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Centrifugal partition chromatography in a biorefinery context: Optimisation and scale-up of monosaccharide fractionation from hydrolysed sugar beet pulp☆

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ABSTRACT

The isolation of component sugars from biomass represents an important step in the bioprocessing of sustainable feedstocks such as sugar beet pulp. Centrifugal partition chromatography (CPC) is used here, as an alternative to multiple resin chromatography steps, to fractionate component monosaccharides from crude hydrolysed sugar beet pulp pectin. CPC separation of samples, prepared in the stationary phase, was carried out using an ethanol: ammonium sulphate (300 g L^{-1}) phase system (0.8:1.8 v:v) in ascending mode. This enabled removal of crude feedstream impurities and separation of monosaccharides into three fractions (L-rhamnose, L-arabinose and D-galactose, and D-galacturonic acid) in a single step. Throughput was improved three-fold by increasing sample injection volume, from 4 to 16% of column volume, with similar separation performance maintained in all cases. Extrusion of the final galacturonic acid fraction increased the eluted solute concentration, reduced the total separation time by 24% and removed the need for further column regeneration. Reproducibility of the separation after extrusion was validated by using multiple stacked injections. Scale-up was performed linearly from a semi-preparative 250 mL column to a preparative 950 mL column with a scale-up ratio of 3.8 applied to mobile phase flow rate and sample injection volume. Throughputs of 9.4 g L^{-1} h^{-1} of total dissolved solids were achieved at the preparative scale with a throughput of 1.9 g L^{-1} h^{-1} of component monosaccharides. These results demonstrate the potential of CPC for both impurity removal and target fractionation within biorefinery separations.

1. Introduction

Sugar beet pulp (SBP) is an abundant low value by-product from the sugar beet processing industry, which, in the UK, utilises around 8 million tonnes of sugar beet per year. SBP is a rich source of carbohydrates, primarily consisting of cellulose and pectin with a low lignin content (1–2%) [1]. Sugar beet pectin has poor gelling properties due to its high degree of esterification [2], limiting its use as a gelling agent to that of a thickener [3]. As a result SBP has generally been dried and pelleted for sale as low value animal feed [4]. However, its abundance, low cost and high carbohydrate content indicates that SBP could be a significant sustainable feedstock for the production of chemical and pharmaceutical intermediates, while simultaneously undergoing waste valorisation within an integrated sugar beet biorefinery.

Previous work, as part of a wider project on sugar beet pulp fractionation and utilisation, has demonstrated that steam explosion, a high pressure steam treatment, can effectively and selectively convert SBP into two fractions [5]: solubilised pectin and enriched insoluble cellulose. We have demonstrated that the latter provides an effective glucose-based feedstock for fermentation into bioethanol after complete enzymatic hydrolysis [5]. This work focuses on the solubilised pectin fraction, which can be sub-

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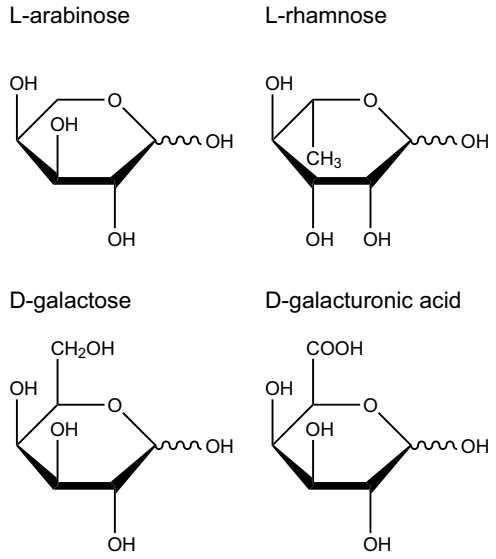

Fig. 1. Structures of the main monosaccharides present in SBP pectin.

jected to complete acid hydrolysis. This is an important first step in the conversion of complex carbohydrate heteropolymers like pectin [6], breaking them down into monosaccharides. In this case, the monosaccharides are primarily L-arabinose (Ara) and D-galacturonic acid (GA), as well as lesser amounts of L-rhamnose (Rha), D-galactose (Gal) and D-glucose (Glu). The structures of these four monosaccharides are shown in Fig. 1.

The pectic monosaccharides have a number of potential applications. GA, as an oxidised sugar, can be used to produce hyperbranched polyesters and plasticisers [7]. Ara can be used to produce biopolymers [8] or reduced to arabinitol, listed as an important value added chemical from biomass for the production of unsaturated polyester resins [7]. Ara can also be upgraded via biocatalysis using transketolase to produce L-gluco-heptulose, which has potential therapeutic applications [9].

