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Review

Role of counter-current chromatography in the modernisation of Chinese herbal medicines

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ABSTRACT

This review focuses on the growing popularity of using counter-current chromatography (CCC), with its liquid stationary phase, as one of the prime methods for isolating compounds from Chinese herbal medicines (CHMs). 198 publications are reviewed covering 108 different plant species from 56 plant families. These describe the isolation of 354 different molecules across a wide range of polarities, chemical classes and molecular weights (in the range 100–1000 Da). The suitability of CCC for the separation of active compounds from CHM, the phase systems used, how CCC has developed in China, compounds isolated, CCC instrumentation, performance, operational issues and innovations, all supported by detailed cross-referencing, are described. It is concluded that CCC is making an increasingly important contribution to the modernisation of Chinese herbal medicines.

Contents

1. Introduction

The signing of the Beijing Declaration on the modernisation and internationalization of Chinese medicines on 29 November 2007 at the Conference on the International Science and Technology Cooperation in Traditional Chinese Medicine in Beijing, was the catalyst for this review of the role counter-current chromatography (CCC) is playing and has played in the modernisation process. The declaration states that:

> "*the combination of traditional Chinese medicine and other schools of medicine may lead to a novel healthcare model for humans, and will effectively lower healthcare costs for both individuals and institutions. Innovation and diffusion of traditional Chinese medicine needs the support of modern science. Newly emerged disciplines, such as genomics, and the steady growth of basic knowledge, in*

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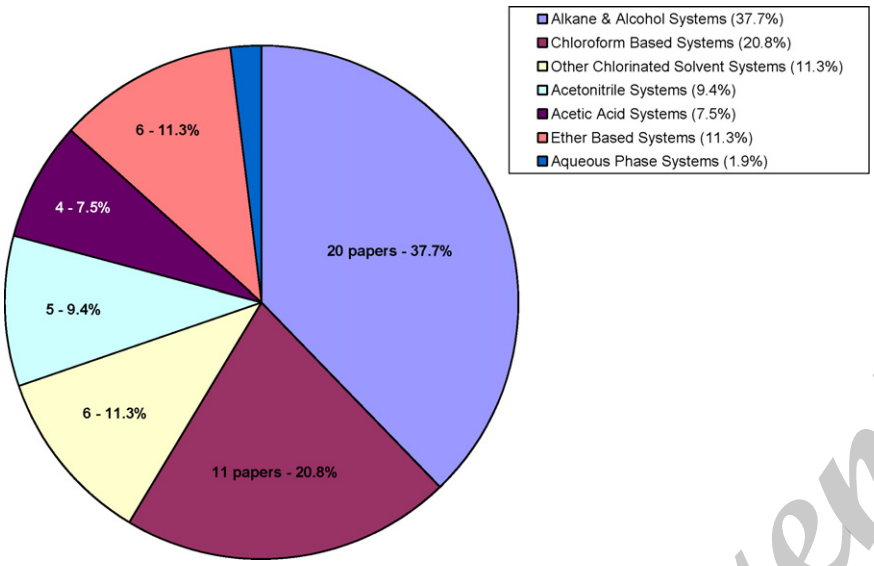

Fig. 1. Pie chart showing the families of phase systems used in the 198 paper reviewed.

particular, bioinformatics, has provided both the means and way forward for interpreting the basic principles of traditional Chinese medicine, and is leading to associated innovation. It is necessary to promote innovations of traditional Chinese medicine through enhanced international cooperation, in an attempt to further enrich its theoretical knowledgebase, improve people's understanding of traditional Chinese medicine, raise the level of safety, effectiveness, and quality of traditional Chinese medicine, and accelerate the modernization and internationalization of the traditional Chinese medicine industry."

The 198 papers reviewed [1–198] cover 354 different molecules from 108 different plant species and involve research collaborations across 109 different organizations. Details of the range of plant species/families studied, the compounds isolated and their numerous medical indications are given. Author, organizational and operational details are contained in a supplement to this review.

2. The growth of interest in Chinese herbal medicines

Since the 1968 Medical Act which deregulated medical practice and allowed Chinese and other herbal practitioners of all kinds to practice without a licence in the UK, the British market for Chinese herbal remedies has been growing at about 20% per annum and there are now well over 1000 registered practitioners in the UK [199]. However, there have been increasing concerns about the safety issues surrounding the unregulated use of traditional Chinese medicine (TCM) approaches, particularly after the mistaken use of a toxic Aristolochia species by a slimming clinic in Belgium resulting in more than 100 cases of renal failure of which some were fatal [200]. The EU Directive on Traditional Herbal Medicinal Products (agreed in April 2004) came into force on 30th October 2005. There will be a transitional period for products legally on the market on 30 April 2004, giving them protection until 2011. The Directive requires traditional, over-the-counter herbal remedies to be made to assured standards of safety and quality and for regulations to be standardised across Europe [201]. This new legislation is making manufacturers simplify, modernise and upgrade their products sometimes using the western reductionist approach and sometimes developing new approaches, which are an interesting synthesis of both Eastern and Western culture. For example Professor Guoan Luo of Tsinghua University, Beijing is using a new "omics" approach, chemomics, where "the phytochemical composition of a herbal formula with demonstrated clinical efficacy is regarded as a global chemome, which can be simplified successively through bioactivity-guided screening to achieve an optimized chemomome with minimal phytochemical composition for further drug development", while maintaining its curative effect for a specific disease [202]. Professor Luo calls this a "modernized composite medicine" or MCM.

3. Counter-current chromatography

Counter-current chromatography is a liquid–liquid partition chromatography process where both the mobile and stationary phases are liquids [203]. The "column" is simply a long length of tubing wound on a drum (the bobbin) which is geared to the main rotor in such a way that it simultaneously rotates at twice the speed of the rotor (planetary motion). This double motion sets up a fluctuating force field which forces one phase (the less dense or lighter

Table 1
A list of phase system families with an analysis of their usage in publications over time.

Phase system family	1988–1999	2000–2003	2004	2005	2006	2007
Alkane and alcohol systems (37.7%)	7	20	16	39	33	33
Chloroform-based systems (20.8%)	14	14	2	6	2	6
Other chlorinated solvent systems (11.3%)	0	0	6	1	1	1
Acetonitrile systems (9.4%)	0	0	0	1	3	3
Acetic acid systems (7.5%)	0	0	1	1	1	3
Ether-based systems (11.3%)	2	3	1	4	0	1
Aqueous phase systems (1.9%)	1	1	0	0	0	0
Total	24	38	26	52	40	47

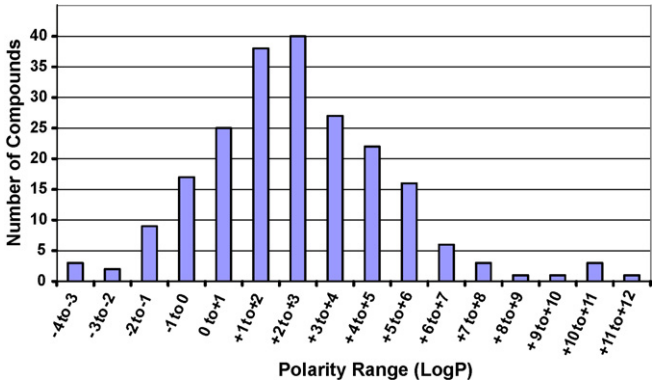

Fig. 2. An analysis of the polarity range (log *P*) of 214 different compounds isolated where log *P* values were available.

