Solvent System Selection Strategies in Countercurrent Separation

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Abstract

The majority of applications in countercurrent and centrifugal partition chromatography, collectively known as countercurrent separation, are dedicated to medicinal plant and natural product research. In countercurrent separation, the selection of the appropriate solvent system is of utmost importance as it is the equivalent to the simultaneous choice of column and eluent in liquid chromatography. However, solvent system selection is often laborious, involving extensive partition and/or analytical trials. Therefore, simplified solvent system selection strategies that predict the partition coefficients and, thus, analyte behavior are in high demand and may advance both the science of countercurrent separation and its applications. The last decade of solvent system selection theory and applications are critically reviewed, and strategies are classified according to their data input requirements. This offers the practitioner an up-to-date overview of rationales and methods for choosing an efficient solvent system, provides a perspective regarding their accuracy, reliability, and practicality, and discusses the possibility of combining multiple methods for enhanced prediction power.

Keywords

countercurrent separation; countercurrent chromatography; centrifugal partition chromatography; solvent system selection

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Introduction

Nature creates abundant molecular structures with diverse pharmacological activities. Bioactive molecules are often present at the level of parts per million in the raw plant material. Countercurrent chromatography and centrifugal partition chromatography, collectively known as counter-current separation (CCS, syn. CS), provide orthogonal and scalable analytical capabilities and, thus, are important chromatography techniques for natural product chemists. Being a liquid-only technique, CCS avoids the loss by degradation or absorption of analytes on a solid support. It is characterized by high recovery and reproducibility and has demonstrated its usefulness in the natural product laboratory [1–3].

Because the stationary phase is a liquid in CCS, the separation resolution of an analyte depends on its partition coefficient (K), i.e., its relative distribution between the mobile and stationary phases of the solvent system. Thus, K is an invariant parameter of any analyte in a particular solvent system and an important characteristic parameter in a CCS experiment. If the analyte greatly prefers the mobile phase, it will be eluted in the solvent front, but if it strongly favors the stationary phase, the analyte will stay in the column, and may be extruded – a mechanism unavailable to solid phase-based LC. However, neither of these extreme K values ($K \approx 0$ or $K \approx \infty$) are likely to provide efficient analyte separation. An ideal solvent system has to deliver an analyte into a distinctive K value range, referred to as the "sweet spot". This range is generally from $K = 0.25$ to $K = 16$ [4].

As most solvent system families used in CCS are ternary or quaternary solvent systems, the names of the multicomponent mixtures are cumbersome. Therefore, an abbreviated naming system for CCS solvent systems (Table 1 S, Supporting Information) was developed which allows for the assembly of pronounceable names that rapidly identify the system.

In CCS practice, solvent system selection may occupy 90% of the time taken in an entire CCS operation [5]. Thus, in the past decade, CCS researchers have sought new methods or models to reduce the effort of determining the appropriate solvent system and have introduced several selection strategies. In the present review, these strategies are classified into, and presented as, three main classes: empirical, semiempirical, and theoretical. Fig. 1 offers a comparison of the primary features of the three different approaches to solvent system selection. The possibility of a fully predictive means of solvent system selection is discussed at the end of the review.

Empirical Solvent System Selection Strategy

Empirical methods require the practitioner to choose the solvent system composition, the solvent system family, and the constituent proportions of the initial solvent system(s). Typical applications may be divided into three categories: 1) the isolation of a single target analyte; 2) the separation and isolation of a group of analytes; and 3) the distribution of a complex mixture in which the target analytes are not explicitly known. As the desired outcomes vary accordingly, researchers attempt to discover a solvent system where: 1) the target analyte has a K value near unity; 2) the target compounds have K values in the sweet spot with a desirable resolution; and 3) an optimal distribution of mass and/or biological activity is observed.

Empirical solvent system selection is an iterative process. Once the results are known for the first set of trials, the second set of trials is planned. Sometimes the optimal solvent may be discovered by adjusting the solvent ratio within a solvent system family (a group of solvent systems that are mixtures of the same solvents). For example, if the target analyte has a high affinity for the organic upper phase of a hexane-ethyl acetate-methanol-water (HEMWat) solvent system, the proportions of hexane and/or methanol relative to ethyl acetate and/or water may be increased in the next set of trials. On the other hand, a solvent system with a different composition than the original solvent system may be attempted if a) there is not a good polarity and/or solubility match between the original solvent system and the target analytes, b) the target analytes are poorly resolved in several formulations of the original solvent system family, c) emulsions form with the extract that may result in poor countercurrent separation behavior. Overall, this approach requires considerable skill and experience on the part of the practitioner to determine what the next steps of the iterative process should be, as well as knowing when the desired objective has been sufficiently attained.

