Pseudo-two-Component Notation for Heptane/Alcohol/Water Systems in Countercurrent Chromatography

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PSEUDO-TWO-COMPONENT NOTATION FOR HEPTANE/ALCOHOL/WATER SYSTEMS IN COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Three-component two-phase systems are widely used in countercurrent chromatography. For chromatographic applications, it is advantageous to specify their composition by volume using pseudo-two-component notation. Thus the composition Heptane/2-PrOH/H_2O-50:20:30 is expressed as Heptane/40% 2-PrOH in H_2O-1:1. The information expressed in a typical phase diagram can be reduced to a table of the phase compositions of several conveniently chosen pseudo-two-component systems. The table facilitates screening partition coefficients in small volume systems and simplifies preparation of large volumes of phases in the quantities desired for preparative countercurrent chromatography.

INTRODUCTION[1]

Three-component two-phase systems provide some of the most versatile approaches to separation by countercurrent chromatography, CCC. Specification of system compositions by volume is adequate and more convenient for routine use in CCC, though it neglects the small contraction which often occurs on admixture of miscible liquids.

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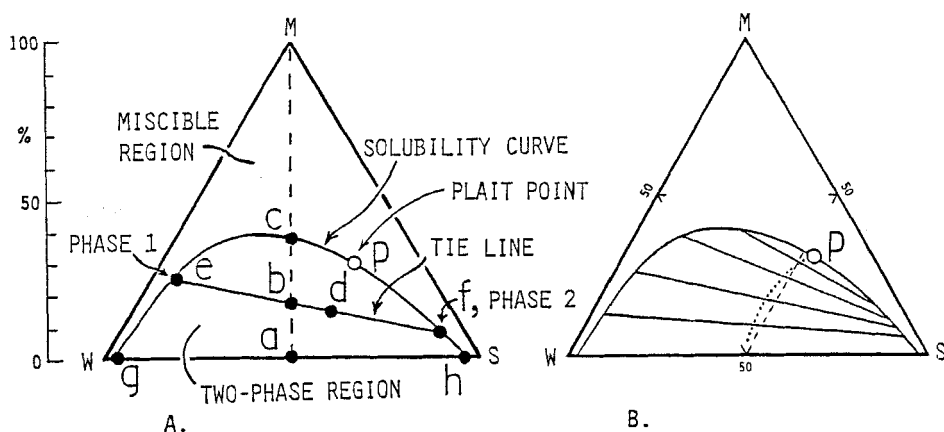
FIGURE 1. General features of ternary phase diagrams

Ternary Diagrams

Phase equilibria of multicomponent solvent mixtures have been extensively studied by Francis.[2] Three-component mixtures are conveniently summarized on an equilateral triangular plot, the general features of which are summarized in Fig. 1. The majority of systems are of the simple binodal or "bite" type illustrated here.[2]

The three system components, W, S and M are indicated at the apexes of the triangle. The two immiscible components, W, water in this example, and S, a solvent essentially immiscible with water, are placed at the base of the triangle. The third component, M, a solvent miscible with both W and S is represented at the upper apex. The distance from one side of the triangle to the opposite apex represents 100% of the component at the apex, e.g. distance aM represents 100% M as illustrated by the scale to the left of Fig. 1A. Any point within the triangle represents a three-component mixture, containing a percentage of M indicated by its distance from the triangle base as measured with this scale. For instance, points f, d, b and c contain respectively 8, 15, 18 and 38% M.

Percentages of components S and W are measured using the same
scale, with zero applied to the side opposite the apex. The sum of
all three percentages will always be 100. Two-component mixtures
lie on the triangle sides. Point a is 50% S and 50% W. Point g
indicates that about 3% S is soluble in W and point h indicates
that about 4% W is soluble in S.

All compositions in the miscible region, above the solubility
curve, form single phase systems. Mixtures below the solubility
curve separate into two phases, having compositions on opposite
sides of the solubility curve. For instance, composition b forms a
phase of composition e containing about 70% water, and a phase of
composition f, containing about 86% S. The phase compositions, e
and f, are connected by a straight line, called a tie line, which
also passes through the system composition point b. The relative
volume of each phase is given by the "lever rule", which states
that each phase volume is proportional to the length of the tie
line segment opposite the point b. In other words, in Fig. 1A,
V_1/V_2 = bf/be, or $V_1/(V_1+V_2)$ = bf/ef. All system compositions,
such as d, which lie on the same tie line generate the same two
phase compositions, albeit in different relative amounts; they
therefore provide identical CCC systems.

