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Developing counter current chromatography to meet the needs of pharmaceutical discovery

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ABSTRACT

Experiments have been carried out to evaluate Counter Current Chromatography (CCC) as an alternative purification technique to preparative Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for small molecule pharmaceuticals. The major drawback of CCC is the extensive time required in selecting the solvents to perform the separation. This is equivalent to choosing both the stationary phase and the mobile phase at the same time. In RP-HPLC it is a simple matter of deciding on the gradient, most samples can be purified on a C18 column with a water:acetonitrile gradient. The majority of the initial work was based on a standard test set of commercially available compounds, developed within our group to evaluate the performance of the HPLC apparatus and the column prior to the start of work each day. The work carried out on CCC has shown that the technique offers similar capabilities and can be carried out using similar protocols to RP-HPLC. CCC also has some advantages over RP-HPLC and can be regarded as a valuable addition to the chromatography toolbox.

1. Introduction

1.1. Historical

The development of Counter Current Chromatography (CCC) has been over shadowed by the more successful and easy to use High Performance Liquid Chromatography (HPLC). CCC can trace its routes back to the Craig-Post Apparatus of 1949. Up to the 1960s, chromatography was a slow and labour intensive process. The 1970s saw significant improvements in many areas of chromatography. In 1970 Csaba Horvath convened the term HPLC which used pumps for packed columns running at 3.5 MPa which quickly developed to 42 MPa. This coincided with the introduction of the reverse phase column packing with improvements in selectivity and reproducibility. 1978 saw the key publication by Clark Still [1] that paved the way for Flash Chromatography which could separate samples in 15 min by low pressure normal phase silica. The 1970s were also significant in the development of CCC with High Speed Counter Current Cromatography (HSCCC) being introduced with the development of the J-type centrifuge using increased gravitational forces (80 *g*) to improve performance [2]. RP-HPLC proved to be a relatively quick, reliable, efficient and cost effective technique both analytically and preparatively and became the technique of choice. 2004 saw the commercialisation of sub 2 μm particle size HPLC columns and equipment with corresponding increases in performance or speed. At a similar time the next significant development in CCC was the development of High Performance Counter Current Chromatography (HPCCC) running at a higher gravitational force of 240 *g*.

1.2. Uptake of CCC

The majority of CCC literature has been in natural product research and of all the possible solvent combinations, the HEMWat system has dominated [3] with run times typically of several hours [4–8]. By comparison the structures in pharmaceutical research tend to be smaller, contain multi functional groups and are of moderate lipophilicity. Of the top 10 selling pharmaceuticals by earnings in 2008, 8 were small molecules. Lipitor, the top selling drug earning Pfizer $12.9 billion revenue [Free Press Release].

In terms of physical properties most pharmaceuticals tend to obey Lipinski's Rule of Five [9], based on the observation that most drugs are relatively small and lipophilic molecules. No more than 5 H bond donors, no more than 10 H bond acceptors, molecular weight of less than 500 and an octanol–water partition coefficient (log *P*) of less than 5. Further enhancements by Ghose et al. [10] added molar refractivity from 40 to 130 and the number of atoms from 20 to 70.

There is very little use of CCC within the pharmaceutical industry [11–13] where RP-HPLC dominates in research and Normal Phase High Performance Liquid Chromatography (NP-HPLC) in process.

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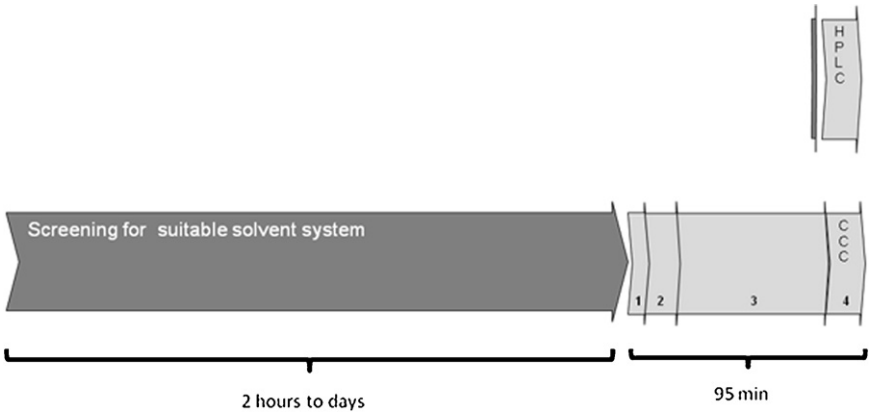


