10]. This allows the residual cellulosic fraction to be used to supplement established routes to bioethanol production, while the pectic sugars have the potential to be converted into chemical and pharmaceutical intermediates.

Various figures are available for the total sugar composition of SBP calculated after complete hydrolysis [5,11]. This work will use the data from Micard et al. [5] assuming removal of the cellulosic fraction as outlined by Leijdekkers et al. [10]. This gives a mass
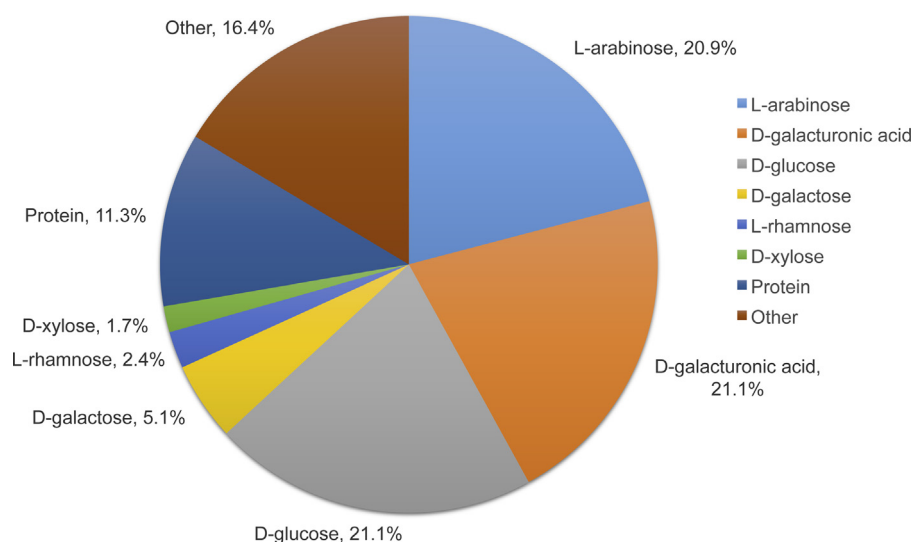
Fig. 1. Overall composition of sugar beet pulp (SBP) (data taken from Micard et al. [5]).

composition of approximately 43% Ara, 41% GA, 11% Gal and 5% Rha.

As the major monosaccharides present in sugar beet pectin, Ara and GA have a number of potential applications. Ara has been shown to be useful for the production of biopolymers after esterification [12]. Ara can also be reduced to arabinitol, highlighted as one of the 12 top value added chemicals from biomass which can be used as a building block for unsaturated polyester resins [13]. Oxidised sugars, such as GA, have also been highlighted as important building blocks from biomass for the production of hyperbranched polyesters and plasticisers [13].

The separation of sugars is traditionally a difficult process. Applying solid–liquid chromatography techniques usually requires the use of strong cation exchange methods to separate sugars by complex formation with the resin counterions [14]. Industrially, separations have been performed using large scale simulated moving bed (SMB) chromatography since the late 1970s [15]. In these cases the intention is to purify a single sugar to high purity,

for example fructose from glucose [16–18] or for the isolation of sucrose from molasses [19–21].

Centrifugal partition chromatography (CPC) is a liquid–liquid separation technique that achieves separation by repeated partitioning of solutes between two immiscible liquid phases [22]. Unlike solid–liquid chromatography, the liquid stationary phase is retained in the column by the application of a centrifugal force along a single rotational axis. The lack of a solid support prevents any irreversible adsorption [23] allowing for very high product recoveries and process flexibility. For example, extrusion of the entire column contents is possible at higher flow rates, allowing strongly retained substrates to be rapidly collected in a lower elution volume [24]. CPC has been used extensively for the separation of natural products [25–29] and we have previously investigated the bioprocess applications of liquid–liquid chromatography for large-scale antibiotic recovery [30,31]. In contrast, there are just a small number of studies on monosaccharide [32,33] and oligosaccharide separations [34,35]. These require the use of highly polar phase systems in order to enable a reasonable partition of the hydrophilic solutes between the two liquid phases. Weak ion-exchange CPC has been used to fractionate sulphated oligosaccharides by adding a lipophilic ion-pairing reagent, allowing charged compounds to partition into the organic phase [36]. CPC allows for the processing of crude hydrolysed SBP without pre-treatment, minimising the processing steps compared to SMB. Furthermore, the ability of CPC to isolate multiple fractions simultaneously is important for biorefinery applications allowing for the separation of multiple sugars in a single operation ready for downstream conversion into value added products.

