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Facilitating the use of counter-current chromatography in pharmaceutical purification through use of organic solvent nanofiltration

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ABSTRACT

This paper demonstrates a combined approach for separating an active pharmaceutical ingredient (API) from a heavily contaminated waste stream. The approach uses organic solvent nanofiltration (OSN) to improve the application of counter-current chromatography (CCC) in an industrial process. OSN provides an efficient route for exchanging solutes from the process solvent into the desired mobile phase for CCC, generating a CCC feed containing less than 0.01% (area % by GC) of the original process solvents. The high solvent burden of CCC was additionally reduced through recovery of mobile phase using OSN, with the recovered solvent containing less than 1% (area % by HPLC) impurities. The recovered solvent was then successfully recycled into a subsequent CCC run with no indication of impurity build-up. Coupling OSN with CCC improved the mass-intensity of the CCC process, reducing the solvent use by 56%. OSN can be a useful tool in facilitating the application of CCC to pharmaceutical process streams.

1. Introduction

Regulators of the pharmaceutical industry, such as the European Medicines Agency and the Food and Drug Administration, are continually evolving their rules and regulations with key focus being placed on quality assurance to ensure patient safety [1,2]. Manufacturing high purity drug products is a challenging responsibility faced by the pharmaceutical industry and increasing demand for higher purity products is often testing the limitations of conventional separation techniques. As a result scientists are more frequently looking to less established methods in order to exploit potential increases in separation efficiency. Two examples of such emerging techniques are counter-current chromatography (CCC) [3–5] and organic solvent nanofiltration (OSN) [6–8].

CCC is a liquid–liquid chromatography technique in which solutes are separated based on their differential solubility between two immiscible phases. Like all chromatography techniques, selection of the correct stationary and mobile phases is a key factor to achieve efficient CCC separation, and the selected solvent systems are highly specific. The most suitable solvent system for a given separation is commonly selected by looking at the partitioning coefficient (K_d) of the compound of interest and related impurities over a range of systems. K_d is defined as the solute distribution between the two immiscible phases of a given solvent system and can be calculated as the ratio between the solute present in the stationary phase divided by the amount present in the mobile phase. Ideally K_d for the target compound should be close to 1 enabling solute elution after one column volume of mobile phase has passed. Additionally the separation factor, defined as the ratio between K_d for the target compound and related impurities, should ideally be large (above 1.5) to enable sufficient separation of target and impurities [9].

To avoid solvent contamination of column, applications of CCC usually begin with a solvent exchange transferring the solute matrix to be separated from the process solvent to the solvent mixture selected for the mobile phase [9,10]. Thermal techniques, such as evaporation, commonly used for this purpose can be time consuming, energy intensive and in certain cases cause product degradation. On a larger scale thermal operation becomes even less viable due to equipment limitations, and an additional draw-back is seen in that distillation is only suitable for exchanging solvent from a low to a high boiling point. This paper demonstrates the potential for using OSN to perform an initial solvent exchange for a crystallisation mother liquor, supplied by GlaxoSmithKline (GSK), into a selected CCC mobile phase system for recovery of the GSK drug component from a crowded impurity profile. An additional limitation of CCC application can be seen with regards to commonly high mass-intensity values calculated to range between 25×10^3 and 124×10^3 for an example CCC separation of cryptotanshinone and tanshinone IIA [11–14]. For calculated example CCC mass-intensity is significantly higher than average mass-intensity values for a full production process of an active pharmaceutical ingredient (API)

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[15]. Mass-intensity is defined as the ratio between the total mass of the material used to generate a quantity of product per unit of target compound produced, and is used as a measurement for process comparison in the pharmaceutical industry [16]. Mass-intensity can be calculated according to Eq. (1) where *MI* is the mass-intensity and *m* is the mass.

$$MI = \frac{m_{\text{solvent}} + m_{\text{crude}}}{m_{\text{API}}} \quad (1)$$

This paper demonstrates how membranes are capable of reducing the typically high solvent burden of the CCC process through recovery and recycle of mobile phase solvent. Both solvent exchange and solvent recovery steps are commonly over-looked when considering CCC applications, but these are critical unit operations if CCC is to be used at anything other than a lab scale.

OSN is a pressure-driven membrane based separation process where an incoming feed stream is separated into two components referred to as the permeate (solvent and solute able to pass through the membrane) and the retentate (solvent and solute unable to pass through the membrane) [17,18]. OSN membrane selectivity is based primarily on steric factors hence separating impurities of a similar molecular weight to the desired product can be challenging when OSN is used alone. In addition to steric exclusion membrane–solvent interactions, pressure, feed concentration, temperature and system charge can be used to fine tune the separation performance [17,19–25]. OSN is a non-thermal technique and is frequently mentioned as offering potential advantages over conventional techniques, with regards to improved energy-efficiency and minimised loss of API due to thermal degradation [7,26,27]. Crucially OSN also allows low boiling point solvents to be replaced with higher boiling points solvents during a solvent exchange.