Fig. 1. Time comparison of purification carrying out by RP-HPLC vs CCC. For information on HPLC run, see Section 1.4. For information on the CCC run and the numbered steps, see Section 2.7.

There are many barriers to the acceptance and use of CCC as a technique, these include:

(A) Limited understanding of CCC by practicing chromatographers
(B) Until recently, build quality of apparatus
(C) Long run time (especially compared to RP-HPLC)
(D) Limited solubility of crude material in chromatography solvents
(E) pH instability of the HEMWat solvent system, the pH decreases over time as the ethyl acetate decomposed to give acetic acid and ethanol, which can result in changes in Kd
(F) Probably the major issue is the time required in choosing the best solvents for the chromatography

1.3. Potential advantages of CCC

The advocates of CCC see the advantages in the use of this technique as [14,15]:

1. Higher recoveries as there is no solid stationary phase for product to irreversibly bind to
2. High capacity due to the large volume of the stationary phase
3. Ability to handle crude extracts and particulates
4. Reduced solvent consumption
5. Milder
6. Wide range of sample polarities can be handled from Colistin peptide antibiotic (octanol/water log P −4.65) to Lycopene (octanol/water log P 17.6)
7. Fresh stationary phase for each run
8. Huge selection of mobile phases and stationary phase known, form Aqueous–Aqueous, through to Non Aqueous–Non Aqueous
9. Can be run in reverse or normal phase mode by the switch of a valve
10. Unlike solid phase chromatography which achieve separation by ever increasing numbers of theoretical plates, CCC which has a relatively very small number of plates, separation is achieved by altering the selectivity of the stationary and mobile phases

1.4. Pharmaceutical requirements of CCC in small molecule research

For CCC to compete with RP-HPLC in chromatography it must be able to meet our requirements of samples for testing: 20–200 mg free flowing solid, purified by chromatography, >95% by LCMS. It must also be able to fit with the current capabilities of our group, the Cancer Purification Group (CPG). We purify the majority of samples synthesised by the Department's 65 chemists. In the first 6 months of 2010 the 4 chromatographers in the group purified over 1700 samples, from 34 different projects. These samples have ranged from 5 mg to 27 g, typically 50–500 mg with an average of 2 injections per sample. A focused gradient is used from a supplied analytical LCMS with a typical injection to injection cycle time of 15 min. Most samples are returned within 3 days from submission, the average turnaround time for purification is 1.76 days (chromatography, fraction dry down, reformatting, freeze drying, quantification and analysis). Most compounds are purified using a C18 stationary phase, acetonitrile/water as the mobile phase and ammonia as the modifier, dissolved usually in any combination of following: acetonitrile, dimethyl formamide (DMF), dimethyl sulphoxide (DMSO), methanol, water. The development of a pharmaceutical drug tends to be an iterative process [16]. The initial hits are analysed to give the basic active core which is then elaborated to optimize activity, desirable physical properties and minimum side effects.

Initially, the time for chromatography by CCC is more than 6 times that of RP-HPLC, Fig. 1, however, the biggest drawback is the considerable time taken for screening for suitable run conditions. For RP-HPLC this is a simple matter of determining the concentration of acetonitrile that the target molecule desorbs from the stationary phase, commonly referred to as the critical elution concentration (CEC) this is related to the retention time. A focused gradient is then run centred on this CEC, the whole process takes seconds to set up and can be automated. This is not the case for CCC where selecting the solvent system is equivalent to choosing both a mobile phase and a stationary phase. Screening just one set of solvents such as HEMWat can take several hours by the shake-split-LCMS method.