Large scale monosaccharide separations are generally performed using simulated moving bed chromatography (SMB) on strong cation exchange resins [10]. SMB can be used to separate fructose and glucose for the production of high fructose corn syrup [11,12], however, SMB is generally limited to binary separations and requires clean input streams. In reality, process streams from biomass tend to contain multiple monosaccharides and a large number of contaminants. Consequently, a series of chromatography steps are required prior to SMB such as deashing and decolourisation [13]. In order to reduce the number of operating steps, a method capable of processing highly complex and contaminated samples is needed.

Centrifugal partition chromatograph (CPC) is a liquid–liquid chromatography technique that partitions solutes between two immiscible liquid phases. One liquid phase is held stationary and retained in the column via centrifugal forces, while the mobile phase is pumped past it in a series of interconnected chambers where the two phases mix and solute partitioning occurs. It has widely been used for the isolation of natural products [14], with only a small number of articles examining the separation of oligosaccharides [15] and glycosides [16], while the separation of monosaccharides has been restricted to the use of counter-current chromatography [17,18]. The lack of a solid stationary phase, simplicity of complete column regeneration and no irreversible adsorption, makes CPC an interesting technology for the separation of crude process streams with little pretreatment, potentially capable of removing impurities and isolating multiple target compounds in a single step.

In our previous CPC work, a synthetic mixture of Ara, GA, Rha and Gal was separated into three fractions (Rha, Ara and Gal, and GA) with an ethanol: ammonium sulphate (300 g L^{-1}): DMSO (0.8:1.8:0.1 v:v:v) phase system [19]. This highly hydrophilic two-phase system is capable of separating these structurally similar hydrophilic monosaccharides. In this study, the previously developed methodology was further optimised and applied for the first time for CPC separation of a fully hydrolysed crude sugar beet pectin fraction in order to remove impurities and fractionate Rha, Ara and Gal, and GA. Sample preparation was optimised for the crude material while throughput was improved by increasing sample volume and operating in elution-extrusion mode. Reproducibility of the elution-extrusion method was demonstrated and successful scale-up was performed linearly from a semi-preparative to preparative column.

2. Materials and methods

2.1. Reagents

L-Arabinose (99%), L-rhamnose (99%), D-galacturonic acid sodium salt (98%), D-galactose (99%), analytical grade ammonium sulphate and ethanol were purchased from Sigma-Aldrich (Gillingham, UK). Water was purified using a Millipore Synergy UV Water Purification System (Watford, UK). Sodium acetate trihydrate (ED grade) was purchased from Fisher Scientific (Loughborough, UK). Sugar beet pulp was provided by AB sugar (Wissington, UK).

2.2. Phase system

The two-phase system used throughout this work was ethanol: aqueous ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v). The phase diagram and the exact composition of each phase was determined as described by Ward et al. [19].

2.3. Sample preparation

Acid hydrolysed sugar beet pectin, referred to here as the 'crude' material, was prepared using steam explosion to fractionate the sugar beet pulp into insoluble cellulose and soluble pectin phases, as described in Hamley-Bennett et al. [5]. Then, the soluble sugar beet pectin was fully hydrolysed with 2.5% (v/v) sulphuric acid, heated to 121 °C for 1 h in an autoclave and then adjusted to pH 6 with NaOH. The crude material contained a total dissolved solids content of ~100 g L^{-1} with a total sugars concentration of ~20 g L^{-1}.

Crude samples were prepared for CPC by using the crude hydrolysate as the water proportion of the lower phase and adding appropriate amounts of ammonium sulphate (332 g L^{-1}) and ethanol (13% v/v). Samples were filtered through a 0.45 μm filter prior to injection.

'Synthetic crude' samples were prepared by dissolving the required masses of pure monosaccharides in water and adding the appropriate amounts of ammonium sulphate (332 g L^{-1}) and ethanol (13% v/v). The prepared monosaccharide composition contained Ara, GA, Gal and Rha at 43, 41, 11 and 5 g L^{-1} respectively, as in our previous work to give a total sugars concentration of 100 g L^{-1} [19]. Samples were filtered through a 0.45 μm filter prior to injection.

2.4. CPC equipment and operating conditions

CPC separations were performed on a Kromaton FCPC-A (fast centrifugal partition chromatography – Rousselet Robatel Kromaton, Annonay, France) on a semi-preparative and a preparative column with experimentally determined total volumes of 250 mL and 950 mL respectively. Both columns feature a twin-cell design

with 840 cells on the semi-preparative column and 800 cells on the preparative column. A puriFlash 450 (Interchim, Montluçon, France) system was attached to the CPC providing a flow controller, pump, injection valve and fraction collector.