Membrane performance is most commonly described with regards to rejection and flux. Membrane rejection is defined as the percentage of a given solute that is unable to pass the membrane, and can be calculated according to Eq. (2) where R_i is the rejection of species *i* and C_i is the concentration in the feed (*f*) and permeate (*p*) respectively [17].

$$R_i = \left(1 - \frac{C_{i,p}}{C_{i,f}}\right) \times 100 \quad (2)$$

The rejection is sometimes defined as the ratio between the permeate and the retentate concentration, rather than the permeate and the feed. For the most accurate rejection measurement the concentration in the feed vessel at the time of permeate sampling should be used for calculations. However lab-scale batch filtration equipment does not usually allow feed sampling during the run, and an estimated concentration based on feed and retentate values is likely to be the most representative available value for the concentration. The permeate flux is defined as the volume of solvent passing through a unit area of membrane per unit time. The flux is calculated according to Eq. (3) where *J* is the flux, *V* is the volume, *A* is the membrane area and *t* is the collection time [17].

$$J = \frac{V_p}{At} \quad (3)$$

An additional parameter often used to describe membrane separation performance is the molecular weight cut-off (MWCO), which is defined as the molecular weight for which 90% of a given solute is rejected by the membrane [28]. MWCO values are often supplied by the manufacturer and provide an initial indication of the membrane operating range. However MWCO is highly dependent on the solvent–solute system used for membrane characterisation, and as varying methods are being employed by different manufacturers, caution must be applied before relying on these values [28,29]. A further inadequacy relating to the application of MWCO is the change in molecular weight required to move the rejection from 90 to 100%. If the membrane rejection curve is not sharp, the molecular weight required to reach full rejection might be significantly higher than indicated by the MWCO. The shortcomings of current membrane characterisation techniques mean that screening a large number of membranes remains an integral part of membrane process development.

Poorly defined MWCO curves have restricted the use of commercial OSN membranes to separations where there is a large difference in molecular weight, commonly ranging from 300 g mol^{-1} [8,29–31] and upwards [32,33]. Despite ongoing research in membrane synthesis the ability to efficiently fractionate similarly sized multi-component solutions solely using OSN remains poor. Thus, developing a purely membrane based approach for the API recovery discussed in this paper is currently unachievable. OSN can however be a useful tool for exchanging the solvent of a solution [34], and can also be used for recovering clean solvent through use of a tight membrane that retains all species [8]. Solvent exchange and high solvent intensities are two challenging process considerations currently facing large scale CCC application, and this paper describes a process where these challenges are overcome using OSN (see Fig. 1). More specifically the separation described here aims to recover pure API (molecular weight ~600 g mol^{-1}) from crystallisation mother liquors (82.0% methanol, 15.9% methyl isobutyl ketone and 2.1% toluene) supplied by GSK containing approximately 4.5 g L^{-1} API and 27 different organic impurities of varying size and molecular properties.

2. Experimental

2.1. OSN filtration

2.1.1. Membrane preconditioning

All membrane discs were washed with a minimum of 40 L pure solvent per m^2 membrane area (*i.e.* 220 mL for a 0.0054 m^2 disc) prior to addition of the feed solution. Washing solvent was selected based on the feed composition with pure ethyl acetate being used for the solvent exchange membranes and a mixture of 30:70% ethyl acetate and heptane being used for the solvent recovery membranes. After washing the filtration system was depressurised and the content changed for the feed solution. The feed was re-circulated through each membrane, at the desired operating pressure, for a minimum of 1.0 h or until a stable flux was reached. When operating at a stable flux the membranes were assumed to have reached close to maximum compaction and tests were started for the membrane screening, solvent exchange and solvent recovery respectively (see Sections 2.1.2–2.1.4). All processing was carried out at 30 bar pressure and ambient temperature (ranging between 25 and 30 °C).

2.1.2. Membrane screening

Membrane screening was carried out using a MET®Cell Cross-Flow system connecting 2–3 filtration cells with individual areas of 0.0054 m^2 in series. Three separate tests were carried out looking at performance in a solution mimicking the CCC mobile phase (screening solution I), pure ethyl acetate (screening solution II) and the mother liquors (screening solution III) respectively. Membrane performance was evaluated through flux and rejection calculations (see Eqs. (2) and (3)) with the permeate being sampled at the end of the pre-conditioning phase and feed and retentate samples being taken at the start and finish of each test. The flux was measured every 0.5 h by collecting permeate into a measuring cylinder over a given period of time.

2.1.3. Solvent exchange

Solvent exchange was conducted in a MET®Cell Dead-End filtration system using a Starmem™122 membrane (batch 9101.4) and