Partitioning experiments

The use of partitioning experiments (also known as "shake flask" experiments) relies on the fact that the partition coefficient (K) obtained by the static equilibrium of an analyte between two phases of a biphasic solvent system will coincide with the K of the analyte in the same solvent system produced in a dynamic CCS experiment. The K is, therefore, a constant parameter to describe the distribution of a given analyte in a specific solvent system. In the partitioning experiment, the K value is calculated by the following equation (Eq. 1):

$$K = [C]_{\text{upper phase}} / [C]_{\text{lower phase}} \quad \text{Eq 1}$$

where $[C]_{\text{upper phase}}$ is the concentration of the analyte in the upper phase and $[C]_{\text{lower phase}}$ is the concentration of the analyte in the lower phase. This distribution corresponds to the dynamic K value obtained from a CCS experiment run with the upper phase stationary (in practise, the CCS-based determination of K values has to be done manually and is unsupported by most CCS instruments). If the lower phase is the stationary phase in the CCS experiment, the ratio must be inverted.

Analytes with different K values in a solvent system may be separated by CCS with that solvent system [5]. The loading material may be a standard sample [6–8] or a crude material [9–16]. Such experiments have been described in detail [6,16]. Simply, the material is distributed to equilibrium, by vigorous shaking, between the two phases of a small amount of the solvent system. This may be done in a separatory funnel or in a vial. Samples of each phase are drawn off and assayed to quantitate the concentration of the desired analyte(s) in the upper and lower phases. The quantitative method is most often HPLC with UV-vis detection [17–19]. However, a wide range of analytical methods have been reported, such as thin-layer chromatography (TLC) with chemical revelation [20], TLC coupled with fluorimetry [21], GC-MS [22], LC-MS [6], and quantitative ^{1}H NMR (qHNMR) [23, 24]. The choice of quantitation method often reflects the properties of the natural product(s) being targeted, the complexity of the sample, and/or targeting of single vs. multiple analytes,

as well as the preferences of the researcher. If the analyte is unknown in an experiment to discover the bioactive component in a crude mixture, *K*-by-bioactivity is a practical method to correlate the distribution of the bioactive principle(s) to the sweet spot of a CCS chromatogram [25].

Table 1 describes 73 compounds belonging to 10 different classes that were isolated and purified using solvent systems selected by partitioning experiments. Terpenoids, flavonoids, and alkaloids are the major target natural product classes. The most commonly selected solvent system is HEMWat (used for 18 of the 73 natural products), because this solvent system provides two-phase systems over a wide polarity range [26].

Although the predetermination of *K* before a CCS instrument operation avoids solvent waste, an examination of several solvent systems takes a large amount of extra experimental effort. In one study, as many as 47 candidate solvent systems have been examined [17]. Automation, with a liquid handling robot, may ease the burden [19], but most laboratories do not have this capability.

Analytical countercurrent separation

Countercurrent separation chromatograms are linearly scalable, providing identical separations (*K* values) with similar efficiency from 20 mL to multi-liter capacity instruments [27–29]. Furthermore, with the development of analytical CCS instruments (Table 2 S, Supporting Information), the runtime has been shortened from days or hours to minutes [30]. Thus, analytical CCS may be used to test different solvent systems and directly observe *K* values, separation resolution, and stationary phase retention. Table 2 provides details of the use of analytical CCS for the separation of 44 natural products from eight classes. HEMWat and chloroform-methanol-water (ChMWat) systems are the main selected solvent systems used for 15 and 9 of the 44 compounds, respectively.

Evaluation of empirical strategies

Empirical solvent system selection strategies have a reputation of being labor intensive and tedious. However, they have several advantages, such as flexibility in the source material used for solvent system selection. Any source material may be employed for these experiments from commercial standards to crude extracts. Depending on the method of analysis, the *K* values of many different analytes in a particular solvent system may be determined simultaneously. In addition, the flexibility of analytical methods to quantitate the composition of upper and lower phases includes the assessment of biological activity [25] and the acquisition of structural data by NMR [24]. Finally, the results from empirical methods transfer well into the larger scale separations in terms of *K* value, resolution, and solvent system suitability for the dynamic CCS process.

Semiempirical Solvent System Selection Strategies

Semiempirical solvent system selection strategies attempt to reduce the number of partitioning experiments. They can identify a suitable solvent system by replacing some or all of the partitioning experiments with a simple method of predicting *K* values and/or using

mathematical relationships to utilize a small number of experimentally determined K values to predict additional data points.