The aim of this work is to establish a CPC method for the separation of the main four sugars present in SBP pectin (Ara, GA, Gal and Rha) following its complete hydrolysis. As SBP consists primarily of Ara and GA, the isolation of these two compounds was prioritised. Highly polar alcohol–ammonium sulphate phase systems are examined along with potential modifiers for improving both retention of the stationary phase and solute partition coefficients. A model separation of the sugars in equal concentration was first performed to demonstrate feasibility of the process followed by the separation of a synthetic crude mixture of all four sugars in their relative proportions in SBP pectin. The fractions collected would provide a starting material for the subsequent synthesis of chemical and pharmaceutical intermediates from this renewable feedstock.
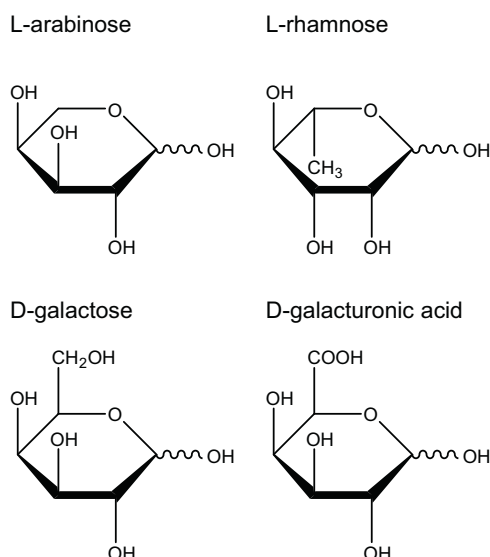

Fig. 2. Pyranose structures of the main monosaccharides present in SBP pectin.

2. Materials and methods

2.1. Reagents

The sugars L-arabinose (99%), L-rhamnose (99%), D-galacturonic acid sodium salt (98%) and D-galactose (99%); and solvents acetonitrile, methanol, n-propanol and n-butanol were purchased from Sigma–Aldrich (Gillingham, UK). Absolute ethanol, dimethyl sulfoxide (DMSO) (99%) and ammonium sulphate (99%) were purchased from Fisher Chemicals (Loughborough, UK). The water used was deionised in house using a Purite Select Fusion purification system (Thame, UK).

2.2. Phase system development and solute partition coefficients

Full details of all of the phase systems considered in this study are presented in Table 1. Two-phase systems were prepared by mixing all components in the specified volumetric ratios to redissolve any precipitated salt and equilibrated for 2 h in a separation funnel prior to separation of the two phases. The ratio of the upper phase volume to the lower phase was recorded and the interface discarded. Settling times were measured by adding 2 mL of each separated phase into a test tube, vigorously mixing and measuring the time taken to settle completely. Three repeats were performed for each phase system.

Saturated ammonium sulphate was prepared by adding 550 g of ammonium sulphate to heated deionised water until it was entirely dissolved. The volume was then made up to 1 L before being allowed to cool to room temperature overnight and the supernatant decanted. Partition coefficients of Ara, Rha, Gal and GA were determined individually by adding 10 mg of the sugar to 2 mL of lower phase. 1 mL of this solution was added to an equal volume of upper phase, thoroughly mixed and allowed to reach equilibrium for 45 min. Concentrations in each phase were determined by HPLC and partition coefficients were calculated as $K_{D\,LP/UP} = C_L/C_U$, where C_L and C_U are the sugar concentrations in the lower and upper phases respectively.

A ternary phase diagram was established for an ethanol: ammonium sulphate:water phase system by adding ethanol, saturated ammonium sulphate and water in various volumetric ratios and observing the phase behaviour and settling time at room temperature. The mass ratios of each component were then calculated, taking into account the saturated ammonium sulphate containing both water and ammonium sulphate. Phase systems were categorised as 'single-phase', 'two-phase' or 'precipitate'. 'Precipitate'

describes any phase system where salt precipitation was observed even if two immiscible liquid phases were still present.

2.3. CPC equipment and operating conditions

Centrifugal partition chromatography was performed on an FCPC-A (fast centrifugal partition chromatography – Roussalet Robatel Kromaton, Annonay, France) with a 200 mL rotor. The column was filled by pumping lower phase in ascending mode at a rotational speed of 600 rpm and a flow rate of 20 mL min^{-1}. In ascending mode, the lower phase is retained as the stationary phase while the upper phase flows through the column as the mobile phase.

Studies on the retention of the stationary phase were performed by flowing upper mobile phase in ascending mode at a set flow rate (2, 4, 8, 12 and 16 mL min^{-1}) and rotational speed (600, 800, 1000, 1500 and 2000 rpm) and observing the volume of stationary phase displaced from the column at initial mobile phase breakthrough and at equilibrium.