Separations were performed in the ascending mode, with the lower phase (LP) stationary and upper phase (UP) mobile. This minimised the volume of lower phase (high in ammonium sulphate) pumped through the system, preventing corrosive damage to the pump seals and making fraction recovery easier as they are in the organic, mobile UP with a lower salt concentration (54 g L^{-1}). Both columns were filled by pumping the lower phase in ascending mode at a rotational speed of 600 rpm and a flow rate of 20 mL min^{-1}. The sample was injected at the start of mobile phase flow into the column without establishing hydrodynamic equilibrium.

On the semi-preparative column, a rotational speed of 1000 rpm, mobile phase flow rate of 8 mL min^{-1} and fraction collection time of 2 min (16 mL per fraction) were used. For sample injection, sample loops of 10, 20, 30 and 40 mL were used.

Scale-up onto the preparative column was performed linearly based on total working volume, as has been reported previously [20]. This working volume was determined experimentally by filling the column with LP, and eluting with UP in the descending mode at 1000 rpm. The process was repeated by filling the column with UP and eluting with LP in the ascending mode at 1000 rpm. The ratio of working volumes between the semi-preparative and preparative columns gave a scale-up factor (3.8) that was used for linear scale-up of the flow rates (from 8 to 30.4 mL min^{-1}) and sample volume (from 40 to 152 mL). Fractions were collected every 1.5 min giving 45.6 mL per fraction.

For elution-extrusion runs on both columns, extrusion of the column contents was performed at 72 min after injection, by switching to pump stationary (lower) phase, at the same flow rate as the mobile phase. Multiple stacked injections (30 mL of crude sample prepared in LP) were performed on the semi-preparative column by injecting successive samples at 28 min after the start of extrusion, coinciding with a switch back to pumping mobile phase. This gave a total run time of 100 min per injection for elution-extrusion runs.

2.5. Analytical methods

Fractions were analysed using a Reagent-Free Ion Chromatography System (ICS) (ICS 5000+, Thermo Scientific, Hemel Hempstead, UK) fitted with an AminoPac PA10 (2 × 250 mm) anion exchange column, with AminoPac PA10 guard column (2 × 50 mm), an eluent generator with a KOH 500 cartridge, and an electrochemical detector (gold electrode). An injection volume of 10 μL was used. The neutral monosaccharides (Ara, Gal and Rha) were analysed using 7.5 mM KOH as the mobile phase with a flow rate of 0.25 mL min^{-1} for 18 min at 30 °C. GA was analysed using a mobile phase of 5% (v/v) 1 M aqueous sodium acetate and 95% (v/v) Milli Q water at 0.25 mL min^{-1} for 5 min at 30 °C. Quantitative analyses were performed by measuring the peak area using the external standard method.

All fractions were diluted with Milli Q water to "break" any two-phase systems that formed as a result of stationary phase bleed from the column, ensuring that the analytical methods were not affected by solute partitioning. For the semi-preparative runs, 2 mL of water was added to 16 mL fractions, while for preparative runs, 4 mL was added to the 45.6 mL fractions.

2.6. Separation targets and quantification

As in a previous article [19], the aim of the separation was to obtain two main pooled fractions, a purified Ara fraction, where the presence of galactose was minimised, and a purified GA fraction. Additionally, a third fraction of Rha could be fractionated. An additional concern here, for the separation of the crude material, was the removal of the other, unknown, contaminating compounds, arising from the full acid hydrolysis of the SBP pectin.

Purity and recovery were calculated from the ICS analysis. Recovery was defined as the mass of the target monosaccharide in a pooled fraction as a percentage of the mass of the same target monosaccharide in all fractions. Purity was defined as the mass of the target monosaccharide in a pooled fraction as a percentage of the mass of all the monosaccharides in that same pooled fraction. Both purity and recovery values are given as percentages (w/w).

3. Results and discussion

3.1. Crude sample preparation and impact on CPC separation

The most straightforward option to access all the monosaccharides present in a complex feedstock like sugar beet pectin is to carry out acid hydrolysis. This was performed on the aqueous pectin fraction following steam explosion of the sugar beet pulp as described in Section 2.3. After full acid hydrolysis in an autoclave and adjustment to pH 6, a dark brown solution is formed with a mass of dried solids (determined gravimetrically) of approximately 100 g L^{-1} and a total monosaccharide concentration of approximately 20 g L^{-1} (Ara, 12 g L^{-1}; GA, 4 g L^{-1}; Gal, 3 g L^{-1}; Rha, 1 g L^{-1}, Glu 1 g L^{-1} as determined by ICS). The colouration could be the result of degradation of the GA [21,22] and neutral monosaccharides, such as fructose [23], during the hydrolysis step leading to the formation of browning products.