Thin-layer chromatography-based and reversed-phase high-performance liquid chromatography-based solvent system selection

TLC separation depends on two mechanisms, principally absorption and, to a lesser extent, partitioning. Although the CCS mechanism is only based on partitioning, both systems provide separations coordinated with analyte polarities. This is demonstrated by the commonly used TLC analysis of CCS fractions, which shows the distribution of analytes as strongly polarity related [31]. This general observation inspired the development of a TLC-based solvent system selection methodology. Marston and Hostettmann proposed that the upper and lower phases of a partitioning experiment be developed on silica gel TLC with the upper phase as the mobile phase [32]. In this way, both the partitioning and the suitability of the compound for a particular solvent system may be assessed.

The Generally Useful Estimate of Solvent Systems (GUESS) method [33] discusses the relationship between the TLC Rf value and the CCS K value. A mixture of 21 natural products with widely varying polarities (the GUESSmix) was prepared [4,33]. The K values were determined by partitioning experiments in both HEMWat and ChMWat solvent systems. These were compared with Rf values from TLC plates developed with the organic phase of that solvent system and/or a simplified formulation of the organic phase [33]. This study demonstrated a relationship between Rf values and K values such that Rf values between 0.29 and 0.71 (optimal value 0.5) could be correlated with K values from 0.4 to 2.5 (optimal value 1).

Representing an exemplary application of the GUESS method, ginkgotoxin, at the parts per million level [23], was enriched from *Ginkgo biloba* L. (Ginkgoaceae) seeds using GUESS-based CCS combined with qHNMR. In another example, the EBuWat (2:1:6, v/v) solvent system, predicted by TLC, led to the separation of isorhamnetin-3-*O*-gentiobioside, rutin, and narcissin [34]. Thus, mobility on TLC may be used as an indicator of CCS selectivity, and the TLC-based GUESS method provides a convenient means of estimating the K value in a solvent system. TLC-based methodology has the added advantage that it may be combined with a bioautography-based evaluation of bioactivity [35]. While the GUESS method simplifies the empirical methodology, its establishment and extension still requires the same iterative process exemplified by partitioning experiments.

Wagenaar et al. described the correlation of synthetic compound groups (libraries) with RP-HPLC (acetonitrile with 0.1% TFA and water gradient) and CCS retention times in selected HEMWat solvent systems [18]. There appears to be a nearly linear correlation between closely related compounds. However, the methodology has not been extended to natural products or beyond the HEM-Wat solvent system.

K value prediction within the HEMWat family

One outcome of the previously mentioned GUESS article was the observation that the logarithmic K value of an analyte, such as umbelliferone, has a linear relationship in 16

predefined HEM-Wat solvent systems [33]. This creates the scenario where if the *K* value in one HEMWat solvent system is known, the corresponding *K* value in a different HEMWat solvent system may be predicted. Ignatova et al. also observed a nearly linear correlation between the Log*K* values of pharmaceuticals and the respective HEMWat solvent system number [36]. The use of the HEMWat solvent system as a test case for solvent system selection methodologies arises, in part, because of the predominance of HEMWat in reported CCS applications [37]. For example, Tables 1 and 2 include a total of 33 compounds from eight different classes that were separated by CCS with a HEMWat solvent system. A significant feature of the HEMWat solvent system is that a wide range of polarities may be covered by the systematic modification of the hexane and ethyl acetate proportions relative to the methanol and water proportions between HEMWat 10:0:10:0 on the nonpolar extremity and HEMWat 0:10:0:10 on the polar extremity.

Han et al. described a method for predicting *K* values for a series of HMWat or HEMWat solvent systems by methodically modifying a pair of parameters. For example, the *K* value of an analyte may be mapped in the HMWat solvent system family by following the formula 5:x:10-x, where "x" is the volume of methanol in the range from 0 to 10. The *K* values of four formulas created a map that may be described by a mathematical equation ($y = ax^b$) that may be used to predict *K* values. The authors also investigated the families created by HEMWat 5:5:x:10-x ("x" again being the volume of methanol) and HEMWat x:10-x:5:5 ("x" being the volume of hexane). The method was applied to determine that HEMWat 5:5:5:5 was the optimal two-phase solvent system for the CCS preparation of pseudolaric acid from *Pseudolarix kaempferi* (Lindl.) Gordon (Pinaceae) [38].

Zhang et al. reported a solvent system selection method that was guided by the calculation of the average polarity (P') of 36 solvent systems. The calculations were based on the solvent proportions and their Rohyschneider Snyder P′ solvent polarity values. The method was employed to isolate racemic tetrahydropalmatine from *Corydalis yanhusuo* W.T. Wang ex Z.Y. Su & C.Y. Wu (Papaveraceae) with HEMWat (4:6:5:5) [39]. Table 3 lists the defined HEMWat solvent systems and those used for the calculation of average polarity.