CPC separation was performed in ascending mode, with the lower and upper phase as stationary and mobile phase respectively, at a rotational speed of 1000 rpm and a mobile phase flow rate of 8 mL min^{-1} supplied by an Agilent G1361A preparative pump. Two types of samples were used for CPC separations: an initial 'model' Rha–Ara–GA sample made up in equal concentrations to determine the separation performance and a 'synthetic crude' mixture comprising the four main sugars in SBP pectin in their respective concentrations. Samples were prepared by dissolving solutes in 20 mL of mobile phase before making up to a total volume of 25 mL. For the model separation, 1.25 g of each sugar was used to prepare 25 mL of the sample, giving a total concentration of 150 g L^{-1}. For the synthetic crude, 1.07 g Ara (43%), 1.04 g GA (41%), 0.28 g Gal (11%) and 0.12 g Rha (5%) were used giving a total concentration of approximately 100 g L^{-1}. For both types of sample 10.81 mL of solution was injected into the CPC column. Fractions were collected every 0.6 min from 15 min after injection in 5 mL test tubes using an Agilent G1364B fraction collector. Samples were immediately sealed and kept at 4 °C until analysis.

2.4. Analytical methods

The concentrations of individual sugars in each phase for partition coefficient calculations were determined by HPLC using a Dionex P680 HPLC pump and ASI-100 autosampler injector fitted with an Aminex HPX-87H column. Isocratic elution was carried out at 0.6 mL min^{-1} for 30 min using 5 mM H_2SO_4 as mobile phase,

Table 1

Summary of highly polar phase systems investigated for fractionation of hydrolysed SBP pectin. Values represent volumetric ratios of solvents used in phase system preparation. Phase systems were formed as described in Section 2.2.

Phase system	EtOH	Sat. AS	2 M AS	Wat	DMSO	ACN	MeOH	PrOH	BuOH
I	3	–	4	–	–	–	–	–	–
II	3	–	5	–	–	–	–	–	–
III	3	–	6	–	–	–	–	–	–
IV	–	1.2	–	1	–	0.5	–	1	–
V	0.5	1.2	–	1	–	0.5	–	0.5	–
VI	0.5	1.2	–	1	–	0.5	–	–	0.5
VII	1	1	–	0.8	–	–	–	–	–
VIII	0.8	1	–	0.8	–	–	–	–	–
IX	0.8	1	–	0.8	–	–	0.1	–	–
X	0.5	1	–	0.8	–	0.5	–	–	–
XI	0.5	1	–	0.8	–	0.5	0.2	–	–
XII	–	1	–	0.8	–	0.5	0.4	–	–
XIII	–	1	–	0.8	0.3	0.5	–	–	–
XIV	0.6	1	–	0.8	0.1	–	–	–	–
XV	0.8	1	–	0.8	0.1	–	–	–	–

EtOH, ethanol; Sat. AS, saturated ammonium sulphate; 2 M AS, 2 M ammonium sulphate; Wat, water; DMSO, dimethyl sulfoxide; ACN, acetonitrile; MeOH, methanol; PrOH, n-propanol; BuOH, n-butanol.

a column temperature of 60 °C, an injection volume of 10 μL and monitored with a refractive index detector (Shodex RI-101 or ERC Refractomax 520). Quantitative analysis was performed by integration of peak areas using the external standard method. This method enabled baseline resolution of the different monosaccharides and the measured retention times for GA, Gal, Rha and Ara were 9.2, 10.1, 10.7 and 11.2 min, respectively.

This HPLC method was also used for the determination of ethanol and DMSO concentrations in each phase; retention times for ethanol and DMSO were 22.0 and 26.4 min, respectively. Ammonium sulphate concentrations in each phase were calculated by gravimetric analysis by drying 0.5 mL of each phase in an oven overnight at 100 °C and measuring the remaining mass.

Quantitative analysis of CPC fractions containing multiple sugar species was performed using a Reagent-Free Ion Chromatography System (ICS 5000+, Dionex, Sunnyvale, CA, USA) with a Carbopac PA1 anion exchange column, an injection volume of 10 μL, an electrochemical detector system, and an eluent generator fitted with a KOH 500 cartridge. Neutral sugars (Ara, Rha and Gal) were analysed using 7.5 mM KOH as the mobile phase with a flow rate of 1.5 mL min^{-1} for 18 min at 30 °C. Retention times for the neutral sugars Rha, Ara and Gal were 7.1, 7.8 and 10.2 min, respectively. GA was analysed with a mobile phase of 25% 0.5 M aqueous sodium acetate (ED grade) at 1 mL min^{-1} for 10 min at 30 °C giving a retention time for GA of 3.0 min. Quantitative analyses were performed measuring peak height or area using the external standard method.

3. Results and discussion

3.1. Phase system development

Fig. 3 shows the ternary phase diagram determined for the ethanol:ammonium sulphate:water phase systems based on the mass of each compound in the system. This demonstrates a relatively small range of compositions under which two-phase systems, suitable for CPC application, form. Binodal curve data of ethanol:ammonium sulphate two phase systems has been published previously [37], however, it does not demonstrate the effective two-phase region for CPC operation, nor the region in which precipitation occurs with further addition of ammonium sulphate or ethanol.