The relationships of tie lines varies among phase systems.
The pattern of progressively increasing slope shown in Fig. 1B is
often seen. The tie lines become shorter as they approach the
solubility curve, until the two phases become identical in
composition at the plait point. The solubility curve becomes
discontinuous at the plait point, where a single phase is obtained.

Variation of K with System Composition

If a solute is partitioned between the phases of a series of
system compositions along the dotted line in Fig. 1B, the partition
coefficient, K, will progress from its value in the binary system W
+ S toward unity at the plait point. K = concentration in one
phase/concentration in other phase. Theoretically, all solutes
should have K nearly equal to unity in the vicinity of the plait

point, because the phase compositions are nearly identical. While, in practice, a general trend toward unity is seen, the selectivity, $\alpha = K_2/K_1$, observed for two solutes is often adequate at particular system compositions for a separation to be achieved by CCC.

MATERIALS AND METHODS

Solvents employed for measurement of phase volumes and partition coefficients were HPLC grade.

The following equations were used to convert phase equilibrium data published on a mole fraction basis to a volume basis:

$$V_w = \frac{X_w M_w}{\rho_w} = 18 X_w, \quad V_m = \frac{X_m M_m}{\rho_m}, \quad V_s = \frac{X_s\, M_s}{\rho_s}$$

where V, X, M and ρ refer to volume, mole fraction, molecular weight and density respectively. The subscripts w, m, and s refer to water, and the miscible and immiscible solvent components respectively. Volume percentages, P^v, were then obtained from

$$P_w^v = \frac{100\, V_w}{V_T}, \quad P_m^v = \frac{100\, V_m}{V_T}, \quad P_s^v = \frac{100\, V_s}{V_T}$$

where the total volume $V_T = V_w + V_m + V_s$. Densities of 0.7812 and 0.6795 were used for 2-PrOH and heptane respectively.

Benzyl alcohol , 2-phenyl ethanol and 3-phenyl propanol were obtained from Aldrich Chemical Co. Stock solutions containing 5 mg/ml of each compound were prepared in aqueous solutions containing various amounts of 2-propanol. Dilutions of the stock solutions were assayed by HPLC; Alltech C-18 econosil-10μ column, 20 μl injection, solvent system 50% MeOH in water, flow 1 ml/min, monitored at 254 nm. Two-ml aliquots of the stock solutions were gently shaken for 30 min. with equal volumes of heptane and clarified by centrifugation. Aliquots, 20 μl, of the aqueous layers were assayed by HPLC. Peak heights for the partitioned aqueous layers were compared to the heights of corresponding peaks for the diluted stock solutions to calculate the partition

coefficients as

$$K_N^u = \frac{H_{a,d}/d - H_{a,p}}{H_{a,p}}$$

where $H_{a,d}$ is the HPLC peak height for the aqueous stock solution
diluted by the factor d = vol. before dilution/vol. after dilution
and $H_{a,p}$ is the peak height for the aqueous phase after
partitioning. The superscript u in K_N^u signifies that the
partition coefficient is not corrected for any volume shift on
mixing the aqueous and organic phases which had not been mutually
saturated previously. The result may be corrected for the volume
shift as follows, using the f_a^f values in Table 1.[3]

$$K_N = \frac{0.5 \, K_N^u + 0.5 - f_a^f}{1 - f_a^f}$$

DISCUSSION
Pseudo-Two-Component Notation
       The phase diagram for Heptane/2-PrOH/H$_2$O, Fig. 2, was
calculated from molar values summarized by Sorensen and Arlt from
data reported by Vorobeva and Karapetyants.[4,5] Its general
features are the same as those discussed for Fig. 1. The line
drawn parallel to the left side of the triangle defines a series of
systems which can be prepared by mixing a volume of heptane with
equal volumes of an aqueous solution of propanol in water. The
numbers along the line specify the composition of the aqueous
solution in units of % 2-propanol by volume.
       An alternate notation for these systems is readily illustrated
with the system designated 40%, as indicated in Fig. 2. In
conventional 3-component notation, this system composition is
specified as Heptane/2-PrOH/H$_2$O-50:20:30 (v/v/v). But, if the 20
volumes of 2-PrOH and 30 volumes of H$_2$O are combined and
represented as 50 volumes of 40% 2-PrOH in H$_2$O, the system
composition can then be expressed as Heptane/40% 2-PrOH in H$_2$O-1:1