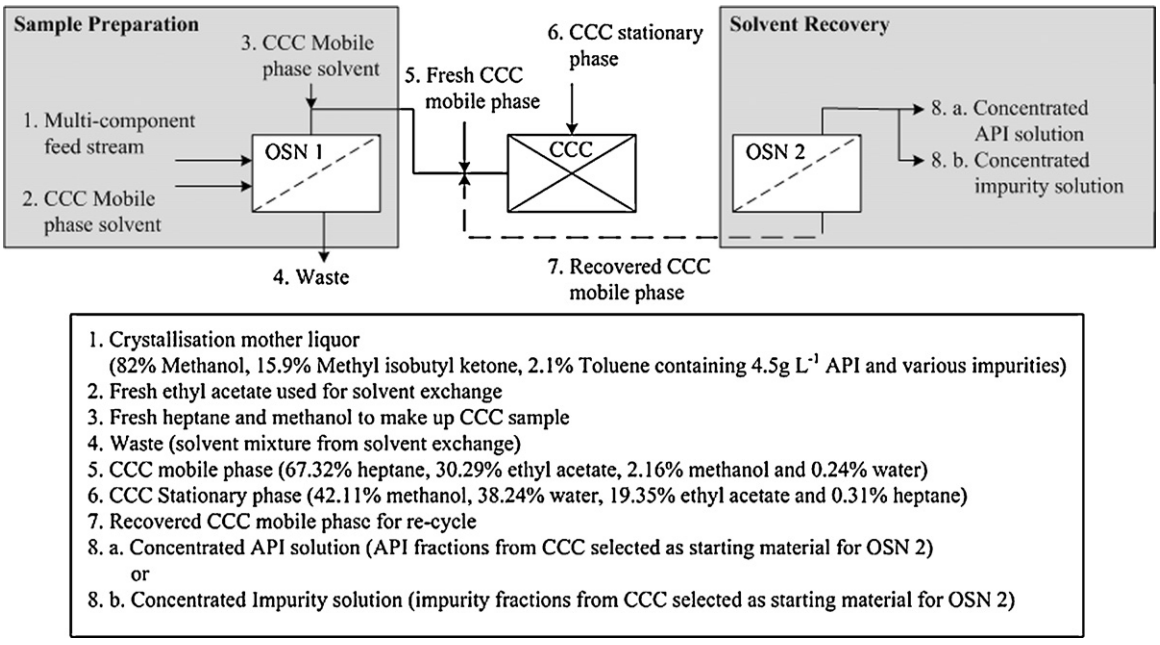

Fig. 1. Process diagram for suggested OSN and CCC hybrid application.

gradual addition of ethyl acetate in a put-and-take diafiltration. For each diafiltration cycle the feed (50:50% crystallisation mother liquor and ethyl acetate) was concentrated through removal of 70% of the original solvent before the system was depressurised and the remaining retentate was mixed with pure ethyl acetate to a volume of 200 mL. Concentration and addition of ethyl acetate was repeated in cycles until the desired solvent composition was reached. To ensure maximum concentration of API in the CCC sample no addition of ethyl acetate was made after the final concentration cycle. Flux was measured every 0.5 h during the pre-conditioning and for every 50 mL permeate passed during the solvent exchange cycles. Permeate samples were taken at the start and finish of each concentration run, in addition to samples of the combined permeate. To minimise API losses the feed and retentate were sampled only at the start and finish of the full solvent exchange and estimated concentrations based on mass-balances were used for rejection calculations at all intermediate stages. To investigate membrane performance over time the same membrane disc was used for the full solvent exchange with the membrane being exposed to nine pressure cycles and over-night storage during two subsequent nights.

2.1.4. Solvent recovery

Solvent recovery was carried out in a MET® Cell Dead-End filtration system using Starmem™ 240 (batch 9217.1) for all processing. To minimise the number of pressure cycles, and as the total volume for each fraction was larger than the equipment operating volume, solvent recovery was operated in a constant volume diafiltration with feed solution being added to the system at a rate equivalent to the permeation. Diafiltration was continued until the full volume had been added to the system after which the feed was concentrated to a level limited by the solubility limit for each fraction. The flux was measured every 0.5 h during the pre-conditioning and for every 50 mL permeate passed during the recovery. Permeate samples were taken at the start and finish of each recovery run, in addition to samples of the combined permeate, and the feed and retentate were sampled at the start and finish of recovery from each fraction. To ensure consistent membrane performance a new disc was used for solvent recovery from each fraction.

2.2. CCC separation

Analytical scale CCC runs were carried out using a Mini centrifuge supplied by Dynamic Extractions Ltd. The Mini equipment contains a centrifuge fitted with a single bobbin 20 mL coil made up of 0.8 mm bore tubing. During CCC operation the coil acts as the column and for the Mini operation the spin rate was set to a constant value of 2100 rpm. After equilibration of the column, a sample volume of 0.9 mL was injected and CCC operation was carried out using a flow rate of 1.5 mL min^{-1} for a total of 35 min collecting 10 fractions of 5.25 mL each. A preparatory CCC run was conducted using a Midi Centrifuge system also supplied by Dynamic Extractions Ltd. The set-up for the Midi equipment was similar to the Mini, with the exception that the centrifuge volume is divided between two bobbin coils of 4.0 mm bore tubing having a combined volume of 925 mL. For Midi scale operation a lower spin rate of 1400 rpm was used to maintain a constant gravitational field to the Mini run. Additional parameters were scaled through volumetric scale-up with a sample size of 41 mL injected using a total processing time of 35 min at a flow rate of 70 mL min^{-1} for the mobile phase, collecting 10 fractions of 245 mL each. Fraction collection for both the Mini and the Midi run were started immediately after the sample was injected. Prior to sample injection on both Mini and Midi scales the column was pre-conditioned by pumping mobile phase through the column, gradually displacing stationary phase. Preconditioning was continued until no more stationary phase was eluting at which point maximum stationary phase retention was assumed. For all CCC runs the outward flow was connected to a diode array detector set at 260 nm to enable in-process monitoring of impurity and API elution.