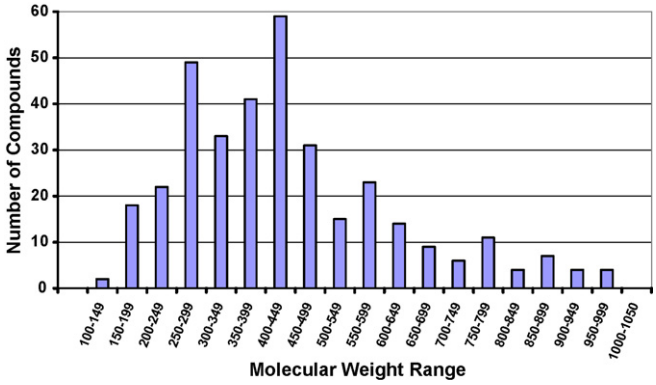

Fig. 3. An analysis of the molecular weights of 354 different compounds isolated where molecular weights and/or molecular structures were available.

phase) to move by definition to the "head" end of the coil/column and the other (the more dense, heavier phase) to go by displacement to the opposite end of the coil/column called the "tail". Changing the direction of motion simply changes the head/tail notation around. Hence the name "counter-current" chromatography. In practice however, the counter-current nature of the process is rarely used. Instead, the column is filled with the phase intended to be the "stationary" phase and the other phase is pumped in the end of the column, which allows the other phase to be retained. So if the heavier phase is the mobile phase (often the aqueous phase) then it is pumped from head to tail in the opposite direction to the way the lighter phase wants to go [204]. By measuring the displacement of the upper (stationary) phase from the column during the equilibration process, it is possible to calculate the amount of stationary phase left in the column and predict exactly when compounds will elute based on their distribution ratio (sometimes referred to as the partition coefficient). The amount of stationary phase left in the column will depend on the speed of rotation (higher "*g*" centrifuges retain a higher percentage of stationary phase), the flow rate of mobile phase (retention is inversely proportional to the square root of flow) and the density difference (the larger this is the better). The distribution ratio is generally defined as the concentration of a compound in the stationary phase divided by the concentration in the mobile phase. A compound with a distribution ratio of zero will not dissolve in the stationary phase and will come out with the solvent front in a time dictated by the volume of mobile phase in the column divided by the flow and one with a distribution ratio of unity will be equally soluble in both phases and therefore elute with the column volume in a time dictated by the column volume divided by the flow rate. Mathematically CCC is very predictable [205]. Also, everything that has been said about the lower phase being the mobile phase above can apply to the upper phase being the mobile phase. In this instance the column is initially filled with the lower phase and then upper phase is flowed from tail to head while the centrifuge is rotating. Once again this will result in a hydrodynamic equilibrium between the two phases. Mass transfer between the two phases is enhanced by mixing and settling zones that travel along the column from tail to head. The liquid nature of the stationary phase means that the compounds retained in this phase can be easily recovered by simply pumping this phase out. Because there is no solid support (as there is in HPLC) there is effectively no adsorption in the usual preparative mode and complete recovery of sample is achieved, an important feature for samples containing polyphenols, which tend to get irreversibly adsorbed in HPLC. The choice of which phase is used as the mobile phase will depend on whether a reverse phase or normal phase separation is required, which will be dependent on the distribution ratios involved and whether the target compound is required in an organic solvent for ease of drying down.

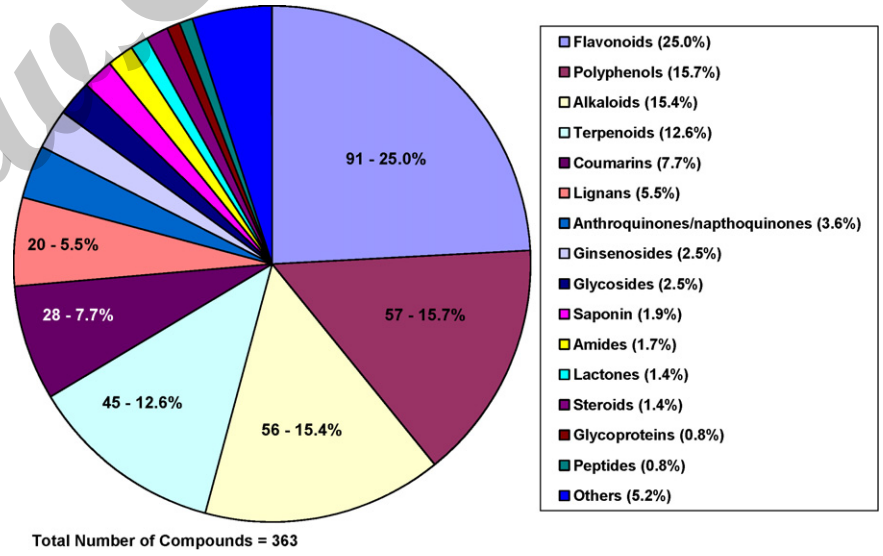

Fig. 4. Pie chart showing the classification of the 363 different compounds isolated.

Table 2
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: alkaloids.

Alkaloids (56 compounds)	Classification	Log *P*	MW	Reference
1-Methyl-2-[(6Z,9Z)]-6,9-pentadecadienyl-4-(1*H*)-quinolone	Alkaloid		365.6	[101]
1-Methyl-2-dodecyl-4-(1*H*)-quinolone	Alkaloid	NA	327.5	[101]
Atisine	Diterpenoid alkaloids	3.634	343.5	[190]
Baccatin-II	Diterpenoid alkaloid	4.06	586.6	[6]
Berberine	Alkaloid	−0.993	336.4	[16,45,184]
Bicuculline	Alkaloid	2.882	367.4	[149]
Caffeine	Alkaloid	−0.131	194.2	[13]
Cephalomannine	Diterpenoid alkaloid	6.586	831.9	[6]
Columbamine	Alkaloid	−1.886	338.8	[116]
Communensin G	Alkaloid	–	471.3	[86]
Communensin H	Alkaloid	–	485.3	[86]
Coptisine	Alkaloid	−0.868	320.3	[16,116,184]
Cyclanoline	Alkaloid	−2.187	342.4	[2]
Dehydrocorydaline	Alkaloid	NA	366.4	[116]
Dihydrotanshinone	Diterpenoid	3.904	278.3	[31,40]
DL-tetrahydropalmatine	Tertiary alkaloids	3.702	355.0	[178]
Epiberberine	Alkaloid	NA	336.4	[16]
Evocarpine	Alkaloid	7.702	339.5	[101]
Evodiamine	Alkaloid	1.636	303.4	[101]
Fangchinoline	Alkaloid	3.776	608.7	[2]
Guanfu base A (GFA)	Diterpenoid alkaloid	0.477	429.5	[190]
Guanfu base F (GFF)	Diterpenoid alkaloid	NA	457.0	[190]
Guanfu base G (GFG)	Diterpenoid alkaloid	NA	473.0	[190]
Guanfu base I (GFI)	Diterpenoid alkaloid	NA	387.0	[190]
Guanfu base P (GFP)	Diterpenoid alkaloid	NA	499.0	[190]
Hyoscyamine	Alkaloid	1.528	289.4	[1]
Isoliensinine	Alkaloid	NA	610.8	[79]
Jatrorrhizine	Alkaloid	−1.886	338.4	[184]
Lappaconitine	Diterpenoid alkaloid	2.126	584.7	[35,43]
Liensinine	Alkaloid	4.842	610.8	[79]
Matrine	Alkaloid	1.44	248.4	[14,175]
N-deacetyllappaconitine	Diterpenoid alkaloid	NA	NA	[43]
N-deacetylranaconitine	Diterpenoid alkaloid	NA	NA	[43]
Neferine	Alkaloid	5.494.	624.8	[79]
Neoplatyphylline	Alkaloid	–	337.4	[7]
Oxymatrine (OMT)	Quinolizidine alkaloid	−0.346	264.0	[175]
Oxysophocarpine (OSC)	Quinolizidine alkaloid	−0.414	262.0	[175]
Palmatine	Alkaloid	−1.117	352.4	[16,116,184]
Platyphylline	Alkaloid	0.811	337.4	[7]
Protopine	Alkaloid	3.762	353.4	[149]
Ranaconitine	Diterpenoid alkaloid	2.788	600.7	[35,43]
Rutaecarpine	Alkaloid	2.4	287.3	[101]
Scopolamine	Alkaloid	0.76	303.4	[1]
Sinomenine	Alkaloid	1.245	329.4	[161]
Sophocarpine	Alkaloid	1.371	246.3	[14]
Squalidine (senecionine)	Alkaloid	0.875	335.4	[7]
Taxol	Diterpenoid alkaloid	7.377	853.9	[6]
Tetrahydropalmatine	Alkaloid	3.702	355.4	[149]
Tetrandrine	Alkaloid	3.55	622.7	[2]
Theophylline	Alkaloid	−0.175	180.2	[13]
Verticine	Isosteroidal alkaloid	4.448	431.0	[177]
Verticinone	Isosteroidal alkaloid	3.902	429.6	[177]
Wilfordine	Alkaloid	5.045	883.8	[195]
Wilforgine	Alkaloid	NA	857.8	[195]
Wilforine	Alkaloid	6.244	867.8	[195]
Wilfortrine	Alkaloid	4.123	873.8	[195]

4. Why counter-current chromatography is so well suited for research on Chinese herbal medicines

The new legislation on traditional herbal medicinal products will require them to be designated as medicines rather than functional foods. They will therefore have to comply with the EU Pharmacopeia, which lists quality standards for herbs permitted for use in the preparation of drugs. These standards will not just apply to Good Manufacturing Practice (GMP) in the supply of the source material but will require information on their composition and toxicity. Most analytical processes require some form of sample preparation before they can analyse the contents unencumbered by particulates or debris. Hence there is a danger that a critical ingredient, whether it is the target active or a toxic contaminant, may be lost. The key advantage of counter-current chromatography is that it has a liquid stationary phase [203] and nothing is lost. Furthermore, the process is scalable [206] so that sufficient quantities for toxicology studies can be obtained.