Being a liquid–liquid separation technique, CPC can cope with a large volume of sample, however, it is important to find a balance between high throughput, purity and yield without disturbing the hydrodynamic equilibrium within the column. It has also been shown that column hydrodynamics and thermodynamic equilibrium between the two phases can be greatly affected by sample preparation [24]. For synthetic crude mixtures, the monosaccharides could be prepared in either of the two phases, however, for the crude hydrolysate, it proved impossible to prepare the crude in the mobile phase (UP 44% v/v ethanol, 54 g L^{-1} ammonium sulphate) without precipitation of ammonium sulphate. There was no such precipitation or solubility difficulties when preparing crude hydrolysate in the stationary phase (LP, 13% v/v ethanol, 332 g L^{-1} ammonium sulphate). Full details of the sample preparation methodology are described in Section 2.3.

It was clear from these initial investigations that sample preparation would be an important consideration for separation of the real crude material. Therefore, CPC separations were first run in the ascending mode with a synthetic crude mixture containing pure monosaccharides, as described in Section 2.3, prepared in either the UP or LP to compare the effect of sample preparation in the mobile or stationary phases. While the crude hydrolysed sugar beet pectin contains only ~20 g L^{-1} of monosaccharides, the other compounds bring the total dissolved solids up to around 100 g L^{-1}. Consequently, a total monosaccharide content of 100 g L^{-1} was used for synthetic crude samples to simulate the total solids loading in the crude.

Table 1 shows the optimised values of purity and recovery for the target monosaccharides in the pooled fractions from the CPC separation of the synthetic crude. When switching from injecting the sample prepared in the UP (mobile) to LP (stationary), the purities and recoveries of Ara and GA are approximately the same, while Rha drops from 91 to 80% (w/w) recovery with purity remaining relatively constant. It is worth noting that injection of a sample made in the stationary phase can overstate the loss of stationary phase, as sample injection will elute a volume of stationary phase equal to

Table 1
Optimised purities and recoveries of target monosaccharides from CPC separations using different feed stream preparations. Feeds were 100 g L^{-1} synthetic crude samples prepared in either the UP or LP, and a crude sample of 20 g L^{-1} total monosaccharides (100 g L^{-1} total solids) prepared in the LP. CPC separation was performed on a semi-preparative, 250 mL Kromaton CPC using an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system at 1000 rpm in ascending mode, with a mobile phase flow rate of 8 mL min^{-1} and 10 mL injection volume, as described in Section 2.4. Purities and recoveries are given as% (w/w) as defined in Section 2.6.

	Sample Phase	S_f (%)	Rha			Ara			GA		
			Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)
Synthetic Crude	UP	58	90	91	28–42	81	94	44–62	97	97	70–118
	LP	64	92	80	28–36	78	95	40–58	95	96	60–110
Real Crude	LP	51	94	96	28–44	88	97	46–66	99	99	78–110

[a] Time refers to the pooled fraction time based on optimised purities and recoveries for target monosaccharides. Fractions were collected every 2 min as described in Section 2.4.

that of the sample injection volume (V_I). Stationary phase retention (S_f) for samples injected in the LP was therefore calculated using Eq. (1), (where V_C is total column volume, V_E is total eluted volume until mobile phase breakthrough and V_D is total dead volume). This equation should be used to take into account V_I when running separations in this manner, particularly when varying the injection volume. Therefore, for the LP sample injection, the stationary phase retention becomes 64%; greater than that of the UP sample injection. Overall these results demonstrate that sample preparation in the stationary phase yields similar separation performance to that of the mobile phase and could be a useful option for crude samples that do not solubilise well in the mobile phase.

$$S_F = \frac{V_C - V_E + V_I + V_D}{V_C}$$

With little difference in recovery and purity between synthetic crude sample preparation in the mobile or stationary phases, separations were attempted with the crude material. As stated previously, sample preparation of the crude sugar beet pectin in the mobile phase (UP) led to precipitation of the ammonium sulphate. Dropping the concentration of ammonium sulphate to 25 g L^{-1} allowed the salts to fully dissolve but led to complete stripping of the stationary phase with a 10 mL sample volume. In an attempt to fully utilise the liquid nature of the stationary phase, 10 mL of the neat crude sample (without mixing in either phase) was injected into the CPC column but also resulted in complete stripping of the stationary phase.