To minimise solvent requirements and to mimic industrial preparation, stationary and mobile phases were made up individually as single saturated phases [14,35]. Based on previous method development [36] the most suitable solvent system was selected as HEMWat 17.5 [35] corresponding to a stationary phase composition of 42.11% methanol, 38.24% water, 19.35% ethyl acetate and 0.31% heptane, and a mobile phase composition of 67.32% heptane, 30.29% ethyl acetate, 2.16% methanol and 0.24% water. To ensure consistency of stationary and mobile phases, a partitioning test was carried out comparing data from the individually made up phases

to HEMWat 17.5 made up as a bulk phase system. As a partitioning test, 1 mg of crude material (fully evaporated mother liquor sample) was dissolved in 0.5 mL of stationary and mobile phase from the fresh solvent system used for CCC run 1, the recovered solvent system used for CCC run 2 and HEMWat 17.5. Samples were mixed and allowed to settle prior to HPLC analysis of each phase.

2.3. Analysis

API and impurity concentrations were monitored using an Agilent 1100 Series HPLC system. No details on analytical technique can be disclosed to ensure confidentiality of API structure and properties.

Solvent levels for ethyl acetate, heptane, methanol, methyl isobutyl ketone and toluene were analysed using a Hewlett Packard HP 6890 Series Gas Chromatograph (GC) system. Samples were analysed using a flame ionization detector with a 10 m long, 200 μm diameter and 1.12 μm film thickness DB-624 GC column (Agilent Technologies, Delaware). The oven temperature was held initially at 240 °C and the column temperature was controlled with a program ranging from an initial value of 35 °C held for 2.0 min, increased to 80 °C at 50 °C min^{-1} and held for 1.0 min and finally increased to 150 °C at 210 °C min^{-1} and held for 1.0 min. The injector temperature was kept constant at 200 °C and the total injection volume was set to 1.0 μL using a split injector mode of 40:1. The detector temperature was set to 250 °C and detection was enabled using a make-up flow of 34.0 mL min^{-1} nitrogen combined with an air flow of 450.0 mL min^{-1} and a hydrogen flow of 40.0 mL min^{-1}. Helium was used as a carrier gas and the flow rate was determined through a pressure ramp ranging from 2.85 to 30.6 psi over 6.3 min. Traces of water in the solvent mixture were measured using volumetric Mitsubishi Karl Fischer moisturemeter CA-100/KF-100.

3. Results and discussion

3.1. Membrane screening

The most important part for successful OSN operation is selection of a suitable membrane and the first stage for an OSN application is commonly a membrane screening. Ideally the selected membrane should have excellent long-term stability in all processing solvents used, and display sharp MWCO curves ranging up to 100% rejection to minimise solvent requirements and solute losses during filtration. Additionally a high solvent permeation rate is desirable and flux must be high enough to enable processing within a reasonable time and membrane area.

For the OSN application discussed here, solvent recovery and a majority of the solvent exchange should ideally be carried out in a solvent composition similar to the CCC mobile phase (67.32% heptane, 30.29% ethyl acetate, 2.16% methanol and 0.24% water). To evaluate membrane performance the mobile phase composition was hence selected as screening solution I and a solution containing 4.5 g L^{-1} API was used for the study. In addition to the API crystallisation liquors contain various concentrations of 27 different impurities. To obtain maximum information prior to membrane selection, all impurities should ideally be included in the screening solution. However impurities are not readily available in dry form and consequently the API was selected as the sole, initial marker for evaluating membrane performance (see Table 1). Membranes selected for screening experiments included membranes from the Duramem™, Starmem™ and Puramem™ series, a range of commercially available OSN membranes suitable for use in organic solvents.

Screening in the CCC mobile phase indicate that the observed rejection for all membranes tested were below the desired value of >99%, with the most promising result being observed for Starmem™240 having a measured rejection of 98.5% and a flux of 48 L m^{-2} h^{-1} (see Table 1). The low rejections observed for Duramem™150, Duramem™200 and Starmem™122 (76.1, 21.7 and 83.1% respectively) are unexpected as all three membranes have MWCOs significantly below the molecular weight of the API. Deviation from the rejection values are likely to be the result of different solvents being used in the membrane screening compared to the MWCO characterisation [20,21,24,37]. Changing results for different solvent–solute combinations indicate that a more universal characterisation method for membrane performance is highly desirable [28,38]. However until such data is available membrane screening remains an important part for any membrane process under development. Additionally the flux for Duramem™200 is increasing from 11 to a semi-stable value of 27–29 L m^{-1} h^{-1} throughout the screening. The flux increase in combination with the low rejection values observed could indicate that this membrane is less suitable for use in heptane containing solvent mixtures.

Starmem™240 has a high API rejection and could potentially be used for recovery of CCC mobile phase through a single or multiple membrane pass (see Section 3.4). However for a solvent exchange multiple permeate passes are not suitable from a processing perspective and the measured rejection of 98.5% for Starmem™240 is calculated to result in API losses of approximately 8% throughout the solvent exchange. Potential losses of API indicate that a solvent exchange directly into mobile phase is not ideal, and another alternative is highly desirable. The second largest component in the CCC mobile phase is ethyl acetate, and in an attempt to improve rejection [28] and hence minimise the overall API losses, a solvent exchange directly into pure ethyl acetate rather than the full CCC mobile phase composition was suggested. Once the components have been exchanged into ethyl acetate the solution can be made up to the correct CCC solvent composition. For an exchange directly into ethyl acetate the CCC sample will be more dilute, however the process offers significant advantages as several membranes stable for use in ethyl acetate are commercially available. Pure ethyl acetate was selected as screening solution II with the API used as a marker for membrane performance (see Table 2). Finally to evaluate potential changes in membrane performance for different solvents,

Table 1
Summary of API rejection and flux data for screening solution I (CCC mobile phase).