The settling time of these two-phase systems is an important metric for understanding stationary phase retention in CPC with shorter settling times giving higher retention [38]. High
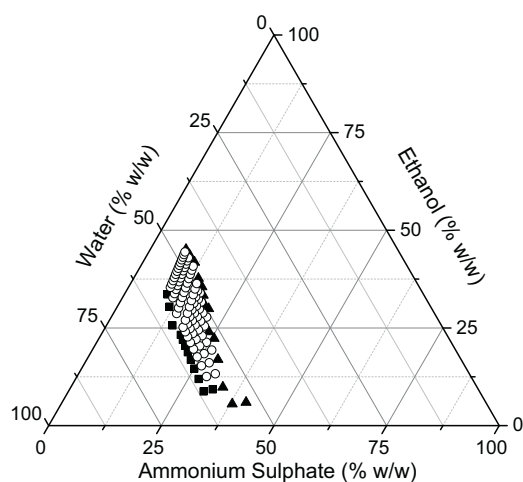
Table 2
Lower phase (LP) volume fractions and settling times of the highly polar phase systems described in Table 1. Experiments were performed as described in Section 2.2. Values represent one standard deviation about the mean ($n = 3$).

Phase system	LP volume fraction	Settling time (s)
I	0.32	19.4 ± 0.3
II	0.38	25.3 ± 0.1
III	0.44	37 ± 1
IV	0.47	16.0 ± 0.3
V	0.44	14.6 ± 0.4
VI	0.51	18.3 ± 0.4
VII	0.44	20.3 ± 0.5
VIII	0.53	29.5 ± 0.4
IX	0.46	29.8 ± 0.5
X	0.76	19.9 ± 0.4
XI	0.43	20.6 ± 0.5
XII	0.58	28.3 ± 0.5
XIII	0.52	41.4 ± 0.8
XIV	0.57	89.8 ± 0.4
XV	0.42	31.4 ± 0.7

stationary phase retentions allow for higher mobile phase flow rates, increasing solute throughput. Here it was observed that systems containing more ammonium sulphate and ethanol (i.e. closer to the precipitate phase boundary) exhibit shorter settling times (Table 2) indicating a likely improvement in retention. Systems with a higher percentage of water (closer to the single phase boundary) exhibit longer settling times, indicating that the retention could be lower.

The partition coefficient is defined as the ratio of solute concentrations at equilibrium between the two liquid phases ($K_{D\,LP/UP} = C_{LP}/C_{UP}$) and gives an indication of the expected elution order [38]. At values close to $K_D = 0$, solutes elute near to the solvent front with little separation while values much above 1 elute after more than 1 column volume of eluent with broader peaks. Partition coefficients around $K_D = 1$ elute around 1 column volume and generally result in sufficient resolution and elution time [39].

Phase systems I–III (Table 1), from Shinomiya and Ito [33], use a constant salt concentration (2 M ammonium sulphate) in varying volumetric ratios with ethanol to demonstrate how the location of the phase system in the two-phase region affects settling time and partition coefficient. With a lower ethanol proportion (phase system III – closer to the single phase boundary in Fig. 3) the partition coefficients are closer to the target of 1 ($K_{D\,LP/UP}$ Ara = 2.2) (Table 3) but the settling time is increased (31 s) (Table 2). A higher ethanol

Table 3
Partition coefficients ($K_{D\,LP/UP}$) of monosaccharides from hydrolysed SBP pectin. K_D values were determined as described in Section 2.2. Values represent one standard deviation about the mean ($n = 2$).

Phase system	Partition coefficient ($K_{D\,LP/UP}$)			
	Ara	Gal	Rha	GA
I	4.3 ± 0.2	5.2 ± 0.1	1.3 ± 0.1	13.0 ± 1.9
II	3.0 ± 0.2	3.5 ± N.D.	1.2 ± 0.0	8.7 ± 0.9
III	2.2 ± N.D.	2.5 ± 0.1	1.2 ± 0.1	4.2 ± N.D.
IV	16 ± 2	24 ± 0	3.9 ± 0.0	33 ± 3
V	7.7 ± 0.0	12 ± 0	2.1 ± 0.1	33 ± 8
VI	10.0 ± 0.5	15 ± 0	2.4 ± N.D.	33 ± 2
VII	4.0 ± 0.2	N.D.	N.D.	N.D.
VIII	2.8 ± 0.0	N.D.	N.D.	7.2 ± 0.0
IX	2.8 ± 0.0	N.D.	N.D.	7.3 ± 0.1
X	5.3 ± 0.1	N.D.	N.D.	N.D.
XI	4.0 ± 0.7	N.D.	N.D.	N.D.
XII	3.7 ± 0.1	N.D.	N.D.	N.D.
XIII	2.9 ± 0.1	N.D.	N.D.	N.D.
XIV	1.8 ± 0.0	2.1 ± 0.1	1.0 ± 0.0	3.6 ± 0.1
XV	2.6 ± 0.0	3.5 ± 0.0	1.2 ± 0.1	7.2 ± 0.2

Ara, L-arabinose; Gal, D-galactose; Rha, L-rhamnose; GA, D-galacturonic acid; N.D., not determined.