Three-dimensional *K* value maps

Dubant et al. described a solvent system screening method that created a three-dimensional map correlating the composition of the upper and lower phases with the partition coefficient of a target analyte [40]. Creating the map required performing partitioning experiments to determine the partition coefficients of the target analyte in at least nine carefully selected solvent systems. Proof of principle was done with a mixture of seven pharmaceutical analytes: reserpine, ninhydrin, chloropropamide, dipropyl phthalate, methyl prednisolone, cortisone, and lidocaine. Further applications have not been reported, and future work will be needed to demonstrate that the primary screening of the *K* values with single factor experiments can be applied more generally.

Predictive models using empirical data to define descriptors

Ren et al. developed a *K* value prediction process employing a NonRandom Two-Liquid Segment Activity Coefficient (NRTL-SAC) model [41] for representing liquid-liquid

equilibria. NRTL-SAC relies on solvent and analyte descriptors that define the liquid non-ideality of analytes and solvent molecules in terms of interactions among three pairwise interacting conceptual segments: the hydrophobic segment (molecular surface area that is adverse to hydrogen bonding), the polar segment (molecular surface area with interactions characteristic of an electron donor or acceptor), and the hydrophilic segment (molecular surface area with interactions characteristic of a hydrogen-bond donor or acceptor). The descriptors for the most common solvents are already known [42]. The *K* value of an analyte may be calculated as follows (Eq. 2) [43]:

$$K = f(X, Y-, Y+, Z, PE) \quad \text{Eq. 2}$$

where X = hydrophobic segment, Y− = polar-attractive segment, Y+ = polar-repulsive segment, and Z = hydrophilic segment. The liquid phase equilibrium composition of a selected solvent system is represented by PE.

It was previously shown that NRTL provided the best thermodynamic equation for the investigation of the *K* value of an analyte [44]. The NRTL-SAC model has been developed for solvent prediction based on a few partitioning experiment results. In order to evaluate the precision of the NRTL-SAC model, acetaminophen, sulfadiazine, cimetidine, sulfamerazine; magnolol, honokiol, 3-hydrophloridzin, and phloridzin were selected as standard analytes [43,45,46].

Another application of using descriptors to predict partition coefficients was reported by Qian and Poole. They determined the partition coefficients for 86 analytes in ChMWat 8:4:3, representing the solvent system for the Folch partition. The empirical data was used to determine the coefficients for a series of five solute descriptors based on excess molar refraction, polarizability, hydrogen-bond acidity, hydrogen-bond basicity, and McGowan's characteristic volume [47].

Other semiempirical strategies

Both step gradients [20] and continuous gradients [36] have been successfully employed for the isolation of selected analytes by CCS. However, the use of gradient CCS chromatography as a solvent system selection technique is still in its infancy. More likely, the solvent system selection approach may induce the researcher to attempt a gradient as the best means of achieving a particular separation goal.

Evaluation of semiempirical strategies

Semiempirical strategies reduce, but do not entirely replace, the need for multiple partitioning experiments for solvent system selection. This reduces not only the workload but also the required expertise of the researcher. The iterative process of partitioning experiments is replaced by allowing the researcher to evaluate *K* values from many different solvent systems simultaneously. The choice of solvent system composition must still be determined by the researcher. However, the exploration of suitability of a particular formulation within the chosen solvent system family is facilitated by semiempirical methods. It may still be necessary to verify the resulting solvent system choice by either an equilibrium partitioning ("shake flask") experiment or by analytical CCS before committing

the experimental sample to the appropriate CCS scale. In addition, the general applicability of semiempirical methods outside of the common solvent system families has not been explored in most cases.

Theoretical Strategies

Theoretical strategies attempt to preclude the need for partitioning experiments altogether by combining solvent descriptors with similar properties attributed to an analyte in order to predict relative solubility. Predictive methods to determine the partition coefficient of a given analyte in the octanol/water solvent system are examples of theoretical strategies [48,49]. The main concept is that the solubility of an analyte in the upper and lower phases of a biphasic solvent system may be calculated independently given the structure of the analyte, the structures of the solvents, and the solvent composition of the phase.