Preparing the crude material in the LP (stationary) (13% v/v ethanol and 332 g L^{-1} ammonium sulphate) led to its full solubilisation, however, a small volume (<10% v/v) of a new phase formed on top of the solution. This new phase was black in colour, indicating a high concentration of impurities; conversely, the rest of the solution was a much lighter brown, indicating a reduction in the level of coloured impurities. It is likely that the impurities causing this colour were acting as phase forming compounds, interacting with the salt in a similar way to ethanol to partition into a new UP. By discarding the new upper phase, a proportion of the impurities were removed with no effect on the concentration of monosaccharides in the sample (confirmed by ICS analysis), effectively utilising the sample preparation step as a form of purification.

The purity and recovery values for injecting crude sample in the LP are also shown in Table 1 with the reconstructed chromatogram shown in Fig. 2. It is clear that the separation is improved relative to the LP synthetic crude separation despite a drop in stationary phase retention from 64% to 51%. This is likely a result of the lower monosaccharide concentration; the crude had a total dissolved solids loading of 100 g L^{-1}, similar to that of the synthetic crude material, but total monosaccharides of only 20 g L^{-1}. This demonstrates that an optimised sample preparation methodology, which provides a partial or complete removal of these impurities prior to CPC separation, could allow similar total monosaccharide loadings.

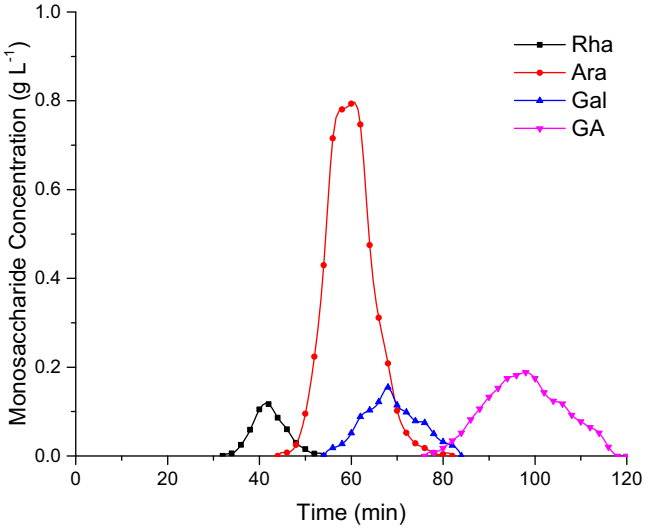

Fig. 2. CPC separation of 10 mL crude hydrolysed sugar beet pulp on the 250 mL semi-preparative CPC column. A rotational speed of 1000 rpm was used with an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system and a mobile phase flow rate of 8 mL min^{-1} in the ascending mode, as described in Section 2.4. The crude sample was prepared in the lower phase for injection, as described in Section 2.3.

3.2. Increasing throughput by increasing injection volume

Throughput is a major consideration for any separation technique used in a biorefinery context where the quantities of feedstocks utilised can be large. Crude separations were therefore performed with increasing injection volumes (from 10 to 20, 30 and 40 mL) to improve CPC throughput. In all cases, crude samples were prepared in the LP. As shown in Table 2, this resulted in an effective quadrupling of throughput with only relatively small reductions (within a 10% range) in purity and recovery of target monosaccharides in pooled fractions due to peak broadening. This broadening of the peaks extended the total elution time from 110 min to 140 min for 10 and 40 mL injections respectively.

Calculated throughputs for increasing injection sample volume are shown in Table 3. It is demonstrated that a four-fold increase in sample volume results in a three-fold increase in throughput, as peak widths increase with higher sample loads, increasing the total run time. Throughput values for elution-extrusion and scale-up are also presented in Table 3 and will be discussed in Sections 3.3 and 3.4 respectively.

3.3. Operation in elution-extrusion mode and reproducibility

One of the advantages of CPC compared to conventional resin based chromatography is that it is possible to manipulate the column contents, particularly the stationary phase, within a run to improve separation performance or reduce separation times [25].