Fig. 3. Ternary phase diagram (% w/w) showing the two-phase region of ethanol:ammonium sulphate:water phase systems. (▲) precipitate, (■) single-phase, (○) two-phase. Experiments were performed as described in Section 2.2.

proportion (phase system I – closer to the precipitate boundary) results in a shorter settling time (21 s) but also partition coefficients further from 1 ($K_{D\,LP/UP}$ Ara = 4.3). There is therefore a trade-off required between the separation achievable (K_D values) and the throughput (settling times). Using a an even lower ethanol proportion (3:7) results in excessive settling times that would result in poor retention while a higher ethanol proportion (3:3) resulted in some salt precipitation. The requirement for short settling times (<30 s) effectively shrinks the practicable two-phase region in Fig. 3, where phase systems with suitable characteristics can be formed.

In an attempt to improve both stationary phase retention and settling times, various modifiers were tested in the ethanol: ammonium sulphate systems. Phase systems IV–VI use a combination of acetonitrile, propanol or butanol as phase system modifiers and have previously been used for the separation of glucosinolates [40,41]. For these phase systems the settling times were shorter than 20 s (Table 2), however, the partition coefficients were much higher than for phase systems I, II and III (Table 3), indicating long and poor separations. It is evident that these systems, while useful for glucosinolate isolation, are not suitable for the separation of sugars, which will partition too strongly into the lower aqueous phase.

All of the phase systems I–VI followed the same order of partition coefficients ($K_{D\,LP/UP}$) for the SBP solutes i.e. Rha < Ara ≤ Gal < GA. It was therefore decided to look primarily at improving the Ara partition coefficient (bringing it closer to 1).

Phase systems VII–XIII examine the effect of methanol and propanol as an alternative to ethanol in forming two-phase systems with ammonium sulphate as well as modifying the partition coefficient in ethanol systems. From these systems, the lower phase volume fractions were generally around 0.5 and settling times between 20 and 30 s (Table 2). Phase system VIII (ethanol:saturated ammonium sulphate:water (0.8:1.0:0.8, v:v:v)) shows a relatively high Ara partition coefficient ($K_{D\,LP/UP}$ Ara = 2.8) but maintains a large separation factor (2.6) between the two main targets (Ara and GA) with a reasonable settling time (30 s). This phase system was used as the basis of further modifications and as a standard for comparison.

Methanol, as a more polar alcohol than ethanol, could provide a way of improving the solute partition coefficients by reducing the polarity difference between the two phases. Phase system IX introduces a small proportion of methanol, which has no effect on the partition coefficients or the settling time. The use of methanol as a modifier is limited as it readily precipitates the ammonium sulphate and so can only be used in low proportions, requiring another compound to stabilise the phase system.

Phase system X examines the addition of acetonitrile to the phase system. While the settling time is reduced to 20 s from phase system VIII (30 s), the Ara partition coefficient almost doubles ($K_{D\,LP/UP}$ Ara = 5.3) as shown in Table 3. To improve the partition coefficient, ethanol and methanol were examined as phase system modifiers (systems XI and XII). Settling times for these systems were 21 and 28 s respectively, however, the partition coefficients were much higher than phase system VIII containing only ethanol as organic solvent.

DMSO was also investigated as a potential phase system modifier to improve partition coefficients of the monosaccharides. It is a polar aprotic solvent which is capable of dissolving a wide range of organic compounds with high loadings [42] and is effective at solubilising even long chain carbohydrates [43]. Adding DMSO to an acetonitrile–ammonium sulphate phase system reduced the partition coefficient from 5.3 (system X) to 2.9 (system XIII) but increases the settling time from 30 s to 41 s.

DMSO was then tested in an ethanol–salt phase system, however, the addition of DMSO readily precipitated the salt and a low concentration was required in order for a two-phase system to form. System XIV uses a lower ethanol proportion and provides
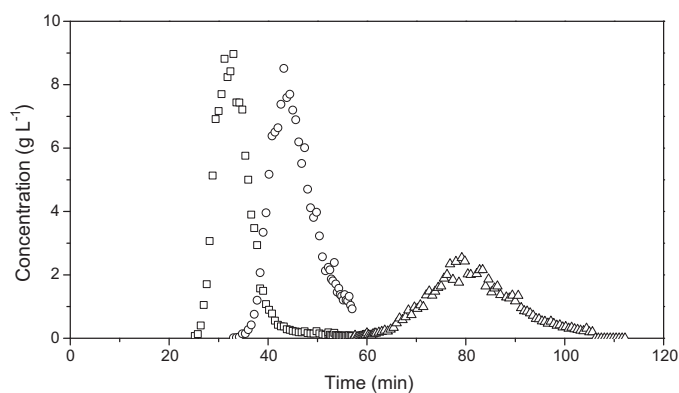