Conductor-like screening model for real solvents

The Conductor-like Screening Model (COSMO) calculates the dielectric screening charges and energies on a van der Waals surface similar to a conductor and optimizes the analyte molecular geometry within a solvent system [50]. COSMO focuses on evaluating a particular analyte's interaction with a few solvent molecules in order to predict the influence of the rest of the solvent on the analyte by an effective solvent continuum. COSMO for Real Solvents (COSMO-RS) has been developed as a quantum chemical and thermodynamic model [51]. It combines both solvent and analyte descriptors in order to calculate K values based on the structural information of analytes and solvents along with the solvent system phase composition. Two steps of the process include the optimization of the analyte's molecular geometry as well as the generation of charge density of the analyte's molecular surface. At this point, the COSMO-RS model is not applicable to ionized analytes. The details on K value calculations by COSMO-RS are described by Hopmann et al. [2,52]. Several case studies have been offered including *n*-alkylbenzenes in HepMWat solvent systems, steroids in HepEMWat solvent systems, benzyl alcohol in HepEMWat, phenols in HepEMWat, and selected GUESSmix analytes in HEMWat. Furthermore, coumarin, piperine, capsaicin, and dihydrocapsaicin were isolated by solvent systems selected with the COSMO-RS model [52,53].

A recent article employs both the COSMO-RS and the UNIversal quasichemical Functional-group Activity Coefficients (UNIFAC) predictive thermodynamic models in calculating K values. Both COSMO-RS and UNIFAC predictions may be calculated with or without partitioning experiment data to inform the descriptor values. Various combinations of UNIFAC, experimental, and COSMO-RS generated data were compared with the ultimate goal of predicting K values that coincide with experimental values. It was concluded that, in most cases, the values obtained from semiempirical and even theoretical methods was sufficient to recommend one or more solvent systems for further analysis with partitioning experiments. It was proposed that these theoretical predictive models may be employed to explore the suitability of a wide variety of solvent system families for countercurrent separation [54].

Evaluation of theoretical strategies

Analyte solubility behavior in solvent systems may be evaluated by software programs. This shifts the iterative solvent system selection process from the laboratory bench to the computer screen. A large number of solvent system formulations may be systematically investigated with minimal effort once the geometric and thermodynamic characteristics of both solvents and analytes have been established. The complete process from molecular structure to K calculation requires at least three different commercial software platforms, which may somewhat limit the overall applicability of the approach. As with the previous methods, the ability of the researcher to input solvent system families and to intelligently guide an iterative selection process is still needed. Because analyte structural information has to be provided, application in natural product discovery is limited in instances of unknown molecules.

Combination of Solvent System Selection Strategies

Navigating the maze of solvent system selection strategies

Fig. 2 represents the workflow divisions that belong to different methods of solvent system selection and how they interrelate to each other. The workflow in Fig. 2 emphasizes the iterative nature of the solvent system selection process. There are several decision points that will indicate whether or not the tested solvent system should be carried forward (confirmed) or reformulated (revised) and retested.

In the beginning, the researcher must decide which solvent system(s) will be attempted. Often, the CCS literature, such as the references in Tables 1 and 2, is helpful to reveal solvent systems that have been successfully employed to isolate certain types of compounds. Another approach is to start with well-known "portal" solvent systems that have established usefulness, such as HEMWat 1:1:1:1 and ChMWat 10:3:7 [32]. In addition, a skilled CCS practitioner may be able to propose a feasible starting point based on the structural characteristics or TLC polarity of the target compound(s).

The solvent system selection method may take four different pathways: "TRANSFER" – the researcher has a high degree of confidence that a literature solvent system will be effective. "MEASURE" – the researcher has a high-throughput quantitative analysis method (chemical or biological) available that may analyze partitioning experiments. Measuring can be approached with "brute force" by testing a large number of solvents systems or a "smart" methodology that uses a limited number of partitioning experiments to predict the analyte(s) K value(s) in other solvent systems. "GUESS" – the compound(s) is readily identifiable by TLC (chemically or biologically), and the researcher prefers to work with TLC data for preliminary testing. "PREDICT" – the structure(s) of the target compound(s) is known and the research team has the hardware as well as software expertise to analyze a large number of potential solvent systems *in silico*.

Once the preliminary testing has finished, a second level of solvent system testing is warranted. A primary solvent system should be tried on a CCS experiment. This will reveal the performance characteristics of the solvent system and extract combination. Adjustments

may need to be made at this level to improve the stationary phase retention volume or reduce column overloading.

The optimal CCS experimental procedure is a culmination of the sample extraction method, preliminary purification, solvent system selection, and appropriate operating conditions which will lead to a desirable separation.

Conclusion

An aspect of solvent system selection in CCS that remains untouched by most solvent system selection strategies is the development of guided decision-making pathways. Current solvent system selection strategies allow the researcher to produce more K value data with less experimental effort, but this still leaves out the question of how to interpret this data. Data interpretation suffers because the comparative polarity and selectivity characteristics of different solvent systems have only been investigated superficially [4,55]. More systematic and in-depth studies of comparative polarity and selectivity characteristics of solvent systems are needed. This is especially important for the development of new solvent system formulations such as the recent work reported on limonene/methanol/water [56] and *n*-hexane/cyclohexane/*tert*-butylmethylether/methanol/water solvent systems [57]. In addition, predictive models for the addition of solvent system modifiers such as ionic liquids or inorganic salts have been only sparingly addressed [58].