5. Methodology

At Brunel we have kept a running bibliography of all papers published on counter-current chromatography. Authors, titles, abstracts and the full reference are stored on a database, which can be searched. The total size of the database is currently standing at about 1500 papers, which are assigned a BIB code, copied and held in our library. This review began with a specific search of this database for papers focusing on Chinese herbal medicines. It was

Table 3
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: amides, anthroquinones/naphthoquinones and coumarins.

	Classification	Log *P*	MW	Reference
Amides (6 compounds)				
(2E,4E)-N-isobutyleicosa-2,4-dienamide	Amide	–	385.0	[80]
(2E,4E,12Z)-N-isobutylocatadeca-2,4,12-trienamide	Amide	–	333.0	[80]
(2E,4E,14Z)-N-isobutyleicosa-2,4,14-trienamide	Amide	–	361.0	[80]
Guineensine, 60 mg of pipernonaline	Amide	–	383.0	[80]
Pellitorine	Amide	3.92	223.4	[80]
Piperine	Amide	2.656	285.3	[80]
Anthroquinones and naphthoquinones (13 compounds)				
2,3,5,4′-Tetrahydroxystilbene-2-β-D-glucoside	Anthroquinone	NA	406.0	[154]
6-Hydroxy-emodin	Anthroquinone	NA	270.0	[154]
Aloe-emodin	Hydroxyanthraquinone	3.382	270.2	[21,34,69]
Aloin A	Anthroquinone C-glucoside	1.858	418.4	[159]
Aloin B	Anthroquinone C-glucoside	1.858	418.4	[159]
Chrysophanol	Hydroxyanthraquinone	5.026	254.2	[21,34,69,154]
Emodin	Anthroquinone	5.027	270.2	[10,21,34,69,154]
Emodin-8-β-D-glucoside	Anthroquinone	NA	432.0	[154]
Mollugin	Naphthohydroquinone	5.155	284.3	[181]
Physcion	Anthroquinone	5.196	284.3	[10,21,34,69]
Polygonimitin B	Anthroquinone	NA	418.0	[154]
Rhein	Hydroxyanthraquinone	4.582	284.2	[21,34,53,69,154]
Shikonin	Naphthoquinone	4.351	288.3	[71]
Coumarins (28 compounds)				
7-Methoxy-daphnoretin	Coumarin	NA	365.1	[139]
Aesculetin	Coumarin	0.978	178.1	[103]
Aesculin	Coumarin	−1.519	340.3	[103]
Bergapten	Coumarin	2	216.2	[65,99]
Daphnoretin	Coumarin	3.326	352.3	[139,153]
Decursidin	Coumarin	5.989	426.5	[104]
Decursitin C	Coumarin	NA	314.4	[104]
Esculetin	Coumarin	0.978	178.1	[194]
Fraxetin	Coumarin	0.59	208.2	[103]
Fraxin	Coumarin	−1.935	370.3	[103]
Imperatorin	Coumarin	3.811	270.3	[65,68,99,150,151]
Isoimperatorin	Coumarin	3.881	270.3	[27,68,150,151]
Isopimpinellin	Coumarin	2.31	246.2	[99]
Nodakenetin	Coumarin	1.687	246.3	[104]
Notopterol	Coumarin	4.254	354.4	[27]
Osthol	Coumarin	3.87	244.3	[77,99]
Ostruthin	Coumarin	5.708	298.4	[104]
Oxypeucedanin	Coumarin	1.738	286.3	[68,150,151]
Pd-C-1V	Coumarin	NA	386.4	[104]
Pd-D-V	Coumarin	NA	386.4	[104]
Pd-Ib	Coumarin	NA	329.3	[67]
Praeruptorin	Coumarin	NA	384.4	[67]
Praeruptorin B (Pd II)	Coumarin	5.989	426.5	[67]
Qianhucoumarin D	Coumarin	NA	344.3	[67]
Scoparone	Coumarin	1.599	206.0	[138]
Umbelliferone	Coumarin	−1.58	162.0	[153]
Xanthotoxin	Coumarin	1.93	216.2	[99]
Xanthotoxol	Coumarin	0.723	202.16	[77]

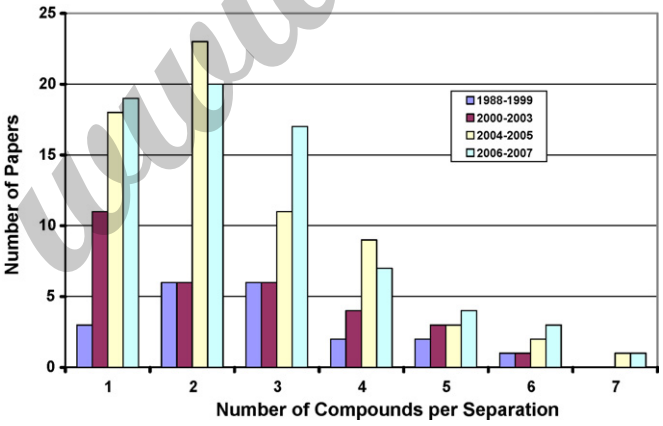

Fig. 5. An analysis of the number of compounds isolated in each paper showing how the number of compounds isolated has increased over the years.

not restricted to China, but included Eastern countries working on their own herbs (i.e. Japan and Korea) and included laboratories in other countries specifically doing research using CCC on Chinese herbs. As each paper was identified and read, key data was entered onto a master Excel Spreadsheet. Fields include the BIB library reference number; the title; journal and author information including the corresponding author; the author's affiliations; the plants separated; their family; classification; medical indicators; the compounds isolated; the log *P* and molecular weights of the compound when known; the phase systems used; their mean polarity and the instruments/centrifuges used for the separation with their operational and performance details. All together there were as many as 170 fields for each paper. Once the information from each paper was entered on the database, it was possible to analyse different aspects of the database by sorting on different fields or correlating between fields. It should be noted that in most cases data was taken from the published data and its accuracy cannot always be guaranteed. We have attempted, however, to make

Table 4
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: flavonoids.