Fig. 4. CPC separation of a model mixture of L-rhamnose, L-arabinose and D-galacturonic acid using phase system XV (Table 1): ethanol:DMSO:aqueous ammonium sulphate (300 g L^{-1}) (0.8:0.1:1.8, v:v:v). Experimental conditions: 50 g L^{-1} of each solute in a 10.81 mL sample loop operating in ascending mode at a flow rate of 8 mL min^{-1} and a rotational speed of 1000 rpm at room temperature. (□) L-rhamnose, (○) L-arabinose, (△) D-galacturonic acid. Experiments were performed as described in Section 2.3.

the lowest achieved partition coefficients for all sugars ($K_{D\,LP/UP}$ Ara = 1.8). The settling time, however, is excessive at 90 s which could lead to significant problems with stationary phase retention in the CPC column. As SBP is a high volume feedstock, increasing CPC throughput is considered to be an overriding consideration and so improvements in the settling time were sought to allow for higher mobile phase flow rates. An increase in the ethanol proportion to 0.8 (system XV) shortened the settling time to 31 s and increased the partition coefficient ($K_{D\,LP/UP}$ Ara = 2.6). Importantly, the separation factor for GA and Ara in system XV (2.8) is higher than both phase system XIV (2.0) and VIII (2.6). Based on these results, phase system XV was selected for subsequent CPC separations.

Analysis of the composition of the two phases formed showed that the ammonium sulphate concentration was 376 g L^{-1} in the LP and 63 g L^{-1} in the UP. Ethanol concentration in the LP and UP was 97 g L^{-1} and 333 g L^{-1}, respectively, while DMSO concentration was 23 g L^{-1} and 56 g L^{-1}, respectively. The saturated ammonium sulphate and water (1.0:0.8, v:v) mixture gave a total aqueous ammonium sulphate concentration of approximately 300 g L^{-1}. This concentration was used for larger scale preparation of phase
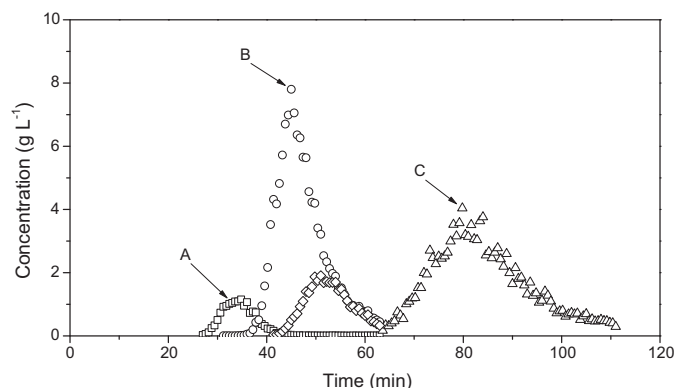

Fig. 5. CPC separation of a synthetic mixture of hydrolysed SBP pectin (43% L-arabinose, 41% D-galacturonic acid, 5% L-rhamnose, 11% D-galactose) using phase system XV (Table 1): ethanol:DMSO:aqueous ammonium sulphate (300 g L^{-1}) (0.8:0.1:1.8, v:v:v). Experimental conditions: 100 g L^{-1} total solute concentration in a 10.81 mL sample loop operating in ascending mode at a flow rate of 8 mL min^{-1} and a rotational speed of 1000 rpm at room temperature. (□) L-rhamnose, (○) L-arabinose, (◇) D-galactose, (△) D-galacturonic acid. Analytical chromatograms at the marked points A, B and C are shown in Fig. 6. Experiments were performed as described in Section 2.3.

systems for CPC to ensure that the concentration was kept constant and not reliant on concentration fluctuations caused by temperature in saturated ammonium sulphate.

3.2. CPC separations

Initial CPC experiments with phase system XV determined the retention of stationary phase (S_F) at different flow rates (F) and rotational speeds. At a rotational speed of 1000 rpm the variation of stationary phase retention with mobile phase flow rate produced a linear relationship ($S_F = -3.7\,F + 86.6$ with $r^2 = 0.996$). An additional retention test at a mobile phase flow rate of $8\,\text{mL min}^{-1}$ gave a stationary phase retention of 57% which was then used for CPC separations.