Of the three classes of solvent system selection strategies, the empirical methods have been most widely used in the literature. However, the semiempirical and theoretical solvent system selection strategies developed recently employ methods or models that show great potential for efficient solvent system selection and guidance to facilitate the CCS purification of natural products. In addition, proper linkage of several solvent system selection strategies may reduce the experimental effort for solvent system selection significantly and lower the experimental burden of CCS applications.

It is important to note that all three types of selection strategies are suitable, in general, as solvent system selection guides for CCS novices. As the semiempirical strategies combine existing expertise with prediction power or recently developed methods, they may offer the best balance of theory and practice in contemporary applications of CCS. As the solvent system selection strategies evolve, such knowledge-based approaches will further simplify the procedure to select the right solvent system or at least provide a starting point with minimal effort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

Aa	acetic acid
Ac	acetonitrile
At	acetone
Bu	*n*-butanol
Ch	chloroform
Di	dichloromethane
E	ethyl acetate
Et	ethanol
H	*n*-hexane or hexane(s)
Hep	*n*-heptane
HFBA	heptafluorobutyric acid
Iso	*iso*-propanol
M	methanol
Pet	petroleum ether
Pro	*n*-propanol
So	DMSO
ter	methyl *tert*-butylether
TFA	trifluoroacetic acid
Wat	water

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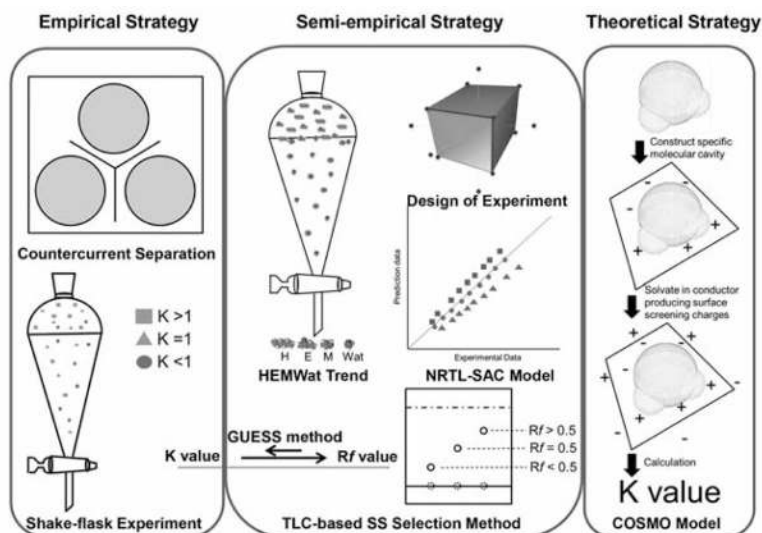
Fig. 1.
A diagrammatic sketch of solvent system selection strategies in CCS. The empirical strategies are comprised of an analytical CCS and a partitioning experiment. The semiempirical strategies involve a TLC-based method, the general HEMWat family trend, three-dimensional K value maps, and the NRTL-SAC model. One representative theoretical strategy is the COSMO-RS model. (Color figure available online only.)
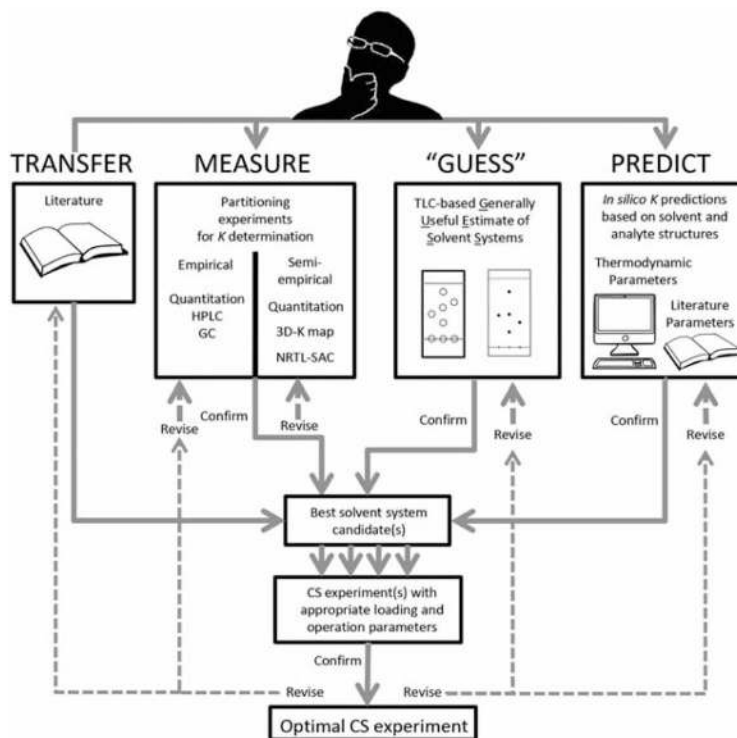
Fig. 2.