Flavonoids part 1 (89 compounds in total)	Classification	Log *P*	MW	Reference
(2S)-4′,5,6,7-Tetrahydroxyflavanone 6-O-β-D-glucopyranoside	Flavanone glycoside	NA	451.1	[123]
(2S)-5,2′,6′-Trihydroxy-2″,2″-dimethylpyrano [5″,6″:6,7] flavanone	Flavonoid	NA	354.0	[141]
(2S)-5,2′,6′-Trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethyl-1-keone-cyclohexadiene)-flavanone	Prenylated dihydroflavonoid	NA	502.2	[185]
(2S)-5,2′,6′-Trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethylpyrano)-3′,4′,-(2,2-dimethyl-1-keone-cyclohexadiene)-flavanone	Prenylated dihydroflavonoid	NA	584.2	[185]
(2S)-5,7,2′,6′-Tetrahydroxy-4′-lavandulylated flavanone	Flavonoid	NA	424.0	[141]
(2S)-5,7,2′,6′-Tetrahydroxy-6,8-di(g,g-dimethylallyl) flavanone	Flavonoid	NA	400.5	[141]
(2S)-5,7,2′,6′-Tetrahydroxy-6-lavandulylated flavanone	Flavonoid	NA	424.0	[141]
(2S,3″S)-5,2′,6′-trihydroxy-3″-g,g-dimethylallyl-2″,2″-dimethyl-3″,4″-dihydropyrano [5″,6″:6,7] flavanone	Flavonoid	NA	424.0	[141]
(3R)-(−)-7,2′-Dihydroxy-3′,4′-dimethyl isoflavan-7-O-glucopyranoside	Flavonoid	–	461.0	[72]
(6aR,11aR) 9,10-Di-methoxypterocarpan-3-O-glucopyranoside	Flavonoid	–	464.0	[72]
2′,3,4,4′-Tetrahydroxychalcone	Flavonoid	–	272.0	[73]
3,5,6,7,8,3′,4′-Heptamethoxyflavone	Polymethoxylated flavones	1.586	432.4	[119]
3′-Methoxy-puerarin	Flavonoid	NA	418.4	[19]
5,6,7,4′-Tetrahydroxyflavone	Flavonoid	–	287.0	[73]
5,7,2′,6′-Tetrahydroxy-6,8-di(g,g-dimethylallyl)-flavanone	Flavonoid	NA	329.5	[114]
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone	Polymethoxylated flavones	–	388.4	[119]
7,3′,4′-Trihydroxydihydroflavone (butin)	Flavonoid	2.157	272.3	[73]
8-C-β-D-(2′-O-(E)-cinnamoyl) glycopyranosyl-2-(2-hydroxy) propyl-7-methoxy-5-methyl-chromone	Chromone	–	540.0	[81]
Apigenin-7-O-glucoside	Flavonoid	−0.388	432.0	[117]
Apigenin-7-O-neohesperidoside	Flavonoid	1.717	578.0	[117]
Baicalein	Flavonoid	3.311	270.2	[47,48,63,82–84,97,127]
Baicalein-7-O-diglucoside	Flavonoid	NA	594.0	[47,83]
Baicalein-7-O-glucoside	Flavonoid	NA	432.0	[47,48,82–84,127]
Baicalin	Flavonoid	0.314	446.5	[47,51,63,121]
Biochanin A	Flavonoid	3.139	284.0	[84]
Bolusanthol B	Flavonoid	4.718	356.4	[114]
Calycosin-7-*O*-β-D-glycoside	Flavonoid	NA	446.0	[52]
Chrysin	Flavonoid	2.879	254.0	[47,48,82–84,127]
Clinopodiside	Flavone glycosides	0.656	959.0	[176]
Diadzin	Flavonoid	0.45	416.4	[19]
Didymin	Flavone glycosides	2.737	594.0	[176]
Epimedokoreanoside I	Flavonoid	NA	910.8	[102]
Formononetin-7-O-β-D-glycoside	Flavonoid	NA	431.0	[52]
Genistein-4-β-L-rhamnopyransoyl-(1-2)-β-D-glucopyranoside	Flavone	NA	578.5	[189]
Genistein-7,4-di-O-β-D-glucoside	Flavone	NA	594.5	[189]
Genistein-7-O-β-D-glucopyranoside-4-O-[α-L-rhamnopyransoyl)-(1-2)-β-D-glucopyranoside]	Flavone	NA	740.7	[189]
Glisoflavone	Flavonoid	4.703	368.4	[3]
Icariin	Flavonoid	2.479	676.7	[102]
Icariside II	Flavonoid	NA	502.5	[102]
Inflacoumarin A	Flavonoid	NA	323.0	[74]
Iisoliquiritigenin	Flavonoid	3.113	256.3	[106]
Isoorientin	Glycosylflavone	1.277	432.4	[113]
Isoquercitrin	Flavonoid	0.824	464.4	[142]
Isorhamnetin	Flavonoid	1.757	316.3	[15,17,55]
Isorhamnetin-3-O-β-D-glucosyl-(1-2)-α-L-rhamnoside	Flavonol glycosides	NA	622.0	[197]
Isovitexin	Glycosylflavone	−0.909	464.4	[113]

this review as informative as possible for those interested in both CCC and its application to the separation of compounds from Chinese herbal medicines with tables for the compounds, the plant species and families with cross references to the papers in which they feature.

To analyse the wide range of polarities of molecules separated by CCC we have used log *P* values as a measure of hydrophobicity and hydrophilicity. High log *P* values indicate compounds of high hydrophobicity. Values for predicted log *P* values were obtained from the eMolecules database (http://www.emolecules.com), which provides calculated log *P* values for millions of compounds. Of the 354 molecules separated by CCC in the papers reviewed, we were able to obtain log *P* values for 214. For phase system polarity we have used Rohrschneider and Snyder polarity parameters for component solvents (i.e. heptane = 0.15, water = 10.2) calculated on a volume basis. In the text we give the Reichardt polarity values in brackets for comparison on a volume basis scaled for water = 10.

6. Phase system selection

While counter-current chromatography is very easy and simple to use, it is often said that one of the aspects, which puts people off using it, is the huge choice of phase systems available and they simply do not know where to start. We analysed the phase systems used in the papers reviewed. From the pie chart in Fig. 1 it can be seen that alkane/alcohol systems are by far the most popular representing nearly 40% of the phase systems used. Table 1 shows how usage has changed over time. Prior to the year 2000, chlorinated systems were used twice as frequently as alkane systems. The decrease in use recently reflects the need to avoid such solvents on health and safety grounds. By 2007 alkane systems were used in 70% of the papers. More recently, papers have been appearing which use acetonitrile systems [47,52,106,155,171,193] and acetic acid systems [72,117,130,163,165,180]. For alkane systems there are prescribed methods for selecting solvents [208,209]. Otherwise, if the plant species is known, then the simplest way

Table 5
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: flavonoids/cont.

Flavonoids/cont part 2 (89 compounds in total)	Classification	Log *P*	MW	Reference
Kaemferol-3-O-rhamnoside-7-O-(6-feruloylgluco-(1-3)-rhamnoside)	Flavonol glycoside	NA	738.0	[197]
Kaempferol	Flavonoid	2.053	286.2	[9,15,17,55]
Kaempferol-3-O-β-D-sophoroside	Flavone	NA	578.5	[189]
Kaempferol-3-O-β-L-ramnopyranosyl-(1-6)-β-D-glucopyranoside	Flavone	NA	594.5	[189]
Kaempferol-7-O-glucoside	Flavonoid	NA	448.4	[117]
Kurarinone	Prenylated flavonoid	NA	438.0	[144]
Kushenol I	Prenylated flavonoid	NA	454.0	[144]
Licoagrochalcone B	Flavonoid	NA	336.0	[140]
Licoarylcoumarin	Flavonoid	NA	368.0	[3]
Licochalcone A	Flavonoid	4.95	338.4	[74]
Liquiritigenin	Flavonoid	2.759	256.2	[106]
Liquiritin	Flavonoid	NA	418.4	[186]
Luteolin-7-O-glucoside	Flavonoid	−0.09	448.0	[117]
Nairutin	Flavone glycoside	NA	580.0	[176]
Naringin	Flavanone glycoside	2.734	581.0	[171,174]
Neoponcirin	Flavanone glycoside	2.723	594.5	[171]
Nobiletin	Polymethoxylated flavone	2.485	402.0	[119]
Orientin	Flavonoid glycoside	1.575	448.0	[125]
Orotinin	Flavonoid	NA	422.0	[140]
Orotinin-5-methyl ether	Flavonoid	NA	436.0	[140]
Oroxylin A	Flavonoid	2.373	284.3	[63,97]
Poncirin	Flavanone glycoside	3.386	594.6	[171]
Puerarin	Flavonoid	1.954	416.4	[19]
Puerarin-2-xyloside	Flavonoid	NA	563.5	[19]
Puerarin-6-xyloside	Flavonoid	NA	563.5	[19]
Quercetin	Flavonoid	2.075	302.2	[9,15,17,55]
Quercetin-3-O-β-L-ramnopyranosyl-(1-6)-β-D-glucopyranoside	Flavone	NA	610.5	[189]
Quercetin-3-O-L-rhamnoside	Flavonoid	2.171	448.0	[107]
Quercetin-3-O-neohesperidoside	Flavonoid glycoside	NA	610.0	[125]
Quercetin-7-O-rhamnoside	Flavonoid	NA	446.0	[142]
Quercitin-3-O-rhamnoside-7-O-(6-feruloylgluco-(1-3)-rhamnoside	Flavonol glycoside	NA	754.0	[197]
Quercitrin	Flavonoid	2.171	448.4	[142]
Rhiofolin	Glycosidic flavonoid	1.717	578.5	[174]
Sarothalin G	Flavonoid	NA	574.7	[142]
Sarothialen A	Flavonoid	NA	534.0	[142]
Sarothralen B	Flavonoid	NA	551.0	[142]
Scutellarin	Flavone glycoside	−0.463	462.4	[130]
Sophoraflavanone G (SFG)	Prenylated flavonoid	6.518	424.5	[144]
Tangeretin	Polymethoxylated flavone	2.663	372.4	[119]
Tetrapterol I	Flavonoid	6.98	392.5	[114]
Vitexin	Flavonoid glycoside	1.277	432.0	[125]
Wogonin	Flavonoid	2.145	284.3	[63,97]
Wogonoside	Flavonoid	1.419	460.0	[121]

is to look up what others have used and use something similar.