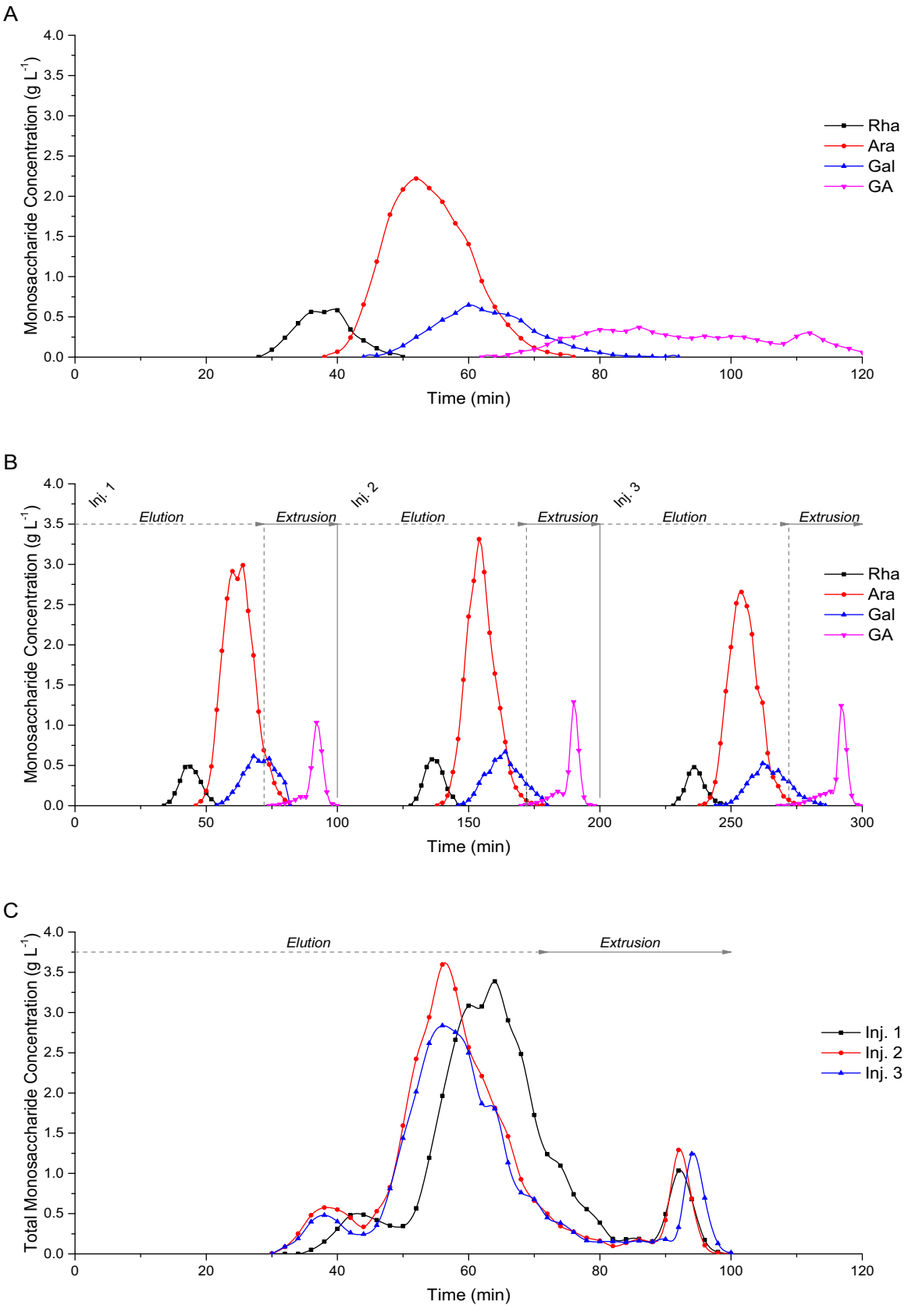

Fig. 3. CPC separation of 30 mL crude hydrolysed sugar beet pulp samples on the 250 mL semi-preparative CPC column. Crude samples were prepared in the lower phase for injection, as described in Section .32. A: elution profile of a single sample with no extrusion. B: Successive elution-extrusion CPC separations with a 100 min cycle time. C: CPC separation as in B with the time reset after each sample injection. CPC separation was performed using an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system at 1000 rpm in ascending mode, as described in Section .42.

Table 2
Optimised purities and recoveries for target monosaccharides from CPC separations with varying sample volumes. Crude samples were prepared in the lower phase for injection, as described in Section 2.3. Purity and recovery values are taken from pooled fractions (times shown). Stationary phase retentions for each sample volume are also shown. CPC separation was performed on a semi-preparative column, 250 mL Kromaton CPC using an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system at 1000 rpm in ascending mode, with a mobile phase flow rate of 8 mL min^{-1}, as described in Section 2.4. Purities and recoveries are given as % (w/w) as defined in Section 2.6.

Sample Volume (mL)	S_f (%)	Rha			Ara			GA		
		Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)
10	51	94	96	28–44	88	97	46–66	99	99	78–110
20	50	98	93	28–42	85	96	44–66	97	98	78–122
30	56	90	88	28–42	79	96	44–66	94	92	76–124
40	57	86	88	28–44	79	96	46–70	92	93	78–140

[a] Time refers to the pooled fraction time based on optimised purities and recoveries for target monosaccharides. Fractions were collected every 2 min as described in Section 2.4.