Workflow highlighting the four methods of solvent system selection. At the top, the chromatographer must decide which solvent system(s) will be tested. On the first level, the researcher may TRANSFER solvent systems from within the CCS literature directly to the second level for best solvent system testing. The researcher may MEASURE the *K* values of target analytes by a series of partitioning experiments. Partitioning experiments may be performed independently in an empirical manner or they can be organized in a semiempirical fashion to maximize the information generated. The researcher may employ the GUESS method, which relies on TLC R*f* values to predict the best solvent system(s) for separation. Finally, the researcher may PREDICT the best solvent system(s) through theoretical modeling of the solubility parameters generated from structural information or gleaned from the literature. The workflow highlights the iterative nature of the solvent system selection process by showing that each solvent system must be evaluated and either confirmed or revised. (Color figure available online only.)

Table 1

Overview of the analytes and their respective solvent systems that were selected through extensive partitioning experiments.

Class and references	Analytes	Selected SS (v/v)
Terpenoids [12, 24,59, 60]	Bilobalide, ginkgolides A, B, C, and J	ChMWat (10: 7: 3) and HEMWatSo (4: 6: 4: 6: 0.5%)
	2,3-*O*-acetylshengmanol-3-*O*-*β*-D-xylopyranoside, cimiracemoside D, 2,5-*O*-acetylcimige-nol-3-*O*-*β*-D-xylopyranoside, and aglycone cimigenol	HAtEIsoEtWat (3.5 :1 :2 :1 :0.5 : 2)
	Triptonide, isoneotriptophenolide, hypolide, triptophenolide, and triptonoterpene methyl ether VI	HEMWat (3 :2 :3 :2)
	Eriocalyxin B	HEMWat (1 :1 :1 :1)
Flavonoids [7, 8,13, 16,61]	Tephrosin, 4′,5′-dimethoxy-6,6-dimethylpyranoisoflavone, deguelin, and 6a,12a-dehydro-deguelin	HEMWat (1 :0.8 : 1: 0.6)
	Amentoflavone, robustaflavone, bilobetin, hinokiflavone, isocryptomerin and apigenin-diglucoside	HepEMWat (2 :3 :2 :3)
	Procyanidins	Methyl Acetate-Water (1 :1)
	Anthocyanins	BuWat (1 :1)
	Catechins	*ter*AcWat (TFA) (2 :2: 3: 0.1%)
Alkaloids [14, 23]	Daurisolin, dauricine, daurinoline, and dauricicoline	PetEEtWat (1 :2 :1 :2)
	Ansamitocin P-3	HEMWat (0.6 : 1: 0.6: 1)
	Ginkgotoxin	ChMWat (10: 5: 5)
Lignans [10, 15,62]	3′-Formylhonokiol, 5-formylhonokiol, and 3′,5-diformylhonokiol	HEMWat (1 :0.4 : 1: 0.4)
	Honokiol and magnolol	HEMWat (1 :0.4 : 1: 0.4)
Quinones [9]	Tanshinone IIA	HDiMWat (4: 0.75: 4: 1) and HEtWat (4: 2: 2)
Organic acids [63–65]	(±)-Cyclohexylmandelic acid	H*ter*Wat (9: 1: 10) with chiral selectors
	Caftaric acid, coutaric acid, and fertaric acid	*ter*AcBuWat (TFA) (2: 2: 1: 5: 0.5%)
	20 Fatty acids	HMWat (350: 175: 2)
Sterols [66]	Sitostanol and *β*-sitosterol	HMWat (silver nitrate) (34: 24: 1%)
Coumarins [67]	Notoptol and divaricatol	HEMWat (1 :1 :1 :1)
Peptides [6]	Enramycin A and B	HBuWat (TFA) (43 :7 :50: 0.05%)
Curcuminoids [17]	Curcumin, demethoxycurcumin and bisdemethoxycurcumin	HChMWat (5 :10 :7.5 : 2.5)

Table 2

Overview of the analytes and their respective solvent systems that were selected and/or isolated by analytical scale CCS instruments.