As the primary constituents of hydrolysed SBP pectin, Ara and GA were studied in initial separation experiments. Rha was also included as its partition coefficient is lower than both Ara and GA while providing a reasonable separation factor with Ara (2.2, based on partition coefficients values). The expected elution order from the partition coefficient data (Table 3) would thus be Rha–Ara–GA for CPC operated in ascending mode, with the upper phase mobile and the lower phase stationary. In order to demonstrate the elution profile clearly, equal concentrations of sugar were used at concentrations of $50\,\text{g L}^{-1}$ each. Fig. 4 shows the CPC elution profile achieved with this 'model' mixture based on ICS analysis of the monosaccharide composition in collected fractions. This matches the expected elution order from partition coefficient data and shows that there is good separation achieved between Rha and Ara, and base line resolution achieved between Ara and GA.
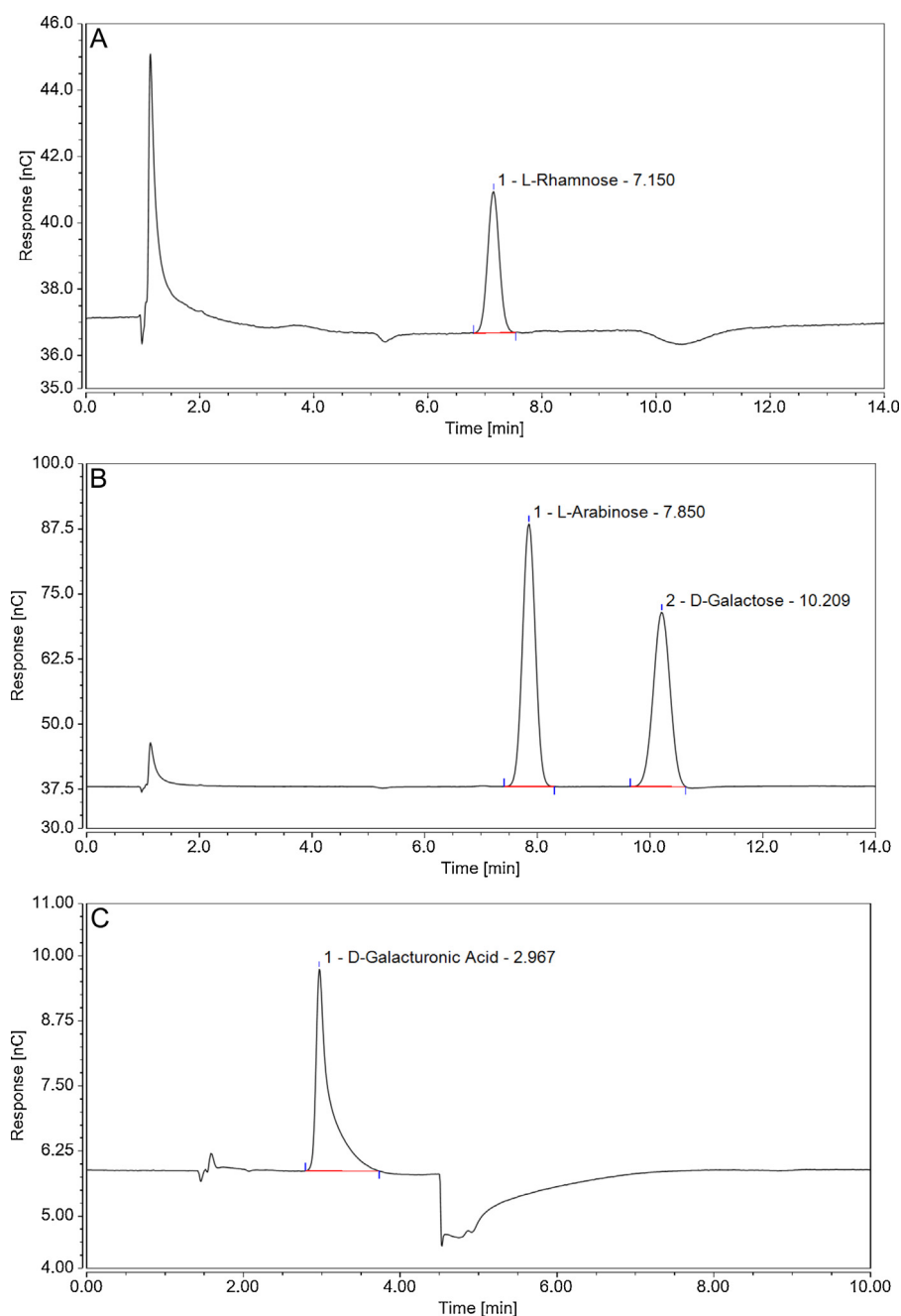

Fig. 6. Analytical chromatograms of CPC fractions from the synthetic crude separation at (A) 33 min; (B) 45 min; and (C) 79.8 min; (as shown in Fig. 5). (A) and (B) show the analysis of neutral sugars. (C) shows the analysis of GA. The two analytical methods used are described in Section 2.4.