TABLE 1. Phase Compositions for Heptane/Aqueous 2-PrOH-1:1(v/v) Systems based on Fig. 2 for the Heptane/2-PrOH/H$_2$O System at 25°C

% 2-PrOH in H$_2$O	f_a^f for Heptane/2-PrOH 1:1 Systems		Conjugate Phase Compositions by Volume					
			Aqueous Phase			Organic Phase		
	Meas. Directly	Est. from Fig. 2	Heptane	2-PrOH	H$_2$O	Heptane	2-PrOH	H$_2$O
0	0.50	0.50	-	-	100	100	-	-
10	-	0.49	-	9	91	99	1	-
20	0.50	0.49	-	18	82	98	2	-
30	0.49	0.48	0.5	27.5	72	97	3	-
40	0.48	0.48	0.5	36.5	63	94	6	-
50	0.46	0.46	1	46	53	92	7.5	0.5
60	0.46	0.44	3	54	43	88	11	1
70	0.46	0.43	6	61	33	82.5	16	1.5
80	0.45	0.44	14.5	64	21.5	77	21	2
85	0.50	0.47	29	58.5	12.5	68	29	3

$f_a^f = V_a^f/(V_a^f + V_o^f)$, final aqueous fraction, after equilibration of initial volumes of heptane and aqueous 2-PrOH solutions in 1:1 ratio.
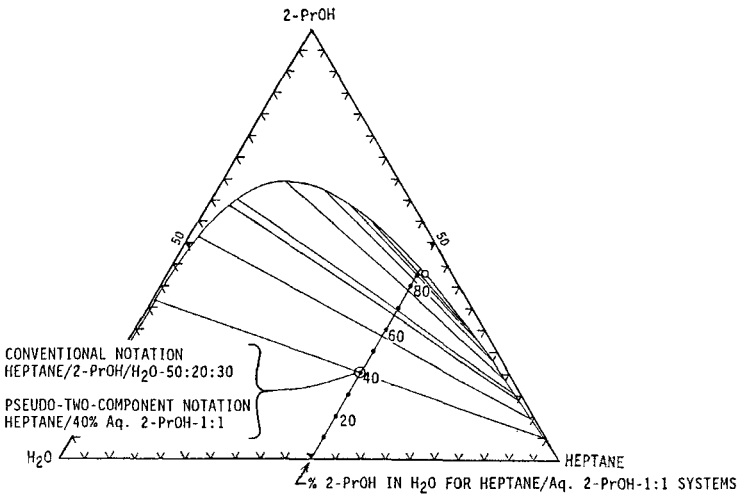
FIGURE 2. Phase diagram for Heptane/2-PrOH/H$_2$O (v/v/v), 25° C.
Calculated from data of Vorbeva and Karapetyants as summarized by
Sorensen and Arlt.[2,3]

(v/v). This alternate terminology may be called pseudo-two-
component notation.

Tabulation of Ternary Systems

Use of pseudo-two-component notation facilitates the
tabulation of ternary systems. On a large-scale plot of Fig. 2,
tie lines passing through a convenient series of pseudo-two-
component systems were drawn by interpolation between known tie
lines. By reading the phase compositions at the point of
intersection of the interpolated tie lines and the solubility
curve, Table 1, listing the phase compositions to an accuracy of
about 0.5%, was constructed. The table effectively summarizes the
information presented in the phase diagram. The table is much
easier to apply to CCC, and its use does not require an
understanding of the phase diagram.