2. Experimental

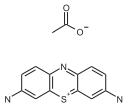
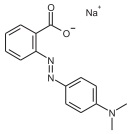
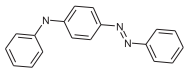
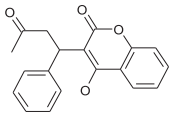
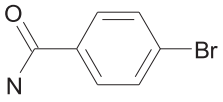
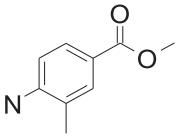
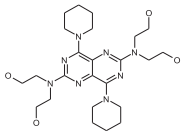
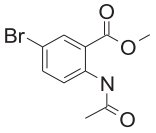
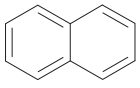
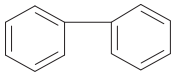
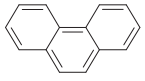
2.1. Reagents

All solvents and ammonia solution were obtained from Fisher Scientific (Loughborough, Leicestershire, UK), water is deionised (Elga PURELAB Maxima, Vivendi Water Systems, High Wycombe, Buckinghamshire, UK). For information on the test compounds see Table 1.

2.2. HPLC

Analytical LCMS: Waters (Milford, MA, USA) AllianceHT HPLC system running MassLynx 4.1 and OpenLynx 4.1 Software and Waters Micromass ZQ mass spectrometer operated under Electrospray conditions. The HPLC system comprised of a Waters

Table 1
Components of the dye and CPG test mixtures.

No.	Compound	MWt	HEMBWat no. for Kd = 1	Structure	Note	Supplier
Dye 1	Thionin		Remains in lower layer		Blue	Fisher Scientific, Loughborough, Leicestershire
Dye 2	Methyl Red, Sodium Salt		14		Red	Acros Organics, New Jersey, USA
Dye 3	4-Phenylazodiphenylamine		22		Yellow	Fluka Chemie GmbH, Buchs, Switzerland
CPG 1	Warfarin	308.34	16.6		Sensitive to pH, drifts significantly if the pH is incorrect, it also is a check that the freeze drying part of the process is working effectively	Sigma–Aldrich Inc., St Louise, MO, USA
CPG 2	4-Bromobenzamide	200.04	16.1			Sigma-Aldrich Chemie, Steinheim, Germany
CPG 3	Methyl 4-amino-3-methylbenzoate	165.19	17.8			Fluorochem, Old Glossop, Derbyshire, UK
CPG 4	Dipyridamole	504.63	14.1		Yellow dye, used as a diagnostic tool to identify leaks and blockages	AK Scientific Inc., Mountain View, CA, USA
CPG 5	Methyl 2-acetamido-5-bromobenzoate	272.10	21.7			Sigma–Aldrich, Gillingham, Dorset, UK
CPG 6	Naphthalene	128.18	19.8			Koch-Light, Colnbrook, Buckinghamshire, UK
CPG 7	Biphenyl	154.21	22.2			Sigma–Aldrich, Gillingham, Dorset, UK
CPG 8	Phenanthrene	178.24	18.4		In conjunction with compound 7 would be an early warning of drop in column performance as the peaks coalesced	Acros Organics, Geel, Belgium

2795 separation module, Waters 2996 diode-array detector and Waters Micromass ZQ mass spectrometer. A Waters XBridge 5 μm, 50 mm × 2 mm C18 column with the following gradient conditions used: 5–95% acetonitrile in water (plus 1% ammonia) over 4 min.

Preparative HPLC: Waters (Milford, MA, USA) preparative HPLC system running MassLynx 4.1 and FractionLynx 4.1 Software and Waters Micromass ZQ mass spectrometer operated under Electrospray conditions. The HPLC system comprised of a Waters 2545 binary gradient module, Waters 2767 sample manager, Waters 2996 diode-array detector and Waters Micromass ZQ mass spectrophotometer. The flow was split between the 2 detectors by an LC Packings WACM-10-50 splitter box (LC Packings (Netherlands) BV, Amsterdam, The Netherlands). A Waters XBridge 5 μm, 100 mm × 19 mm C18 column, with the following gradient conditions used: 20–80% acetonitrile in water (plus 1% ammonia) over 10 min.