Membrane	MWCO (g mol^{-1}) [39–41]	Flux (L m^{-2} h^{-1})	Rejection (%)
Duramem™150	150[a]	0.2	76.1
Duramem™200	200[a]	28	21.7
Starmem™122	220[b]	8	83.1
Starmem™240	400[b]	48	98.5
Puramem™280	280[c]	9	86.7

[a] Based on rejection of styrene oligomers dissolved in acetone.
[b] Based on rejection of alkanes dissolved in toluene.
[c] Based on rejection of styrene oligomers dissolved in toluene.

Table 2
Result summary for screening solution II (ethyl acetate) and III (crystallisation mother liquor).

Membrane	Screening solution	Flux (L m^{-2} h^{-1})	Rejection (%)
Duramem™150	II	5	99.1
Duramem™200	II	29	91.6
Starmem™122	II	84	99.8
Starmem™240	II	88	99.5
Puramem™280	II	77	99.6
Duramem™150	III	16	99.2
Duramem™200	III	55	96.5
Starmem™122	III	59	98.4
Starmem™240	III	48	98.9
Puramem™280	III	53	98.2

and to get an estimation of the rejection of the impurities present, the crystallisation mother liquor was selected as screening solution III (see Table 2).

For screening in ethyl acetate (solution II) the strongest membrane performance was observed for Starmem™122 having an API rejection of 99.8% combined with a high flux of 84 L m^{-2} h^{-1}. When using the crystallisation mother liquor (solution III, 82.0% methanol, 15.9% MiBK, 2.1% toluene containing ~4.5 g L^{-1} API and 27 different organic impurities) the API rejection for Starmem™122 was however reduced to 98.4%, and the most suitable membrane performance was observed for Duramem™150 having a rejection of 99.2% in combination with a flux of 16 L m^{-2} h^{-1}. A similar decrease in rejection was also observed for Starmem™240 and Puramem™280 when comparing data from screening tests in ethyl acetate and the crystallisation mother liquors, whereas for Duramem™150 and Duramem™200 the rejection remained constant or increased during screening in the mother liquors compared to data in ethyl acetate. Changes in rejection are likely to be a result of the changing solvents influencing the membrane performance [28,29], however additional factors such as solvent–solute interactions could also be contributing to the observed changes. The majority of the solvent exchange discussed here will use solutions composed mainly of ethyl acetate and only the initial concentration stage will be carried out using a feed that is closer to the crystallisation mother liquor composition (see Section 3.2). Based on this information Starmem™122 was selected as the most suitable membrane candidate for the proposed solvent exchange.

3.2. Solvent exchange

Solvent exchange through nanofiltration can be carried out through a continuous or discontinuous diafiltration process. In a discontinuous diafiltration, also called a put-and-take process, the feed solution is concentrated to a pre-determined level before the system is depressurised and the feed volume adjusted to the original value using fresh solvent. Process steps are repeated in cycles until the desired solvent composition is reached. For a continuous diafiltration, fresh solvent is added to the feed vessel using an HPLC pump at a rate equivalent to the permeation. Continuous operation adds potential advantages with regards to reduced need for process monitoring and manual operation. However the solvent requirement for a continuous process is generally higher than for a put-and-take process, and for the solvent exchange described in this paper, calculations indicate that for continuous operation an additional 3.5 diafiltration volumes (1 diafiltration volume = 200 mL or the feed volume) of solvent are required to reach the desired solvent composition. In order to conserve mass-efficiency, a put-and-take diafiltration using a 400 mL 50:50 mixture of crystallisation mother liquor and ethyl acetate as feed and a concentration level of 70% for each cycle, was selected for the solvent exchange presented in this paper.

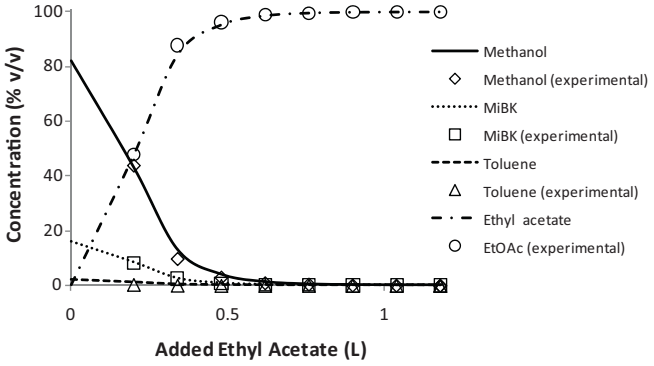

Fig. 2. Summary of calculated and experimental solvent levels obtained during put-and-take diafiltration of crystallisation mother liquor using ethyl acetate.