7. Compounds isolated

There are 354 different compounds isolated in the papers reviewed. Where possible the log *P* and molecular weight have been given. Fig. 2 shows the polarity range (log *P*) of 211 compounds where the log *P* could be found. It can be seen that there is a wide range of polarities covered from −4 to +12 peaking between +2 and 3. An example of a compound separated in a low polarity phase system is lutein with a log *P* of +11.8 [54] using heptane/chloroform/acetonitrile (10/3/7, v/v/v) with a mean polarity 2.72 (2.06). An example of a compound separated in an intermediate polarity phase system is quercetin with a log *P* of 2.075 [9,15,17,55] using chloroform/methanol/water (4/3/2, v/v/v) with a mean polarity of 5.79 (5.91). Finally, an example of a compound separated in a high polarity phase system is gastrodin with a log *P* of −1.853 [66] using butanol/ethyl acetate/water (2/3/5, v/v/v) with a mean polarity of 7.2 (7.44). Fig. 2 shows that most attention has been paid to compounds of intermediate polarity and less to those of high polarity (negative log *P* values) and those of low polarity (high log *P* value). All of the most frequently studied molecules have intermediate polarities—tanshinone IIA with a log *P* of 5.471 [25,31,40,42,50,59,60,131]; baicalein with a log *P* of 3.311 [47,48,63,82–84,97,127]; cryptotanshinone with a log *P* of 4.931 [25,31,40,50,60,131]; tanshinone I with a log *P* of 4.443 [25,31,40,50,59,60,131]; chrysin with a log *P* of 2.879 [47,48,82–84,127]. Baicalein-7-O-glucoside has been widely studied by Chen et al. [47,48,82–84,127] but we have not been able to find its log *P*. A possible explanation for the low number of examples of polar compounds is that such molecules require polar two-phase systems. These all tend to have low interfacial tension and low-density difference, which can lead to poor retention of the stationary phase and low separation efficiency. However, it must be noted that peptides, which are often quite polar molecules, have been separated in polar phases such as butanol/acetic acid/water, for example by Knight [210].

The molecular weight range (Fig. 3) is not so Gaussian but does illustrate that counter-current chromatography separations, and the aqueous organic phase systems they use, favour the lower end of the molecular weight range with peaks between 250 and 500. Interestingly, however, high molecular weight glycoproteins (11,300–65,700 mol. wt.) have been isolated by CCC from *Morchella esculenta* (L.) by Wei et al. [32] using an aqueous–aqueous two-phase system of polyethylene glycol (PEG) 8000 (12.5%, w/w) and potassium phosphate (25%, w/w) with the compounds eluting in

Table 6
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: ginsenosides, glycoproteins, glycosides and lignans.

	Classification	Log *P*	MW	Reference
Ginsenosides (9 compounds)				
Ginsenoside F4	Ginsenoside	8.317	766.0	[166]
Ginsenoside Rg3	Ginsenoside	5.269	785.0	[166]
Ginsenoside Rg5	Ginsenoside	NA	766.0	[166]
Ginsenoside Rk1	Ginsenoside	NA	766.0	[166]
Ginsenoside-Rb1	Dammarane saponin	4.654	1109.0	[46,49]
Ginsenoside-Rd	Dammarane saponin	3.108	947.2	[46]
Ginsenoside-Re	Dammarane saponin	4.746	947.1	[46,49]
Ginsenoside-Rg1	Dammarane saponin	1.657	801.0	[46,49]
Notoginsenoside-R1	Dammarane saponin	4.423	933.2	[46,49]
Glycoproteins (3 compounds)				
Glycoprotein 1	Glycoprotein	NA	65,700	[32]
Glycoprotein 2	Glycoprotein	NA	22,400	[32]
Glycoprotein 3	Glycoprotein	NA	11,300	[32]
Glycosides (9 compounds)				
2′-Acetyl acteoside	Phenylethanoid glycoside (PhG)	NA	666.6	[29]
4′-O-β-D-Glucosyl-5-O-methylvisamminol	Xanthene glucoside	NA	438.0	[146]
Acteoside	Phenylethanoid glycoside	2.437	624.6	[29,100]
Amygdalin	Glycoside	−0.36	457.0	[152]
Curculigoside	Phenolic glucoside	−0.024	466.4	[143]
Curculigoside B	Phenolic glucoside	0.407	452.4	[143]
Gastrodin	Glycoside	−1.853	286.3	[66]
Isoacteoside	Phenylethanoid glycoside (PhG)	2.576	624.6	[100]
Prim-O-glucosyl-cinmifugin	Xanthene glucoside	NA	438.0	[146]
Lignans (20 compounds)				
4′-Dimethyl-podophyllotoxin	Lignan	NA	414.0	[9]
Arctigenin	Lignan	2.466	372.0	[133]
Arctiin	Lignan	0.235	534.6	[118,191]
Clemastanin B	Lignan	NA	684.0	[111]
Deoxyschisandrin	Lignan	5.867	416.5	[5,91]
Gomisin A	Lignan	4.77	416.5	[112]
γ-Schisandrin	Lignan	6.456	400.5	[91]
Indigoticoside A	Lignan	NA	522.0	[111]
Liriodendrin	Lignan	−3.912	742.7	[163]
Matairesinol	Lignan	1.697	358.0	[133]
Meso-dihydroguaiaretic acid	Lignan	NA	330.0	[5]
Phillyrin	Lignan glucoside	−0.693	520.5	[98]
Pinoresinol diglucoside	Lignan	−2.924	682.7	[163]
Podophyllotoxin	Lignan	1.598	414.4	[9]
Pregmoisin	Lignan	NA	390.0	[5]
Schisandrin	Lignan	4.18	432.5	[92,112]
Schisanhenol	Lignan	5.336	402.5	[4]
Schisanhenol acetate	Lignan	NA	444.5	[4,5]
Schisanhenol B	Lignan	–	444.0	[5]
Schisantherin	Lignan	6.383	536.6	[92]

order of decreasing molecular weight (65.7, 22.4 and 11.3 K) using a PEG-rich stationary phase and eluting with the phosphate-rich mobile phase.

Compounds have been classified (often by the author) into groups. It can be seen from the pie chart in Fig. 4 that flavonoids is the largest group (89 papers: 25%). Flavonoids (25%), alkaloids (16%), polyphenols (14%), terpenoids (13%) and coumarins (8%) account for over 75% of all of the compounds isolated. These compounds with the classification that appeared in the paper, their log *P* and molecular weight if known are given with their references in Table 2 for alkaloids; Table 3 for amides, anthroquinones/naphthoquinones and coumarins; Tables 4 and 5 for flavonoids; Table 6 for ginsenosides, glycoproteins, glycosides and lignans; Table 7 for polyphenols; Table 8 for peptides, lactones, saponins, steroids and other classifications and Table 9 for terpenoids. Molecular weights of compounds isolated have, where possible, been taken from each paper. If it was missing we have obtained the molecular weights from tables or calculated them for new compounds from the chemical formulae given. The data is for guidance rather than guaranteed accuracy as we have not checked published data.