Table 2
Arizona/HEMBWat solvent system compositions of Berthold/Garrard [20,21].

Letter	Number	Heptane/ hexane	Ethyl acetate	Methanol	Butanol	Water
	1	0	0	0	5	5
	2	0	1	0	4	5
	3	0	2	0	3	5
	4	0	3	0	2	5
	5	0	4	0	1	5
A	6	0	1	0	0	1
B	7	1	19	1	0	19
C	8	1	9	1	0	9
D	9	1	6	1	0	6
F	10	1	5	1	0	5
G	11	1	4	1	0	4
H	12	1	3	1	0	3
J	13	2	5	2	0	5
K	14	1	2	1	0	2
L	15	2	3	2	0	3
M	16	5	6	5	0	6
N	17	1	1	1	0	1
P	18	6	5	6	0	5
Q	19	3	2	3	0	2
R	20	2	1	2	0	1
S	21	5	2	5	0	2
T	22	3	1	3	0	1
U	23	4	1	4	0	1
V	24	5	1	5	0	1
W	25	6	1	6	0	1
X	26	9	1	9	0	1
Y	27	19	1	19	0	1
Z	28	1	0	1	0	0

2.3. Preparation of CCC solvents mixtures

Premixed solvents were prepared by mixing the required components of the HEMWat solvent mixtures (Table 2) and shaken to equilibrate the phases. The resulting two layers were separated using a separating funnel into individual bottles. Unused solvents were disposed of after 24 h.

2.4. Determination of Kd

10 mg of samples were made up into 1 ml of DMSO; 0.02 ml of this solution was added to 0.9 ml of each layer of the HEMWat solvent mixture. The mixture was vigorously agitated on a Bibby Stuart SA8 vortex mixer (Bibby Sterilin Ltd., Stone, Stafford, UK), allowed to settle and the two layers were separated off and each layer analysed using the analytical LCMS system. The integrated UV peak area for the target molecule in each of the two layers was determined and used to calculate the distribution ratio Kd.

2.5. Counter Current Chromatography apparatus

All Counter Current Chromatography was performed on an Armen CPC (Armen Instruments, ZI Kermelin-16, rue Ampere-56890 Saint Ave, France) with a 250 ml rotor, Armen 50 ml/min quaternary pump, automated injection and mode valves, Gilson 108 UV detector (Gilson Inc., Middleton, WI, USA), Isco Foxy 200 fraction collector (Teledyne Isco Inc., Lincoln, NE, USA) running on Armen Gilder 2.9 Software, or a Gilson 206 which was controlled via Gilson Unipoint 3.3 Software. The conditions for the run were adjusted, see Section 3. The dye experiments were carried out with the Armen CPC connected to the Waters Preparative HPLC system as described in Section 2.2 but without the splitter box and mass spectrophotometer.

2.6. Test mixture components

The test mixture consisted of 8 compounds, the criteria for the initial choice of the components was that they should be inexpensive, readily available, low hazard and cover the range of retention times. They should also be visible by either UV or mass spectra (positive or negative). The components chosen are described in Table 1.

2.7. Initial CCC run conditions

There are many ways to perform a CCC run; most of the work presented here has been in reverse phase using the elution–extrusion protocol developed by Berthod [17,18]:

1. Load, 7 min at 50 ml/min, 500 rpm
2. Equilibrate, 13 min at 12 ml/min, 1800 rpm
3. Elution, 60 min at 12 ml/min, 1800 rpm
4. Extrusion, 15 min at 12 ml/min, 500 rpm

This process generates a new "column" of stationary phase equilibrated with mobile phase for each run, after injection and elution of the target sample any remaining material is then displaced off the system.