The solvent target composition was set to 99.99% (% volume) ethyl acetate with trace levels of solvents from the mother liquor (methanol, methyl isobutyl ketone and toluene) restricted to a maximum level of 0.01% (% volume). The level for mother liquor solvent traces was set to a very low value to limit potential contamination of the CCC stationary phase. The solvent level for each cycle was calculated using a mass-balance assuming 0% rejection for all solvents present (see Fig. 2). Mass-balance was calculated based on Eq. (4) where C is the concentration of solvent component i in the feed (f), permeate (p), added diafiltration volume (d) and retentate (r) respectively, and V is the volume. Important to note is that the retentate volume refers to the volume after the put-and-take cycle has been completed, and is hence equal to the feed volume.

$$C_{i,f} = \frac{V_f C_{i,f} - V_p C_{i,p} + V_d C_{i,d}}{V_r} \quad (4)$$

Solvent levels were monitored for each put-and-take cycle of the solvent exchange using GC. Data indicate that the desired solvent composition was reached after 8 additions of ethyl acetate making up a total of 5.9 diafiltration volumes, for a starting volume of 400 mL containing 200 mL mother liquor (see Fig. 2 and Table 3). The measured solvent composition correlated well with the calculated level from the mass-balance indicating that the assumption of a 0% solvent rejection holds true for the given system, and for a well mixed solution the solvent composition should be maintained over the membrane (see Fig. 2).

Analysis of API concentrations in the feed, permeate and retentate showed that the API rejection ranged between 99.3 and 99.9% for all put-and-take cycles, resulting in an overall API loss of 2.3% (see Table 3). Observed rejections were consistent with data measured during membrane screening for all stages except the initial

Table 3
Summary of the observed rejection, API losses and solvent composition for each put-and-take solvent addition.

Cycle	Added volume[a] (–)	Rejection (%)	API losses (%)	Solvent composition			
				Methanol (% v/v[c])	MiBK[b] (% v/v[c])	Toluene (% v/v[c])	Ethyl acetate (% v/v[c])
1	1.0	99.3	1.4	43.6	8.1	0.4	48.0
2	1.7	99.7	1.6	9.7	2.4	0.08	87.8
3	2.4	99.9	1.7	3.0	0.8	0.01	96.3
4	3.1	99.9	1.7	0.9	0.2	0.01	98.8
5	3.8	99.9	1.8	0.4	0.06	0.001	99.5
6	4.5	99.9	1.8	0.1	0.003	<0.001	99.9
7	5.2	99.9	1.8	0.07	<0.001	<0.001	99.9
8	5.9	99.4	2.2	0.01	<0.001	<0.001	99.99
Retentate	5.9	-	2.3	0.01	<0.001	<0.001	99.99

[a] 1 volume = 200 mL (feed volume).
[b] Methyl isobutyl ketone.
[c] % Volume.

Table 4
API recovery from CCC runs 1 and 2.

	CCC run 1	CCC run 2
Recovered API (mg)	3.51	3.56
Total API added (mg)	3.51	3.51
Overall recovery (%)	99.9	101.4

concentration of the mother liquor (see Section 3.1). Prior to the first put-and-take cycle, ethyl acetate was added to the crystallisation mother liquors to a level of 50% (% volume). For the mixed feed the API rejection was observed to increase from the expected value of 98.4% to a value of 99.3% (see Table 3) and though some losses of API will still occur, this study show that the overall losses can be minimised through early addition of ethyl acetate. The observed increase in API rejection for addition of ethyl acetate is further consistent with trends observed during the membrane screening (see Table 2), and strongly indicates that the performance of Starmem^{TM}122 is dependent on membrane–solvent–solute interactions.

3.3. CCC

Prior to the CCC separations K_d values of the individually prepared stationary and mobile phases were evaluated. Partitioning samples were analysed with HPLC and data indicates that for both the individually prepared phases and a bulk prepared system of HEMWat 17.5, K_d values were equal to 1.06. Consistent K_d values indicate that there is no significant difference in the partitioning between solvent systems made up as single phases compared to HEMWat 17.5 made up as a bulk phase. Additionally calculated K_d ratios for the API and related impurities indicate that the separation factor is above 1.5 for all impurities except two where the values are 1.1 and 1.3 respectively. Separation factors are consistent between single phases and HEMWat 17.5 and correspond to previous data indicating that HEMWat 17.5 is the most suitable solvent system for API and impurity separation with only minor co-elution of impurities [36].

The resulting solution from the solvent exchange (see Section 3.2) was mixed with fresh methanol, heptane and water to make up CCC samples with the desired mobile phase composition of 67.32% heptane, 30.29% ethyl acetate, 2.16% methanol and 0.24% water. CCC was then used to separate the API from impurities present in the incoming feed stream. Two CCC Mini runs were carried out on a 0.9 mL sample scale using fresh (CCC run 1) and recovered solvent (CCC run 2, see Section 3.4) respectively as mobile phase. For both Mini runs the stationary phase retention was measured to 83% and each separation was run for a total of 35 min with fraction collection starting immediately after sample injection and continuing for a total of 10 × 3.5 min intervals. Individual CCC fractions from Runs 1 and 2 were analysed with HPLC and data indicate that the separation profile for CCC runs 1 and 2 operating on fresh and recovered mobile phase respectively, are almost identical (see Fig. 3). For both CCC runs 1 and 2 the majority of the impurities were eluted between 5 and 15 min with only trace amounts visible at higher elution times (see Fig. 3). The API eluted between 20 and 30 min after the initial impurity block and HPLC data indicate that 77.5–80.0% of the added API eluted in fractions with purities ranging from 91.6 to 100%. All additional API containing fractions ranged in purity between 27.0 and 85.2% with the overall API recovery adding up to approximately 100% for both runs (see Table 4). Though a 90% purity is not sufficient for the final product, API is recovered from the fractions through crystallisation generally resulting in a purity >99%. Important to note is that consistent performance was observed for CCC runs 1 and 2 with no indication of impurity enrichment in the API containing fractions when recovered solvent was used as mobile phase for separation. The relatively high purity for the API fractions further indicates sufficient separation performance for the CCC and feasibility for initial API purification can be considered proven.