Class and references	Analytes	Selected SS (v/v)
Terpenoids [68, 69]	Linalool, terpinen-4-ol, *α*-terpineol, and *p*-anisaldehyde	HEMWat (5: 5: 2: 2)
	Anethole and foeniculin	HM (1: 1)
	Lycopene	HDiAc (10 :3.5 : 6.5)
Flavonoids [70–73]	Hispidulin, nepetin, homoplantaginin and nepetin-7-glucoside	HChMWat (0.5: 4: 3: 2) and ChMWat (4:3 :2)
	Patuletin-3-*O*-glucoside, hyperoside, 6-methoxykaempferol-3-*O*-gal-actoside, and astragalin	EMWat (10: 1: 10)
	Quercetin, kaempferol, and isorhamnetin	DiMWat (5: 3: 2)
	Amygdalin	EBuWat (5: 2: 5), (5: 1: 5), (10 :1 :10), (50: 1: 50) and BuWat (1: 1)
	Taxifolin-3-*O*-glucoside and hyperoside	EMWat (25: 1: 25), (10: 1: 10) and (5: 1: 5)
	Baicalein-7-*O*-diglucoside, baicalein-7-*O*-glucoside, baicalein and chrysin	HEMWat (1: 1.2: 1: 1)
	Isoflavan glycoside and pterocarpan glycoside	EEtWatAa (4 :1 :5 :0.25)
Alkaloids [74–77]	Betalain	ProAcWat-(sat'd($NH_4)_2SO_4$) (1 :0.5 :1 :1.2) and *ter*BuAcWat (HFBA) (2: 2: 1: 5: 0.7)
	Betacyanin	*ter*BuAcWat (HFBA) (1: 3: 1: 5: 0.7)
	Dehydrocavidine	ChMWat (0.3 M HCl) (4: 0.5 :2)
	Lappaconitine, ranaconitine, *N*-deacetyllappaconitine and *N*-deacetylranaconitine	ChMWat (0.3 M or 0.2 M HCl) (4: 1.5: 2)
Phenylpropanoids [78,79]	Salvianolic acid B	HEMWatAa (1: 5: 1.5: 5: 0. 596%)
	Eleutheroside E	ChMIsoWat (5: 6: 1: 4)
Coumarins [80,81]	Imperatorin, oxypeucedanin and isoimperatorin	HEMWat (1: 1: 1: 1) and (5 :5 :4.5 :5.5)
	Osthol and xanthotoxol	HEMWat (1: 1: 1: 1) and (5 :5 :6: 4)
Iridoids [82]	Geniposide	EBuWat (2: 1.5: 3)
Anthraquinones [83]	Rhein	HEMWat (3: 7: 5: 5)
Benzoquinones [84]	Coenzyme Q10	HepDiAc (12 :7 :14)

Table 3

The surveyed HEMWat trends and polarities. The polarity gradient and HEMWat family trend may be obtained by experimental methods (e.g., the GUESS method [33]) or by calculation of the average of polarity [39].

Defined HEMWat SSs HEMWat SS No.	HEMWat volume ratios (v/v)	HEMWat SSs vs. average polarities HEMWat volume ratios (v/v)	Polarity
HEMWat − 7	HEMWat (9: 1: 9:1)	–	–
–	–	HEMWat (5: 1: 5: 1)	3.38
HEMWat − 6	HEMWat (8: 2: 8:2)	–	–
HEMWat − 5	HEMWat (7: 3: 7:3)	–	–
HEMWat − 4	HEMWat (7: 3: 6:4)	–	–
–	–	HEMWat (6: 4: 5: 3)	4.13
HEMWat − 3	HEMWat (6: 4: 6:4)		
–	–	HEMWat (5: 4: 5: 4)	4.69
HEMWat − 2	HEMWat (7: 3: 5:5)	–	–
HEMWat − 1	HEMWat (6: 4: 5:5)		4.74
HEMWat 0	HEMWat (5: 5: 5:5)		4.95
HEMWat + 1	HEMWat (4: 6: 5:5)		5.16
HEMWat + 2	HEMWat (3: 7: 5:5)		5.38
HEMWat + 3	HEMWat (4: 6: 4:6)	–	–
–	–	HEMWat (3: 5: 3: 5)	5.54
HEMWat + 4	HEMWat (3: 7: 4:6)	–	–
–	–	HEMWat (1: 2: 1: 2)	5.73
HEMWat + 5	HEMWat (3: 7: 3:7)	–	–
–	–	HEMWat (2: 5: 2: 5)	5.96
–	–	HEMWat (3: 10: 3: 10)	6.22
HEMWat + 6	HEMWat (2: 8: 2:8)	–	–
–	–	HEMWat (1: 5: 1: 5)	6.52
HEMWat + 7	HEMWat (1: 9: 1:9)	–	–
HEMWat + 8	HEMWat (0: 10:0 :10)	–	–