3. Results and discussions

3.1. Terminology

There is not an agreed way to write CCC. Alternatives include Counter Current Chromatography, CounterCurrent Chromatography, Countercurrent Chromatography, Counter-Current Chromatography. There has been a suggestion of calling it countercurrent separation (CS) [3]. The term counter current would imply flow of 2 liquids in opposite directions, in most cases only one liquid is flowing in one direction at any one time. There are essentially two main types of modern system in common use: hydrodynamic (changing *g* force) or hydrostatic (constant *g* force). The term CPC refers to centrifugal partition chromatography which is a hydrostatic system but still uses the same basic liquid–liquid interactions of a liquid mobile phase and a liquid stationary phase. HSCCC can now be considered as CCC as most systems operate normally at high speed, indeed HPCCC may follow to be the norm as systems develop.

There is also a variety of similar tables for the HEMWat solvent system; Hydrocarbon (Hexane/Heptane):Ethyl Acetate:Methanol:Water, e.g. Oka [19] keeping the water volume constant, Arizona (Berthold, using letters) [20] and HEMBWat (Garrard, using numbers) [21] both using equal polarity steps and Freisen & Pauli keeping the total volume constant [22].

3.2. Understanding the process

The key property of a compound in CCC is the Partition Coefficient (Kd) which is the concentration ratio of the compound in the two phases. How does one relate a CCC run to HPLC? In order to gain a better understanding of the process, 3 dyes were selected to visualise the CCC process, Table 1. They were portioned between the 2 layers of the HEMWat solvent systems 7, 12, 17, 21 and 27 (A, H, N, T and Y). The blue dye remains in the lower layer in all solvent systems, the red dye has a Kd = 1 around solvent system K and the yellow dye has a Kd = 1of solvent system T. The elution order by thin layer chromatography on silica is yellow (running with the solvent front), red (intermediate retention depending on the ethyl acetate content), and then blue (which remains on the base line). This is the opposite for RP-HPLC which is blue, red, and then yellow.

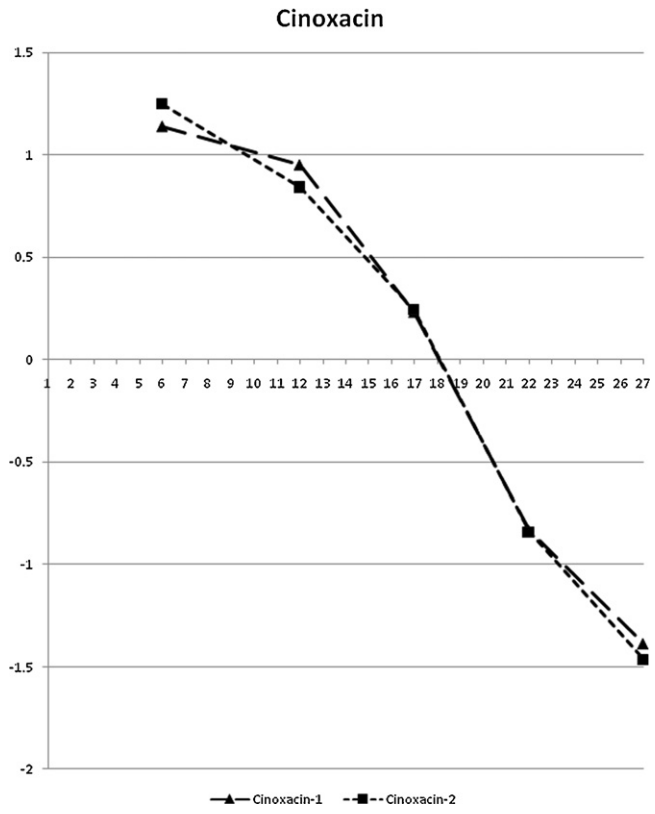


Fig. 2. A typical plot of log Kd vs HEMWat solvent number.