Table 3
Monosaccharide and total solids throughputs for CPC separations with various operating strategies. Crude samples were prepared in the lower phase for injection, as described in Section 2.3. Throughputs are given in grams of monosaccharide processed, per litre of total column volume, per hour of run time. Run time is defined as the time taken for full elution of all solutes. CPC was performed on a Kromaton CPC using an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system at 1000 rpm in ascending mode as described in Section 2.4. Elution-extrusion mode was performed, where stated, by switching to pump mobile phase 72 min after sample injection as described in Section 2.4.

Column volume (mL)	Sample Volume (mL)	Mode	Run Time (min)	Total monosaccharide throughput (g L^{-1} h^{-1})	Total solids throughput (g L^{-1} h^{-1})
250	10	Elution	110	0.44	2.2
250	20	Elution	122	0.79	3.9
250	30	Elution	124	1.2	5.8
250	40	Elution	140	1.4	6.9
250	30	Elution-extrusion	100	1.4	7.2
950	152	Elution	117	1.6	8.2
950	152	Elution-extrusion	102	1.9	9.4

Fig. 3A shows the elution profile of a 30 mL sample loaded onto the CPC column (Glu coelutes with Ara and so is not shown on the elution profiles). It is clear that the GA peak is excessively broad, taking one hour to fully elute. This band broadening primarily occurs as solutes leave the column as noted by Berthod et al. [26]. The use of elution-extrusion mode allows for shortening of the elution time of strongly retained compounds by rapidly extruding them from the column once the narrower, earlier fractions have eluted. Extrusion was performed by simply switching to pump stationary phase as described by Berthod et al. [25].

Extrusion also has the benefit of refreshing the stationary phase, an important aspect of improving throughput and reproducibility as separation will always occur on a regenerated column. Fig. 3B demonstrates the performance of CPC separation using the elution-extrusion mode, with three stacked 30 mL injections of crude sample prepared in LP. The extrusion step was started 72 min after sample injection and lasted for 28 min, at which point the flow was switched back to the mobile phase (UP) and the next injection was started. This gave a total cycle time of 100 min per sample. Fig. 3C demonstrates the good reproducibility of the method by overlaying the profiles for total monosaccharide concentration for each of the three injections based on ICS analysis.

Throughput values are presented in Table 3 and show that throughput was increased from 1.2 g L^{-1} h^{-1} to 1.4 g L^{-1} h^{-1} using the elution-extrusion method, relative to full elution, based on total run time. As confirmed in Fig. 3B, this operating strategy had the added benefit of being able to perform consecutive injections immediately without the need for further column regeneration.

3.4. CPC process scale-up

Linear scale up of the CPC column provides a further option for increasing throughput based on the volume ratio of the two columns. While this method can provide an effective method of scaling up, the separation behaviour is not expected to be strictly linear [27]. Improvements in separation performance can often be seen at the larger scale [20], and alternative scale-up methodologies have been developed to take advantage of this, including the free space between peaks method [28] and the use of global mass transfer coefficients and the stationary phase retention as scale-up invariants [29].

Table 4
Conditions used for scale-up of CPC separations from the semi-preparative column to the preparative column.

Operating Conditions	Semi-preparative	Preparative
Working volume (mL)	250	950
Mobile phase flow rate (mL min^{-1})	8	30.4
Injection Volume (mL)	40	152

The linear method presented here uses the working volumes of the columns, including extra volume before and after the column to scale up from a semi-preparative to a preparative scale CPC machine. It is therefore important that this extra volume is minimised when calculating the working volumes of the columns. The working volumes of the columns were determined to be 250 mL and 950 mL, respectively, giving a scale-up factor of 3.8 using the method described in Section 2.4. This scale-up factor was used to linearly scale-up the mobile phase flow rate, injection volume and stationary phase flow rate in the extrusion step. The extrusion time was maintained at 72 min between scales and rotational speed was kept constant at 1000 rpm to maintain the same g-force as the columns had the same diameter. A summary of the scale-up parameters is detailed in Table 4.

The scaled-up process showed a similar separation profile, as indicated in Fig. 4, with the same elution sequence of Rha, Ara and Gal, followed by GA in the extrusion. There was also comparable separation performance, as indicated by the calculated purity and recovery data in Table 5, between the semi-preparative (250 mL) and preparative (950 mL) scale machines. Although there is a drop in stationary phase retention with increasing scale (from 57 to 50%), the negative impact of this could be compensated by improved mixing within the larger cells of the preparative column. Nevertheless, the performance is similar, demonstrating process

Table 5
Optimised purities and recoveries for target monosaccharides for CPC separations at semi-preparative (250 mL) and preparative (950 mL) scales. Crude samples were prepared in the lower phase for injection, as described in Section .32. Scale-up parameters are outlined in Table 4. Extrusion was performed after 72 min in elution-extrusion mode at the preparative scale. CPC separation was performed using an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system at 1000 rpm in ascending mode and 10 mL injection volume, as described in Section 2.4. Purities and recoveries are given as% (w/w) as defined in Section 2.6.