Subsequently a CPC separation was performed using all four main sugars present in SBP pectin. This 'synthetic crude' material contained the monosaccharides at a concentration ratio expected in a hydrolysed SBP pectin fraction assuming the removal of cellulose, i.e. 43% Ara, 41% GA, 11% Gal and 5% Rha [5]. A total solute concentration of $100\,g\,L^{-1}$ was used. As shown in Fig. 5, elution of Rha started after 22 min and the whole separation process was completed within 2 h. The 'synthetic crude' material was separated into three main fractions; a highly pure Rha fraction (>90%, w/w) eluted between 27 and 37 min, a mixed Ara–Gal fraction eluted between 38 and 63 min (comprising 76% Ara and 22% Gal) and a highly pure GA fraction (>90%) eluted between 67 and 111 min. Fig. 6 shows example analytical chromatograms for specific points in the CPC elution profile demonstrating the sugars present in each fraction: Fig. 6A, Rha fraction at 33 min; Fig. 6B Ara and Gal fraction at 45 min; and Fig. 6C, GA fraction at 79.8 min. Two analytical methods are required to fully analyse each fraction: one for neutral sugars and one for GA (as described in Section 2.4). Fractions A and B showed no presence of GA and so only the neutral sugar analyses are shown. Similarly, fraction C showed no presence of neutral sugars and only the GA analysis is shown. It is important to note that for biorefinery applications complete fractionation of all four sugars into separate fractions is not necessarily required, for example, selective enzymatic modification of Ara in the presence of Gal.

A comparison of Figs. 4 and 5 showed that the monosaccharide elution profiles and retention times did not change between the 'model' and 'synthetic crude' separations. This indicates that the selected phase system (XV, Table 1) is stable to fluctuations in solute concentrations and the introduction of Gal into the feed stream. While the peaks appear in their expected order, the compounds eluted earlier than expected based on their partition coefficient values (Table 3). This is probably a result of the stationary phase stripping that occurs shortly after injection, resulting in a smaller stationary phase retention and compounds with K_D values higher than 1 eluting faster [44].

In terms of CPC operation these results show that Rha could be collected as a first fraction with high purity prior to the elution of Ara. While Ara has good separation from Rha and baseline separation from GA, it co-elutes with Gal in the second fraction. GA can be taken as a third and final fraction with high purity. However, it elutes as a broad peak, taking approximately 60 min (480 mL) for full recovery. In order to decrease the time of this separation process, and therefore increase the throughput, extrusion of the column contents after the Ara and Gal fraction could be considered. This would have the benefit of increasing the concentration of GA in the fraction, ensuring complete recovery and preparing the column for further injections. In the case of this elution–extrusion mode [24], the estimated throughput based on the current operating conditions at this semi-preparative scale would be $0.9\,g\,h^{-1}$ with 0.62 L of solvent per gram of total sugars. This is based on an extrusion flow rate of $16\,mL\,min^{-1}$ for 12 min after 1 h of elution at $8\,mL\,min^{-1}$.

With regards to further scale up and economic potential of the method, a number of areas would need further investigation. Firstly the application of the method described needs to be applied to the processing of the real crude hydrolysed SBP pectin. Secondly, a reduction in solvent usage needs to be sought by increasing the throughput, for example by increasing the injection volume and concentration. Thirdly, solvent recycling would need to play an important role in the industrial viability of this method. Ammonium sulphate could be recovered by precipitation by adding ethanol or DMSO, which are already present in the phase system, or by reverse osmosis [45]. Ethanol could be recovered by distillation. Furthermore, as it could be recycled and remixed with water, it would not be required to fully dehydrate the ethanol by breaking the azeotrope, simplifying the distillation step. DMSO could then be recovered by vacuum distillation and recycled. Finally the process economics would need to be addressed, taking into account solvent usage and recycling, particularly in comparison to resin based chromatography techniques. The overall process economics would also need to consider the value of the potential synthetic products from the selected Ara and GA fractions, which are envisaged to be chiral aminopolyols [46–48] for use as pharmaceutical intermediates.

4. Conclusion

This work represents an important first step in demonstrating the capability of CPC as a separation technology suitable for use within a whole-crop biorefinery context. It demonstrates the ability to separate compounds in a low value feedstock like SBP, after hydrolysis, into various fractions for subsequent conversion into higher value products. A highly polar ethanol:DMSO:aqueous ammonium sulphate $(300\,g\,L^{-1})$ (0.8:0.1:1.8, v:v:v) phase system was developed for CPC separation in ascending mode. The presence of DMSO was found to improve the separation factor of the solutes. 1.08 g of total sugars, in proportion to the concentrations in hydrolysed pectin derived from SBP, was loaded on a 200 mL CPC column and separated in under 2 h. Specifically, the four predominant monosaccharides from SBP hydrolysis were separated into three fractions (Rha, Ara and Gal, and GA). Future work will address the processing of crude material from actual SBP hydrolysis and further optimisation of operating conditions to achieve higher throughputs and yields while reducing solvent utilisation.

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