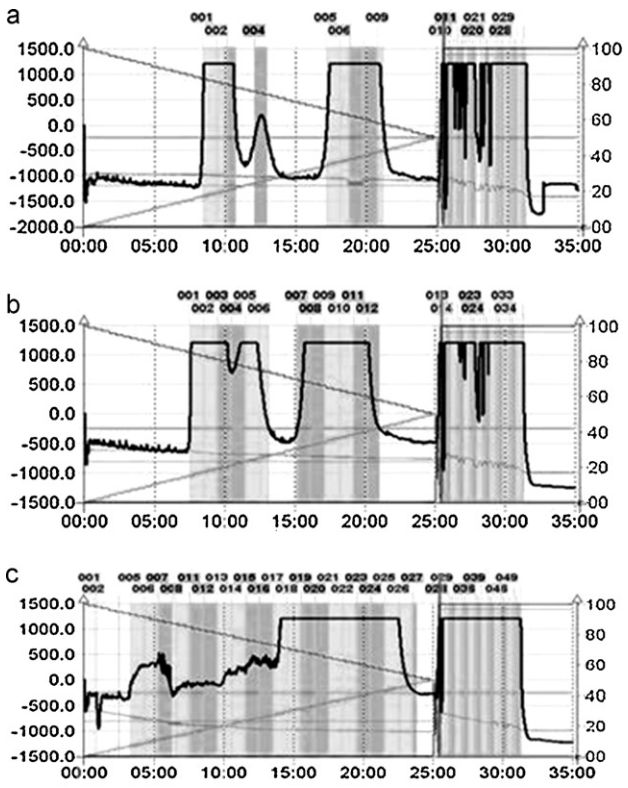


Fig. 3. CCC of test mixture using equilibrated solvents with varying injection volume and load: (a) 1 ml, (b) 3 ml and (e) 4 ml. Experiment carried out on Armen 250 ml CPC, stationary phase was lower N, mobile phase was a linear gradient over 25 min from upper N to 50:50 upper N: upper W followed by extrusion with lower N. Elution was at 12 ml/min at 1800 rpm, loading and extrusion was at 50 ml/min at 500 rpm.

A typical CCC has 2 phases, an upper and lower phase. Depending on the direction of flow, the upper or lower phase is stationary. If the upper phase is stationary and the lower phase is mobile, this is regarded as reverse phase, as the lower phase is aqueous. Alternatively if the lower phase is stationary and the upper phase is mobile, this is regarded as normal phase, as the upper phase is hexane/ethyl acetate for HEMWat. Therefore in a reverse phase run the blue dye would be in the mobile lower aqueous phase and would therefore elute first, in normal phase it would stick to the stationary phase and would only elute off the column on extrusion.

In a similar method to above, the Kd of a colourless substrate can be found by separating the two phases and measuring the area of the UV absorption of an LCMS of each layer. It is usual to run the Counter Current Chromatography where Kd of the target compound = 1, therefore a graph was produced to plot log Kd vs solvent system, the intercept of the log Kd = 0 was determined to give the solvent system for Kd = 1 (Fig. 2).

It has been reported that there is an approximate relationship of RP-HPLC proportional to Kd [23]. This may be useful as a starting point, however, in a series of compounds investigated within our group initial results suggests there is no significant relationship.

3.3. Solvent compatibility

Due to the diverse nature of the compounds submitted to the group for purification, there is a need to use a single solvent that can dissolve the majority of samples. Solvents frequently used are DMSO or DMF, 1–2 ml which is usually sufficient to dissolve 100–200 mg of crude material. It is normal in CCC purifications to dissolve the crude material in the solvent mixture that the chromatography will be run in. There is a mention of the use of polar solvent [11] but the details are limited, the loading profile was investigated to determine the compatibility of polar solvents with CCC.

The test mix containing 12.5 mg of each of the 8 component in 1 ml of DMSO was loaded in increasing volumes onto the column. As can be seen in Fig. 3a, b, the system is able to take more than 1% of the total column volume of polar solvent (DMF gives similar results) without any detrimental effect on the chromatography. This is approximately 2.5% of the mobile phase volume, however, when the amount of DMSO is increased to 4 ml, resolution of the desired peak is lost, presumably due to loss of phase separation (Fig. 3c).

3.4. Searching for the best solvent

There are several commonly used approaches to solvent selection.

1 Literature searching for a similar compound and it's solvent system
2 Systematic searching using solvent tables to discover the solvent system where Kd for the target molecule is near 1, a trial and error approach using shake-split methodology or with the assistance of a robot [21]
3 G.U.E.S.S. method a TLC based method [22]
4 "Best solvent" approach, starting from a solvent that the mixture is soluble in and choosing the other solvent to make up a 2 phase system [24].