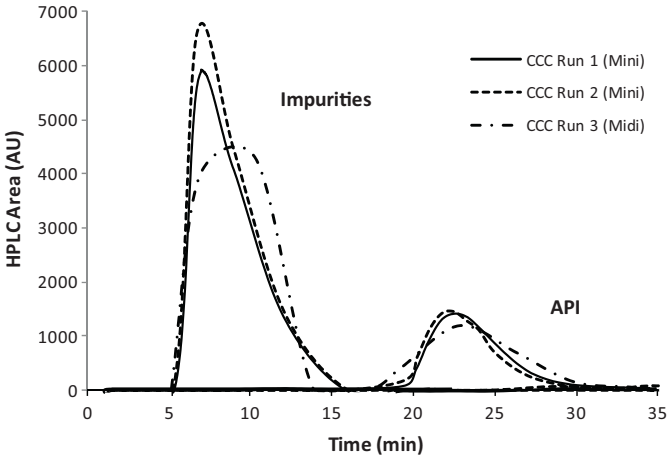

Fig. 3. Comparison of HPLC re-constructed fractograms for CCC run 1 (Mini, fresh solvent for mobile phase), run 2 (Mini, recovered solvent for mobile phase) and run 3 (Midi, fresh solvent for mobile phase – material for solvent recovery).

In addition to small scale CCC runs for demonstration of feasibility, one run was carried out on a larger scale to generate sufficient mobile phase for recovery and reprocessing in subsequent CCC operation (used in CCC run 2). For the larger Midi scale run the stationary phase retention was measured to 80% and the CCC separation was operated using a 41 mL sample and fresh solvent to make up the mobile phase and a total of 2450 mL solvent was eluted in 10 separated CCC fractions. HPLC data indicated that the API was eluting in the final 5 fractions with impurities ranging between 62.2 and 100.0% (see Fig. 3). Of interest is that the fractions with lower purities have low API concentrations and in absolute values the impurities present are minimal and likely to wash away during API recovery through crystallisation. The elution profile for the Midi run differs slightly from that observed during the Mini operations (see Fig. 3). Though the impurities and API are still eluting within approximately the same time interval as for the Mini runs, the API peak is broader resulting in API eluting over a larger range of fractions. Minor differences in elution profiles could potentially be a result of small differences in phase compositions indicated by the lower stationary phase retention for the Midi run, in combination with minor differences in mixing in the various size equipment. Important to note however is that API elution time as well as purities of API fractions from this large scale CCC run were similar to data observed for the smaller scale runs, and CCC performance was observed to be consistent at the two scales tested.

3.4. Solvent recovery

Feasibility of solvent recovery was investigated for the mobile phase collected from larger scale CCC run (see Section 3.3). The eluted mobile phase can be divided into 4 fractions depending on content (see Table 5), and feasibility of solvent recovery was investigated separately for each fraction. Fractions were studied separately to enable recovery of API and to minimise the risk of including low molecular weight impurities, which are not easily removable by OSN, into the recovered solvent.

For recycle of solvent into subsequent CCC operations the selected solvent specification states that the recovered solvent must be within 0.5% (% volume) of the desired solvent composition and contain a total of no more than 1% (area % by HPLC) impurities. HPLC data indicate that for fractions F0 and F3–F5 the overall

Table 5
Summary of CCC run 3 mobile phase fractions investigated for solvent recovery potential.

Fraction	Organic content	Volume (mL)	Rejection (%)	OSN recovery
F0	Low concentration, 2 impurities	505	13–47	Yes
F1–F2	High concentration, 20 impurities	490	0–100	No
F3–F5	Low concentration, 7 impurities	735	17–100	Yes
F6–F10	Intermediate concentration, API only	1225	98.5	Yes
Total	–	2955	–	–

Table 6
Solvent composition based on GC and Karl Fisher data.

Fraction	Heptane (% v/v[a])	Ethyl acetate (% v/v[a])	Methanol (% v/v[a])	Water (% v/v[a])	Impurities (% a/a[b])	Volume (mL)
F0	66.6	30.6	2.2	0.3	0.46	435
F3–F5	67.8	29.8	2.1	0.3	0.47	660
F6–F10	67.6	30.4	1.7	0.3	0.45	890
Combined permeate	67.7	30.2	1.9	0.3	0.46	1985
Desired composition	67.32	30.29	2.16	0.24		

[a] % Volume.
[b] % Area by HPLC.