In addition to the phase compositions, values of the phase
volume ratio, f_a^f, are tabulated. This is a measurement of the
final aqueous fraction, $V_a^f/(V_a^f + V_o^f)$, after initial volumes, V_a^i
and V_o^i, of aqueous 2-PrOH and heptane are equilibrated. The
values in the column headed "meas. directly" were obtained by
direct measurement of the final volumes after equilibration of 5-ml
volumes of aqueous propanol and heptane. Under the heading "from
Fig. 2" are listed values of f_a^f calculated from the tie-line
segment lengths for interpolated tie-lines on Fig. 2. There is
good agreement between the two series of values. Not all phase
systems lend themselves to expression as 1:1 systems. The
$CHCl_3/MeOH/H_2O$ system is more conveniently summarized as $CHCl_3/Aq.$
$MeOH-4:6$ systems.[3,6]

Use of Table 1 for Solvent Screening

For CCC with the multilayer coil planet centrifuge, it is
convenient to adjust the partition coefficient for the component of
interest to lie in the range of about 0.5 to 5, ideally in the
vicinity of 1.0. To survey the effect of solvent system
composition on a small scale, a few mg of solute can be partitioned
between one or two ml of each phase, followed by assay of the
phases by spectrophotometry or other means.[7] For the Heptane/2-
PrOH/H_2O system, a series of equal volumes of aqueous 10, 20 and
30% 2-PrOH, and heptane can be used. Since the final aqueous
fractions, f_a^f, range from 0.45 to 0.50, the effect of the volume
shift can be neglected for practical work. If more accuracy is
desired, either correction factors can be applied,[3] or a slightly
larger volume of each solution can be mutually presaturated and
exactly 1-ml of each equilibrated phase removed for the
partitioning studies.

Use of Table 1 to Mix Large Volumes for CCC

On a large scale, it is convenient to use Table 1 to prepare
appropriate volumes of each phase for chromatography. For example,
to operate a 300 ml column with a stationary organic phase, it may
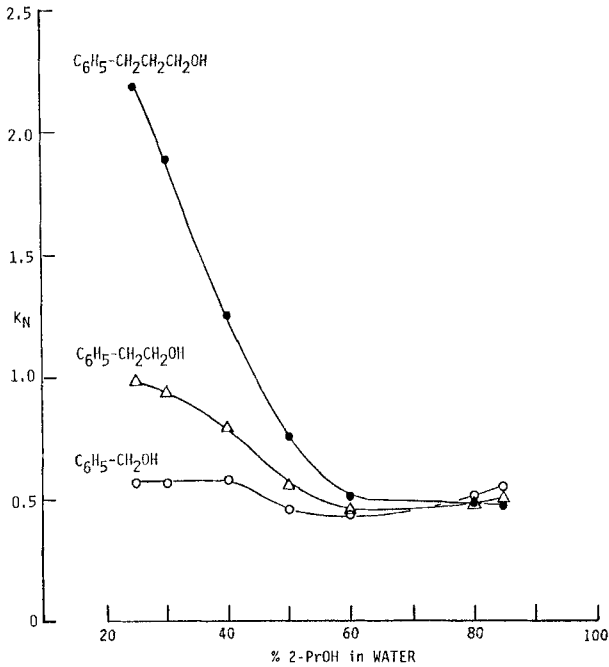be desired to prepare 500 ml of organic phase and 1000 ml of

FIGURE 3. Partition coefficients for phenylalkanols in the
Heptane/Aqueous 2-PrOH-1:1 systems. Aqueous phase assayed by HPLC
before and after equilibration with heptane. K_N = conc. in nonaq.
phase/conc. in aq. phase. Values corrected using f_a^T values from
Table 1.

aqueous phase. Using the 30% 2-PrOH in water/Heptane-1:1 system as
an example, the phase compositions from Table 1 are multiplied by 5
and 10 respectively to prepare the desired volumes of each phase as
summarized here:

Component	Aqueous Phase		Organic Phase		Total
	Table %	ml	Table %	ml	ml
Heptane	0.5 x 10 =	5	97 x 5 =	485	490
2-PrOH	27.5 x 10 =	275	3 x 5 =	15	290
H_2O	72.0 x 10 =	720	0		720
Total	100	1000	100	500	1500

The volumes in the total columns, when mixed in a separatory funnel, will separate to give 1000 ml of aqueous phase and 500 ml of organic phase. One should not presume that perfectly equilibrated individual phases can be mixed directly from the table without actual equilibration with the other phase. To allow for errors in constructing the table and in measuring the volumes and for variations in temperature, the total mixture should be equilibrated.

Should additional mobile phase be needed during the run, it is sufficient to prepare more mobile phase from the tabulated composition and equilibrate it against excess stationary phase prepared earlier or displaced from the column in the initial stage of development.

ACKNOWLEDGMENTS

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