8. Number of compounds isolated by counter-current chromatography

In CCC a phase system is selected to provide a distribution ratio of between 0.2 and 5 to elute the compound within a reasonable time period. Compounds with higher distribution ratios will be retained progressively in the stationary phase in the CCC column, while compounds with lower distribution ratios will elute fast with the mobile phase, with little space for separation. Given these constraints, CCC does not resolve a mixture into all its components in a single run, unlike HPLC. Generally several phase systems are needed to obtain all components isocratically. Alternatively there are a number of ways of operating the CCC process to retrieve the compounds in the stationary phase. No papers report complete separations of all the compounds and, in general, the procedures used isolate selected compounds in a single CCC run using a single phase system. We have analysed the number of compounds separated in the papers reviewed. The result is plotted in Fig. 5 and shows how the distribution of the number of compounds separated has changed over time. It can be seen that more recent separations are isolating more compounds. This is due to improvements in the techniques used.

Table 7
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: polyphenols.

Polyphenols (49 compounds)	Classification	Log *P*	MW	Reference
3,4-Dihydroxyphenyllactic acid	Polyphenol	−0.289	198.2	[165]
(−)-4′-O-methylnyasol	Polyphenol	NA	266.3	[147]
1-O-(4-Hydroxybenzoyl)-glucose	Polyphenol	NA	299.0	[162]
1-O-Caffeoyl-β-D-glucose	Polyphenol	NA	341.0	[162]
1-O-Feruloyl-β-D-glucose	Polyphenol	NA	355.0	[162]
1-O-p-Coumaroyl-β-D-glucose	Polyphenol	NA	341.0	[162]
2,3,5,4′-Tetrahydroxy stilbene-2-O-D-glucoside	Polyphenol	NA	390.0	[11]
3,4-O-Dicaffeoylquinic acid	Polyphenol	NA	514.0	[187]
3,5-O-Dicaffeoylquinic acid	Polyphenol	NA	514.0	[187,192]
4,5-O-Dicaffeoylquinic acid	Polyphenol	NA	514.0	[187,192]
Anthraglycoside A	Polyphenol	NA	434.4	[33]
Anthraglycoside B	Polyphenol	NA	432.4	[33]
Astilbin	Polyphenol	3.938	266.3	[88,89]
Catechin	Polyphenol	0.491	290.3	[22,89,94,95]
Catechin gallate	Polyphenol	2.668	442.4	[95]
Chebulagic acid	Tannin	2.253	954.7	[132]
Chebulinic acid	Tannin	4.139	956.7	[132]
Chlorogenic acid	Polyphenol	−0.356	354.3	[70]
Cis-hinkiresinol	Polyphenol	NA	252.3	[147]
Corilagin	Tannin	2.342	634.4	[38]
Ellagic acid	Tannin	0.518	302.2	[38]
Epicatechin	Polyphenol	0.491	290.3	[95,145]
Epicatechin gallate	Polyphenol	2.668	442.4	[95,145]
Epigallocatechin	Polyphenol	−0.096	306.3	[95]
Epigallocatechin gallate	Polyphenol	2.082	458.4	[95,145]
Epigallocatechin-3,5-di-O-gallate	Polyphenol	NA	598.5	[95]
Eugenol	Polyphenol	2.204	164.2	[164]
Ferulic acid	Phenolic acid	1.641	194.0	[135]
Gallic acid	Polyphenol	0.911	170.1	[23,24,179]
Honokiol	Polyphenol	4.199	266.3	[75,137,160]
Hyperoside	Polyphenol	1.745	464.0	[158]
Lithospermic	Polyphenol	1.453	538.0	[126]
Magnolol	Polyphenol	3.938	266.3	[75,137,160]
Mangerferin	Polyphenol	0.127	422.3	[134,147,198]
Methyl gallate	Polyphenol	1.537	184.1	[108]
Naringenin	Polyphenol	3.192	272.0	[89]
Neomangiferin	Polyphenol	NA	584.5	[147,198]
Parthenocissin A	Polyphenol	NA	454.5	[169]
p-Coumaric acid	Polyphenol	1.876	164.2	[162]
Piceid	Polyphenol	1	390.4	[28,85]
Protocatechualdehyde	Polyphenol	1.137	138.1	[165]
Punicalagin	Polyphenol	2.943	1084.7	[180]
Quadrangularin A	Polyphenol	5.425	454.5	[169]
Resveratrol	Polyphenol	3.139	228.2	[28,33,85]
Rosmarinic acid	Polyphenol	1.698	360.0	[126]
Salidroside	Polyphenol	1.234	300.3	[30]
Salvianolic acid B	Polyphenol	2.14	714.6	[39,126,157,165]
Salvianolic acid E	Polyphenol	NA	718.0	[126]
Trans-3,5,4′-trihydroxystilbene-4′-O-β-D-glucopyranoside	Polyphenol	–	390.4	[94]

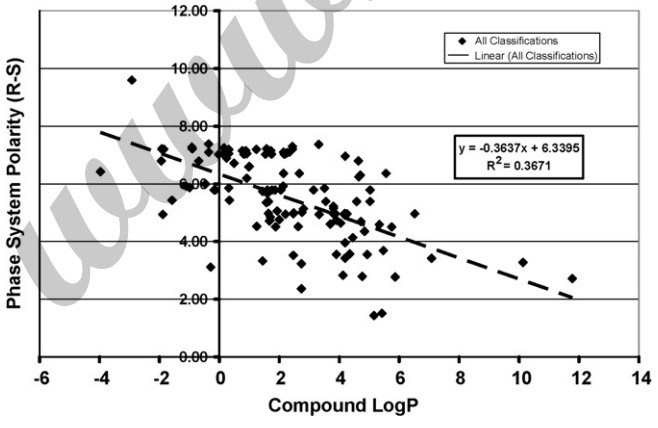

Fig. 6. A plot of the log *P* of each isolated compound first eluted against the mean polarity of the phase system used. A total of 123 compounds were analysed. The data is only partially controlled for distribution ratio by taking the first compound to elute which has a distribution ratio in the range 0.2–1.0.

These will be discussed in more detail in Section 11 dealing with performance and operational issues. The full potential, though, of CCC in China has still to be realised as discussed in the final section on the future.

9. Phase system polarity

A wide range of phase compositions has been used. These are obtained by the use of a selection of solvents of varying polarity (Table 10). The selection of appropriate phase systems has been discussed widely [208,209]. A method we have developed at Brunel has been to measure the distribution ratios of all components of a sample in a range of phase systems, prepared using a liquid handling robot, determining the concentration of each component in the two phases by an appropriate analytical method (usually HPLC or GC) [208]. Given the complexity and variety of the phase systems reported, we have used a "polarity" estimate to provide a qualitative and relative measure of phase systems used by calculating "polarity" from a sum of individual polarities of the components, on a

Table 8
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: lactones, peptides, saponins, steroids and others.

	Classification	Log *P*	MW	Reference
Lactones (5 compounds)				
11b,13-Dihydrolactucin	Sesquiterpene lactone	NA	278.0	[193]
Costunolide	Sesquiterpene lactone	4.049	232.3	[96]
Dehydrocostuslactone	Sesquiterpene lactone	3.398	230.3	[96]
Lactucin	Sesquiterpene lactone	0.334	276.3	[193]
Lactucopicrin	Sesquiterpene lactone	NA	399.4	[193]
Peptides (3 compounds)				
Aurentiamide acetate	Peptide	NA	444.5	[110]
Heterophyllin B	Cyclopeptide	NA	778.0	[44]
Pseudostellarin B	Peptide	NA	683.0	[167]
Saponins (7 compounds)				
3-O-β-D-Glucose-β-sitosterol	Saponin	NA	576.0	[196]
Astragaloside I	Saponin	NA	869.0	[168]
Astragaloside II	Saponin	NA	827.0	[168]
Astragaloside IV	Saponin	1.957	785.0	[168]
Glycyrrhizin	Saponin	4.637	822.9	[62,186]
Lucyoside H	Saponin	NA	780.0	[128]
Lucyoside Q	Saponin	NA	634.0	[128]
Steroids (5 compounds)				
24-Methylene cycloartanol	Steroid	NA	440.0	[196]
3-O-Palmitoyl-β-sitosterol	Steroid	NA	652.0	[196]
Lupenone	Steroid	10.399	424.7	[196]
α-Spinasterol	Steroid	10.133	412.7	[196]
β-Sitosterol	Steroid	10.729	414.7	[196]
Others (19 compounds)				
(E)-4-(3′,4′-Dimethoxyphenyl)but-1,3-diene	Phenylbutenoid	NA	238.3	[105]
(E)-4-(3′,4′-Dmethoxyphenyl)but-3-enyl acetate	Phenylbutenoid	NA	204.3	[105]
1-[2′,4′-Dihydroxy-3′,5′-di-(3″-methylbut-2″-enyl)-6′-methoxy] phenylethanone	Aryl ketone	–	316.0	[61]
132-Hydroxy-(132-S)-phaeophytin-a	Porphyrins	NA	887.0	[170]
3,6′-Disinapoyl sucrose	Sucrose ester	–	752.0	[93]
3-Butylidenephthalide	Phthalide	NA	188.2	[155]
3-Butylphthalide	Phthalide	NA	186.2	[155]
Chlorophyll b methoxylactone	Porphyrin	NA	952.0	[170]
Chuanxiongzine	Pyrazine	1.561	136.2.	[64]
Eleutheroside E	–	NA	742.7	[41]
Isoastilbin	–	–	450.0	[88]
Isopsoralen	Psoralens	2.012	186.2	[76]
Lutein	Carotenoid	11.778	568.9	[54]
Neocnidilide	Phthalide	NA	253.0	[155]
Psoralen	Psoralen	1.67	186.2	[76]
Salicin	–	−1.851	457.4	[87]
Senkyunolide A	Phthalide	2.484	192.0	[155]
Tenuifoliside A	Sucrose ester	–	682.0	[93]
Z ligustilide	Phthalide	2.63	190.0	[155]