Scale	Mode	S_f (%)	Rha			Ara			GA		
			Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)	Purity (%)	Recovery (%)	Time[a] (min)
Semi-prep.	Elution	57	86	88	28–44	79	96	46–70	92	93	78–100
Preparative	Elution	50	82	89	36–43.5	84	98	45–63	90	87	67.5–120
Preparative	Elution – Extrusion	50	92	93	33–45	84	97	46.5	96	95	78–100.5

[a] Time refers to the pooled fraction time based on optimised purities and recoveries for target monosaccharides. On the semi-preparative scale fractions were collected every 2 min, and on the preparative scale every 1.5 min as described in Section 2.4.

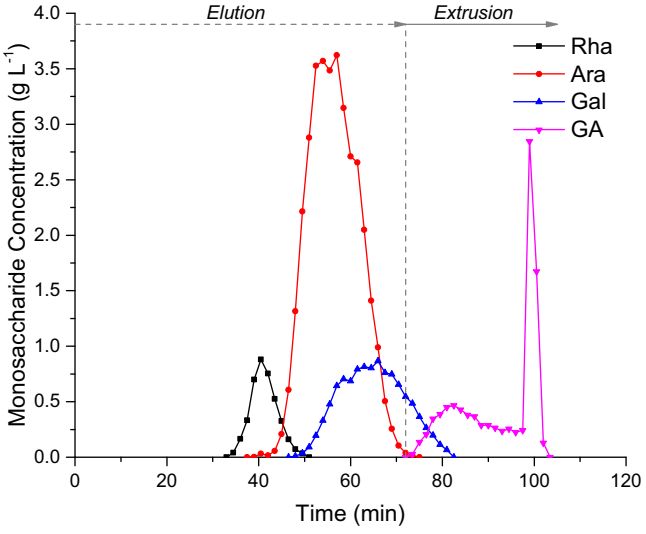

Fig. 4. CPC separation of 152 mL crude hydrolysed sugar beet pulp on the 950 mL semi-preparative CPC column. A rotational speed of 1000 rpm was used with an ethanol: ammonium sulphate (300 g L^{-1}) (0.8:1.8 v:v) phase system and a mobile phase flow rate of 30.4 mL min^{-1} in the ascending mode, as described in Section 2.4. Extrusion was performed 72 min after sample injection by switching to pump stationary phase as described in Section 2.4. The crude sample was prepared in the lower phase for injection, as described in Section 2.3.

stability between scales with the elution-extrusion mode outperforming the full elution method in terms of product purity and recovery. Furthermore, throughput values, shown in Table 3, are improved beyond the linear scale-up factor of 3.8; when normalised per litre of column volume, the results give a throughput of monosaccharides of 1.4 g L^{-1} h^{-1} at the semi-preparative scale and 1.7 g L^{-1} h^{-1} at the preparative scale under full elution conditions. Under elution-extrusion conditions, this throughput is increased even further to 1.9 g L^{-1} h^{-1}.

4. Conclusions

This work demonstrates the application of CPC to the separation of pectin monosaccharides from crude hydrolysed sugar beet pulp. This is a representative biorefinery feedstock with a large number of impurities and a high dissolved solids loading. The CPC separation method developed here simultaneously removes impurities and isolates three target monosaccharides (Rha, Ara and GA) into separate fractions when using the crude material. It is shown that crude samples must be prepared in the stationary phase, allowing the material to solubilise without precipitation, with a total monosaccharide concentration of 20 g L^{-1} and a total solids loading of 100 g L^{-1}.

Increasing the sample volume from 4 to 16% of the column volume at the semi-preparative scale, provided a three times improvement in throughput with only small losses in separation performance. Extrusion of the column contents during the elution of the final GA fraction reduced the total run time by 24% and allowed successive injections to be performed without further regeneration of the stationary phase. Finally, a linear scale-up from the semi-preparative (250) mL to preparative (950 mL) scale resulted in comparable separation performance while further increasing the throughput from 6.8 g L^{-1} h^{-1} to 8.2 g L^{-1} h^{-1} total solids in elution mode. This was further improved to 9.4 g L^{-1} h^{-1} total solids in elution-extrusion mode. This represents an increase in monosaccharide throughput from 1.4 g L^{-1} h^{-1} to 1.9 g L^{-1} h^{-1}, normalised by column volume, by employing scale-up and elution-extrusion without a significant change in product purity or recovery.

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