impurity content and concentration was low (see Fig. 3 and Table 5), and despite rejection values ranging between 13 and 100%, impurity removal was expected to be sufficient to attempt solvent recovery in a single membrane pass. Fractions F6–F10 contained the API and concentrated material was intended for API recovery through crystallisation. The rejection of API is high at 98.5%, indicating that solvent could potentially be recovered in a single membrane pass. However to ensure minimal losses of API a dual membrane stage was used for solvent recovery with the retentate from each stage collected separately. Finally HPLC data for fractions F1–F2 indicate that the overall impurity content and concentration was high, with impurity rejections ranging between 0 and 100%. The low rejections measured in combination with high starting concentrations indicate that even if multiple membrane passes were to be used, the recovered permeate would still be far from the solvent specification. Fractions F1–F2 were hence considered unsuitable for solvent recovery and were discarded as waste. The solvent composition for each recovered fraction was analysed with GC and Karl Fisher prior to combining the solvent into the final recovered mobile phase (see Table 6). The composition of the combined solvent was estimated to 67.7% heptane, 30.2% ethyl acetate, 1.9% methanol and 0.3% water hence deviating from the desired composition by a maximum of 0.4% (% volume) for heptane. Partition testing reveals that the K_d of API in the recovered mobile phase is 1.06 which is consistent with values observed for HEMWat 17.5 and fresh solvent phases used for CCC run 1 (see Section 3.3). Consistent API K_d values indicate that minor differences in the solvent composition have no significant impact on the solute partitioning. HPLC analysis of the recovered solvent further indicated that the impurity trace in the combined permeate was 0.46% (area % by HPLC) which was significantly lower than the set target of 1% (area % by HPLC). The recovered solvent was hence within the solvent specification and was considered suitable for re-cycling into subsequent CCC operation. Feasibility for solvent recovery was further confirmed by consistent CCC performance, with no indication of impurity build-ups in the API containing fractions, when operating using recovered solvent (CCC run 2, see Section 3.3).

3.5. Solvent mass-intensity

Eq. (1) was used to calculate the solvent mass-intensity for CCC operation with and without solvent recovery and OSN solvent exchange respectively (see Table 7). As expected mass-intensity data indicate that the solvent intensity for CCC operation alone is high at a value of 29×10^3, however when combining CCC operation with solvent recovery the solvent mass-intensity is calculated to decrease by 60% for a solvent recovery level of 70% (obtained recovery level from CCC run 3). Solvent mass-intensity data further indicate that when the solvent requirement for the OSN solvent exchange is included for comparison, the overall solvent intensity increases. This is consistent with expected behaviour however of interest is that the OSN solvent exchange only results in a relative mass-intensity increase of 5% for the CCC process with no solvent recovery and 10% for the CCC process with solvent recovery. When comparing the overall CCC process this further means that even when the relatively solvent intensive OSN solvent exchange is used, the overall solvent mass-intensity for CCC operation with and without solvent recovery can be reduced by 56%.

Table 7
Calculated mass-intensity for CCC run 1 and 2.

Process	Solvent exchange	Solvent recovery[a]	Solvent mass-intensity
CCC run 1	Not included	0%	29×10^3
CCC run 1	Included	0%	30×10^3
CCC run 2	Not included	70%[b]	12×10^3
CCC run 2	Included	70%[b]	13×10^3

[a] Solvent recovery is limited to the mobile phase.
[b] Equivalent to recovery level obtained in CCC run 3.

4. Conclusion

The work presented in this study demonstrates that separations using CCC can be far more efficient when coupled with OSN technology. OSN solvent exchange generated CCC feed within specification and feasibility was demonstrated through successful CCC operation. Although solvent exchange using OSN diafiltration is a relatively solvent intensive process (see Table 7) alternative thermal routes can be a significant challenge particularly if azeotropic mixtures are present, or the swap is from a high boiling point solvent to a low boiling point solvent. Additionally OSN avoids potential thermal degradation of API and may provide benefits with regards to improved energy-efficiency. Throughout the solvent exchange presented here the API rejection remained >99% but despite high API rejection the overall API loss added up to 2.3%. Losses of API highlight that membranes with 100% rejection are desirable and membrane development remains an important area of research. Improvements of the overall process mass-intensity was demonstrated through OSN solvent recovery and recycle of CCC mobile phase. Feasibility for solvent recovery was demonstrated through generation of solvent within the stated solvent specification and with consistent partitioning values to the mobile phase

prepared using fresh solvents. Recovered solvent was used for successful CCC separation demonstrating consistent performance to operation carried out using fresh solvent. Comparison of mass-intensity data show that even when the solvent intensive OSN solvent exchange was used for sample preparation, recycle of the mobile phase resulted in a 56% improvement of the mass-intensity of this process indicating a significant potential for improving overall CCC mass-efficiency.

Acronyms

API	active pharmaceutical ingredient
CCC	counter-current chromatography
GC	gas chromatography
GSK	GlaxoSmithKline
MET	Evonik Membrane Extraction Technology
MiBK	methyl isobutyl ketone
MWCO	molecular weight cut-off
OSN	organic solvent nanofiltration

List of symbols

A	membrane area (m^2)
C	Concentration ($g\,L^{-1}$, $mL\,mL^{-1}$ or %)
J	flux ($L\,m^{-2}\,h^{-1}$ or $m^3\,m^{-1}\,s^{-1}$)
K_d	partitioning coefficient (–)
m	mass (g)
MI	mass-intensity (–)
R	rejection (%)
t	time (h or s)
V	permeate volume (L or m^3)

Subscripts

d	added diafiltration volume
f	feed
i	component
p	permeate
r	retentate

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