All of these approaches can be very time consuming. A simple alternative is to run the chromatography on a small injection and analyse the results starting from a polar solvent system then decreasing the polarity in subsequent runs. This approach would

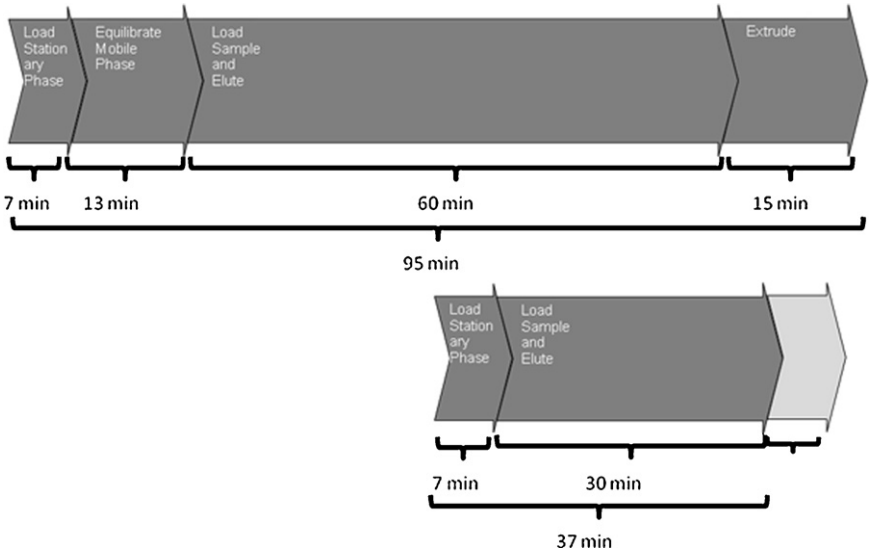


Fig. 4. Time comparison of Initial CCC purification vs optimized CCC purification run time and protocol for elution–extrusion CCC.

obviously benefit from automation and the addition of a mass spectrophotometer to assist in peak identification.

3.5. Reducing solvent waste and solvent preparation

Typically in CCC the solvents for the chromatography are made up by mixing the individual solvents together and equilibrated by shaking and then the 2 layers separated. The stationary phase is then loaded onto the column to displace any solvent/air then the mobile phase is introduced until the column is equilibrated. The sample is injected, the components are eluted and any late running components are extruded off. This usually requires differing amounts of upper and lower phase. The instability of the HEMWat system is known [20] as the ethyl acetate hydrolyses to acetic acid and the pH of the phases decreases. This can have considerable effect on the Kd of any component that has an acid or base functionality, therefore mixed solvents cannot be kept for any prolonged time. Thus, the mixing of the solvents is both wasteful of time and solvents.

The Armen CPC system has 4 solvent inlet lines. This allows each line to be dedicated to each one of the 4 solvents of the HEMWat system. As the solvent compositions have been measured for each phase of the various combinations of the HEMWat system [21] these values can be used to generate the solvent required for the stationary and mobile phases for the chromatography. The test mixture was rerun using the composition tables and the chromatography is very similar to that of the classic separated solvents approach. Mixing the solvent at the pump, results in only the required amount being made, without the issues of pH variation.

3.6. Optimisation of run time

One of the main perceptions and issues with CCC is the long time spent in order to achieve any separation. The Craig-Post system often required days or weeks to achieve the separation. It is still common to see run time measured in hours for example 5 h [4] and 10 h [8], this does not fit the needs of our research department. The first runs were typically about 1½ h (Fig. 4).

As we typically only wish to isolate the target compound the solvent system is chosen where Target Kd = 1 thus the sample should elute around 20 min after injection hence the elution time can be halved. Common practice in CCC is to equilibrate the mobile phase; one of the main advantages is that the detector gives a better, smoother baseline. This is not necessary, indeed Ito's Golden Rules covers this point [25]. If the conditions for the next run are known then the extrusion time can actually be the loading step for the subsequent run for example in solvent screening or multiple injections of the same sample. Preparative run times are still longer than RP-HPLC but they are more comparable (Fig. 4).