volume basis. We have used the Rohrschneider and Snyder (R–S) polarity parameters. A comprehensive list of solvent polarities is given in [211]. A plot of compound log *P* against phase system polarity for 123 compounds eluting as the first peak is given in Fig. 6. Although there is considerable scatter there is a very significant correlation ($P < 0.001$) between the two.

10. Counter-current chromatography instrumentation

When CCC was first used in China in 1988 [1,2] two commercially available instruments from the United States were used—a PC Inc. instrument [1] and a PharmaTech instrument [2]. However the climate at that time in China was not one that could support investment in western commercial technology, so they made their own instruments in well equipped laboratories. Supplementary Table S17 shows how in the early years after publication of the first few papers using commercial instruments, probably on working visits, the majority of papers were from Beijing Institute for New Technology Application using their newly made centrifuge. Their instruments were sold to other establishments until TautoBiotech (www.tautobiotech.com) took over the license to make these instruments from the Beijing Institute. It is only recently that the more robust high-*g* instruments from Dynamic Extractions (www.dynamicextractions.com) are starting to find their way into China now that laboratories in China are able to afford high performance western instrumentation. Supplementary Figure S5 shows the instruments used in the papers reviewed. The field is dominated by the Beijing Institute and TautoBiotech accounting for 61% of all the usage. Only PharmaTech made an impact on the market and sadly the premature death of the company's founder, Edward Chou, has led to a decline in their uptake in China recently. In 2006/2007 Shanghai TautoBiotech were the manufacturers of nearly 50% of the instruments in use.

11. Performance and operational issues

Table 11 gives a list of the CCC instruments used with their operational details for the papers studied in this review. The CCC instruments used are mainly centred in China and are the TautoBiotech 300A, the Beijing Institute GS10A2 and the PharmaTech CCC1000 accounting for nearly 75% of the compound isolations reported in this review. All are low-*g* instruments operating

Table 9
Lists of compounds isolated with their published classification, log *P*, molecular weight and references grouped by type or family: terpenoids.

Terpenoids (46 compounds)	Classification	Log *P*	MW	Reference
10-Deacetylbaccatin + others	Diterpene	3.508	544.6	[12]
24-Hydroxy-ursolic acid	Pentacyclic triterpene	NA	472.0	[129]
8-Hydroxy-10-hydrosweroside	Iridoid glycoside	−3.965	376.4	[173]
Atisine	Diterpenoid alkaloid	3.634	343.5	[190]
Atractylenolide III	Sesquiterpene	NA	248.0	[156]
Atractylon	Sesquiterpene	5.566	216.3	[156]
Baccatin-III	Alkaloid (diterpenoid)	4.06	586.6	[6]
Barbinervic acid	Pentacyclic Triterpene	5.751	488.0	[129]
Celastrol	Triterpenoid	7.078	450.6	[78]
Cephalomannine	Alkaloid (diterpenoid)	6.586	831.9	[6]
Cryptotanshinone	Diterpenoid	4.931	296.4	[25,31,40,50,60,131]
Curdione	Sesquiterpene	3.121	236.0	[122]
Danshenxinkun	Diterpenoid	NA	281.3	[31]
Geniposide	Iridoid glucoside	−1.92	388.4	[124,172]
Germacrone	Sesquiterpene	4.762	218.0	[122]
Goyaglycoside-a	Triterpene saponin	–	656.0	[90]
Goyaglycoside-e	Triterpene saponin	–	788.0	[90]
Guanfu base A (GFA)	Diterpenoid alkaloid	0.477	429.5	[190]
Guanfu base F (GFF)	Diterpenoid alkaloid	NA	457.0	[190]
Guanfu base G (GFG)	Diterpenoid alkaloid	NA	473.0	[190]
Guanfu base I (GFI)	Diterpenoid alkaloid	NA	387.0	[190]
Guanfu base P (GFP)	Diterpenoid alkaloid	NA	499.0	[190]
Harpagoside	Iridoid glocoside	−0.917	494.0	[148]
Isofraxidin	Triterpene	1.314	222.2	[8]
Lappaconitine	Diterpenoid alkaloid	2.126	584.7	[35,43]
Methylenetanshiquinone	Diterpenoid	NA	278.3	[31]
Momordicose L	Triterpene saponins	NA	642.0	[90]
Momordicoside K	Triterpene saponins	NA	656.0	[80]
N-deacetyllappaconitine	Diterpenoid alkaloid	NA	NA	[43]
N-deacetylranaconitine	Diterpenoid alkaloid	NA	NA	[43]
Oridonin	Diterpenoid	1.44	364.0	[136,182]
Paeoniflorin	Monoterpine glycoside	0.161	480.5	[56]
Ponicidin	Diterpenoid	3.109	362.0	[182]
Ranaconitine	Diterpenoid alkaloid	2.788	600.7	[35,43]
Rotungenic acid	Pentacyclic Triterpene	NA	488.0	[129]
Rupestonic acid	Azulene sesquiterpene	2.801	248.3	[109]
Scopoletin	Triterpene	1.285	192.0	[8]
Swertiamarin	Iridoid glycosides	−3.15	374.3	[173]
Tanshinone I	Diterpenoid	4.443	276.3	[25,31,40,50,59,60,131]
Tanshinone IIA	Diterpenoid	5.471	294.3	[25,31,40,42,50,59,60,131]
Taraxeryl-acetate	Triterpene	NA	468.8	[8]
Taxol	Alkaloid (diterpenoid)	7.377	853.9	[6]
Trifloroside	Iridoid glycoside	0.685	782.7	[173]
Tripdiolide	Diterpenoid triepoxide	0.169	376.0	[120]
Unknown compound	Iridoid glycoside	NA	NA	[173]
Ursolic acid	Pentacyclic triterpene	9.011	456.7	[129]

between 50 and 60 g with long operating times (from 2 up to 10 or even 13 h) and have maximum throughputs of between 0.2 and 0.6 g/h. New higher "*g*" technology is now coming on to the market [206,212] with separation times of minutes rather than hours. Also CCC has been shown to be easy to scale up [87,160,206] either using the latest Dynamic Extractions Maxi Centrifuge, which can process 103 g/h with cycle times of less than an hour [160], or the Zhejiang Gongshang Slowly Rotating Coiled (SRC) tube which can process up to 24 g/h over a period of a few days [87].

CCC is primarily used to isolate and purify compounds. Using a particular phase system, only a few compounds can generally be separated isocratically (Fig. 5). The most we have found for Chinese herbal medicines is 7 [95]. In this case 7 catechins were obtained using one form of dual mode CCC. 5 target compounds were eluted, pumping the aqueous phase of a hexane/ethyl acetate/methanol/water (1/7/1/7, v/v/v/v) phase system as the mobile phase (in the head to tail direction) and then a further 2 target compounds (among others) were eluted from the stationary phase by changing the mobile phase to the organic phase and pumping in the other direction (tail to head). This illustrates how improved separations can be achieved by better use of the potential of CCC.