3.7. Where next?

Table 3 shows how the issues around CCC adoption have been addressed and what further work needs to be done to enhance the technique.

Table 3
Future work for CCC development.

Area	Issue	Solution	Future
Terminology	Confusion and conflicting terms	Needs to be standardised	
Understanding the process	How does it relate to standard chromatography	Hopefully the information in this has helped	You need to try it!
Solubility	Solvent compatibility	Can accommodate over 1% of column volume of DMSO/DMF	Will this be more of an issue for large scale systems?
Solvent selection	Very time consuming	Can be automated	Smarter ways needed
Making up mobile and stationary phases	Time consuming and wasteful	At pump mixing	Only available for HEMWat
HEMWat/Arizona type solvent	pH instability	At pump mixing	Effect of buffers
Run time	Not comparable to HPLC	Still longer than HPLC but more comparable	Limited room for further improvement by its very nature
Why use CCC		Can provide solutions where HPLC has failed	Can provide better options to HPLC

3.8. CCC in the toolbox

CCC has proved to be a valuable addition to the group. It is still used as a last option when RP-HPLC has failed:

(A) Low solubility sample, as the flow is essentially along a tube any low soluble samples will give a better recovery compared to RP-HPLC as the solid will not be trapped by frits, etc. We have successfully used CCC to purify a crude insoluble sample which despite various attempts failed to give a solution and therefore was not suitable purification by HPLC.
(B) pH sensitive compounds. RP-HPLC is commonly run with buffers to improve peak shape, this is mainly due to secondary interactions with the solid stationary phase support. Some compounds can react with the modifier and decompose. CCC can give much better peak shape without the need of modifiers CCC has been used successfully in our laboratory to purify these problematic samples.
(C) A series of compounds when purified by HPLC gave low recoveries. The product interacted with the packing and slowly leached off the column over the next few injections resulting in low recovery and contaminating subsequent samples. In a direct comparison of one sample CCC gave 4 times the recovery of RP-HPLC.

3.9. Scale-up

We have successfully outsourced a large scale CCC purification of a candidate drug, to Dynamic Extractions Ltd, 890 Plymouth Road, Slough, UK. The purification was carried out on the 18 l Maxi instrument using a hexane:toluene:acetone:water (3:2:10:5) solvent system with the lower phase as the mobile phase with a 70 min cycle time. The sample could have been purified internally by RP-HPLC or SFC but not in the required time scale due to other commitments. The sample considered of three separate batches and was mixture of diastereoisomers, the required product being the major component of approximately 90% purity. In total 420 g of crude material was processed to give 332 g of target compound with a purity of >99.7% by UV-HPLC (254 nm). This represents a 88% recovery for the separation. There were some mixed fractions which were reprocessed. The separation overall required 20 injections (the maximum single injection size was 34.3 g) and used a total of 115 l of hexane, 84 l of toluene, 497 l of acetone and 236 l of water.

4. Conclusion

The practical aspects of running Counter Current Chromatography have been explored and developed to sit alongside the normal protocols used for small scale preparative RP-HPLC. Out of the box solutions are now becoming available to reduce the learning curve in working with CCC. This also avoids the need to take a HPLC system out of use to be tied to the CCC. The biggest issue in the technique becoming a main stream tool for purification is the selection of a suitable solvent system. This is our next big hurdle to overcome. As with any other chromatography technique it is not a solution for every separation. Polar organic-water systems currently have poor stationary phase retention and work is in its infancy (Ito CCC2010). The use of ionic liquids has an issue with excessive back pressure which is incompatible with these low pressure systems (Berthod CCC2010). There is a huge potential for the technique to be used especially in complimenting RP-HPLC, for example in the purification of reactions which use heavy metal catalysts such as Suzuki/Heck/Negishi/Buchwald. These reactions often require a cleanup step prior to purification to avoid contamination or damage to the column from the catalysis or ligands. This is not the case with CCC as a "new column" is made for each run.

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