Table 10
List of solvents used in making of the phase systems used in the 198 papers reviewed in the Rohrschneider and Snyder (R–S) polarity order with the Reichardt polarity values for comparison.

Solvent	R–S value	Reichardt
Light petroleum ether	0.10	–
Hexane	0.15	0.009
Heptane	0.15	0.012
Trichloromethane/carbon tetrachloride	1.60	0.052
Diethyl ether	2.80	0.117
Dichloromethane/methylene chloride	3.10	0.309
Dichloroethane	3.50	0.327
n-Butanol	3.90	0.586
Isopropanol	3.90	0.546
n-Propanol or 1-propanol	4.00	0.617
Tetrahydrofuran (THF)	4.00	0.207
Chloroform/trichloromethane	4.10	0.259
Ethanol	4.30	0.654
Ethyl Acetate	4.40	0.228
Methanol	5.10	0.762
Acetonitrile	5.80	0.460
Acetic acid	6.00	0.648
Water	10.20	1.000

Table 11
The CCC instruments listed with details of the rotor radius (*R*, cm); their minimum and maximum coil/column volumes (Vc, ml); the tubing internal diameter (*d*, mm); rotor speed range (*N*, rpm); "*g*" field range (number of times earth's gravitational field); flow range (*F*, ml/min); range of sample loading by weight (mg); run time range (min) and range of throughput (g/h).

Centrifuge	*R* (cm)	$Vc_{min/max}$ (ml)	*d* (mm)	$N_{min/max}$ (rpm)	$g_{min/max}$	$F_{min/max}$ (ml/min)	Sample-min/max (mg)	Run time (min)	Throughput (g/h)
Beijing Institute (GS10A2)	8	190/300	1.6	800/800	57.2/57.2	0.8/2.0	150/3,000	90/690	0.007/0.6
Beijing Institute (GS20)	5	30/40	0.85	1,500/1,800	125/181	1.0/1.0	2/40	90/390	0.001/0.016
Brunel University (Milli)	5	4.6	0.8	2100	246	0.5/1	4	14	0.017
Brunel University (Midi)	11	50	1.6	800/1,100	78/148	1	10/20	110/120	0.005
Dynamic Extractions (Mini)	5	4.6/18	0.8	1,800/2,100	181/246	2.5	4/80	25	0.001/0.01
Dynamic Extractions (Maxi)	30	4,600	10	600	121	600	43,000	25	103.2
PC Inc	10	238/385	1.6	650/830	47/82	2/3.3	30/10,000	160/930	0.015/0.91
PharmaTech (CCC-1000)	7.5	325/850	1.6/2.6	800/1,000	53.7/92	1/5	5/160	160/930	0.02/0.2
Sanki (LLB-M)	N/A	230	N/A	800/1,200	–	2	150/957	75/180	0.064/0.2
ShanghaiTautoBiotech (TBE-300A)	5/13	119/350	1.6/2.6	670/950	48/50.4	1/4	100/2,000	120/780	0.004/0.48
ShanghaiTautoBiotech (TBE-1000A)	–	1,000	–	450/500	–	5	200/1,000	300/500	0.09/0.142
ShanghaiTautoBiotech (TBE-60A)	–	60	–	1,000	–	0.6	5	120	0.0025
Zhejiang Upright CCC	9	1,600	4	300/500	9–25	4/5	200/2,500	150/600	0.05/0.44
Zhejiang Gongshang (SRC)	7/20.5	700/40,000	5.7/17.0	60/80	0.5/1.1	1/50	3,000/500,000	1250/2,100	0.086/24.0
Zhejiang Ito HSCCC	8/10	146/700	1.6/3	700/800	54.8/71.5	1.5/3.5	258/2,000	150/475	0.075/0.4

12. Operational innovations

It has long been appreciated that CCC is an excellent sample preparation process as it can handle very crude extracts ahead of preparative HPLC final stage high purity clean up. Peng et al. [184] have taken this one stage further by injecting the powders of a raw material without any preparation. They demonstrate this for an isolation and separation of four alkaloids, jatrorrhizine, coptisine, palmatine and berberine from *Coptis chinensis* Franch with high recoveries of over 92% as determined by HPLC.

A number of methods have been used to improve CCC separations. In multidimensional CCC, a peak is taken out of one CCC centrifuge and run in a second centrifuge to further resolve it [15,40,120,150,182] sometimes with a change in phase system. In stepwise gradient elution, the mobile phase is changed progressively to elute compounds [67–69,77,94,99,116,131,189,193]; in the latter case [193] the fraction was evaporated and rerun. Faster elution of slowly eluting compounds can be achieved by stepwise increase of the flow rate [125,139–142,144,151,173,196].

In a few cases, for charged compounds such as alkaloids or rhein (an anthroquinone with a carboxylic acid substituent), pH dependent methods have been used, such as pH zone refining [14,35,149] and pH modulated stepwise elution [21,34]. Given the large number of alkaloid CCC studies (56 compounds in 66 papers) it is interesting that so little advantage of pH dependent methods has been taken.

Compared with HPLC, CCC does not separate a large number of components in a single run and as such is generally not seen as an analytical technique. Nevertheless there are several reports of CCC being used for fingerprint analysis of Chinese herbal medicines. Examples of fingerprinting using *Salvia miltiorrhiza* Bunge are given in [59,60,131] and another using *Arctium lappa* L., *Magnolia officianalis* Rehd. Et Wils. and *Psoralea corylitolia* L. given in [191].

13. Counter-current chromatography–are there limitations?

The main limitations in CCC have not been so much in its operation and performance, which has numerous advantages, but in the availability of reliable instrumentation which is both robust, easy to use and, above all, quiet. However, the latest instruments on the market are robust, have belt drives to make them quiet and can run at high gravitational fields which lead to better performance with separations in minutes rather than the hours that are typical of the papers in this review. Another limitation holding up its use as a competitive technology is that the user base in industry and academia are not trained in the use of the technology, a situation that will slowly change, as users become more aware of its advantages.

Operationally, people familiar with solid phase chromatography, find the broad range of solvents available for counter-current chromatography applications very challenging. Easy solvent select protocols are now emerging but more are required before the technology becomes more widely accepted. Also care has to be taken when using the process preparatively that any surfactants in the sample are allowed for, as any large change in the physical properties of the liquid phase systems can lead to a loss of stationary phase and hence resolution.

The main difference between CCC and solid phase chromatography is that CCC focuses on a relatively narrow polarity range. This is ideal for the preparative separation of a target compound but not as powerful as HPLC for example if being used analytically for fingerprinting. The fact that most chromatographers at the moment are unfamiliar with CCC means that it tends only to be used "when all else fails". However, this review clearly shows that the use of the technology is growing, particularly in China (Supplementary Figure S1) and at conferences on large scale chromatography there is talk of liquid–liquid chromatography being the new emerging technology.

14. Supplementary material

The Supplementary Material section appended to this review illustrates the growth of CCC in China from its introduction in 1988 (section S1) to the present day and gives an analysis of the publication record of the leading authors using the technology, their affiliations (sections S2 and S3) and the journals they publish in (section S4). The plant species they investigate (section S5) with the medical indicators claimed by the authors (section S6) are listed in such a way that references on particular plants or particular medical indicators can be easily found. Finally a selective case study is given [213] in section S7, which illustrates how isolated compounds can be then synthesized into new analogues with more powerful activity against specific diseases.

15. The future

There is no doubt that there is huge activity in China and elsewhere to isolate and identify actives in Chinese herbal medicines. Today, at the Institute of Materia Medica (IMM) in Beijing (director Wang Xiaoling–organization 7 in Supplementary Table S3) their priority is the discovery and development of novel compounds from natural products to treat commonly occurring diseases both in China and internationally with a growing emphasis on the global

market [214]. The Herbalome Project at the Dalian Institute of Chemical Physics (DICP) has just won a $5 million start up grant to develop purification methods for traditional Chinese medicines [215]. Organizations worldwide have shown growing interest in China as a location for drug discovery and development. Countercurrent chromatography is playing a major role in this process. The new high performance scalable CCC technology now emerging in the west will help reduce the number of processing steps, speed up the optimization process and throughput as well.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2008.11.095.

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