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### Standard Solutes for Classification of Solvent Systems in Countercurrent Chromatography

Walter D. Conway<sup>a</sup>; Richard L. Hammond<sup>a</sup>; Anthony M. Sarlo<sup>a</sup>

<sup>a</sup> Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, New York

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## STANDARD SOLUTES FOR CLASSIFICATION OF SOLVENT SYSTEMS IN COUNTER- CURRENT CHROMATOGRAPHY

Walter D. Conway, Richard L. Hammond,  
and Anthony M. Sarlo

*Department of Pharmaceutics  
School of Pharmacy*

*State University of New York at Buffalo  
565 Hochstetter Hall  
Amherst, New York 14260*

### ABSTRACT

A series of p-dinitrophenylamino alcohols have been synthesized for use as standard solutes for evaluating the polarity of solvent systems used in countercurrent chromatography. The rank order polarity of the DNP-amino alcohols as well as p-nitrophenyl glucosides were determined by measuring their partition coefficients in 1-pentanol/heptane/water systems containing from 2% pentanol in heptane to 100% pentanol. The standard solutes were used to establish the rank order polarity of eleven chloroform/methanol/water systems which are widely used in countercurrent chromatography.

### INTRODUCTION

The capability of choosing from a very large number of solvent systems is a major advantage of countercurrent

chromatography (CCC). As a general rule, a solvent system is desired in which the component of interest has a partition coefficient in the range of 0.2 to 5, expressed as concentration in stationary phase/concentration in mobile phase (2). The lower portion of this range (0.2-1) is best for use in the multilayer coil planet centrifuge (3), in which the stationary phase typically occupies 80% of the column volume. The upper range (1 to 5) is better suited for instruments such as the horizontal flow-through coil planet centrifuge (4), where only 40% of the column volume is filled with stationary phase.

The sheer number of solvent systems from which to choose, coupled with the scarcity of data comparing the partitioning behavior of typical solutes in the systems, necessitates a largely empirical approach to developing a solvent system for a specific application. To facilitate the acquisition of partitioning data, which would allow comparison of many solvent systems, a series of 2,4-dinitrophenylamino alcohols (DNP-amino alcohols) has been prepared and evaluated for use as standard solutes. The use of p-nitrophenylglycosides, which are commercially available, has also been examined.

## EXPERIMENTAL

### Chemicals

All solvents and chemicals were either HPLC grade or reagent grade.

### DNP-Amino Alcohols

N-2,4-dinitrophenylamino alcohols (Table 1) were synthesized by the method of Sanger (5) by reaction of 0.1 to 1 g of amino alcohol or its hydrochloride salt with a 25% molar excess of 2,4-dinitrofluorobenzene in a 75% aqueous ethanolic solution at room temperature in the presence of an excess of

sodium bicarbonate. The reaction mixture was typically stirred overnight, filtered and the filtrate evaporated to dryness. The residue was taken up in ethanol, precipitated with water and the solid removed by filtration and dried. The crude product was crystallized from ethyl acetate, except DNP-tris (hydroxymethyl) aminopropane which was crystallized from methyl ethyl ketone, and DNP-glucosamine which was crystallized from ethanol. Yields were in the range of 60 to 88% except for DNP-glucosamine which was 34%. Products were examined by TLC to ensure the absence of 2,4-dinitrophenol and any yellow impurities.

Absorption spectra of the products were recorded in methanol, water, 0.1 N HCl and 0.1 N NaOH.

#### Partition Coefficients

Partition coefficients were measured in systems ranging from 2% pentyl alcohol in heptane to 100% pentyl alcohol vs water using a modification of a method described earlier (6). The method entails equilibration of about 0.1 mg of solute with 1 ml each of organic and aqueous phases, mutually saturated prior to use. After equilibration and centrifugation, the lower phase is transferred quantitatively to a second tube using a Pasteur pipet attached to a 2-ml hypodermic syringe. Both phases are diluted with 5 ml of 2-propanol and 1 ml of fresh "other" phase (i.e. the one not contained in the initially diluted solution) is added to ensure identical solvent composition of the diluted phase solutions. The absorbance,  $A$ , of each diluted phase was measured (at 348 nm for the DNP-amino alcohols and at 294 nm for p-nitrophenyl glucosides). A blank was also prepared from 5 ml of 2-propanol plus 1 ml of each phase but absorbance readings on this was negligible at these wavelengths. The partition coefficient

was calculated as  $K_S = (A_{\text{organic}} - A_{\text{blank}})/(A_{\text{aqueous}} - A_{\text{blank}})$ . The range of measurable partition coefficients was extended beyond that reported earlier by reducing the volume of one of the phases to 0.25 ml when  $K_S$  fell outside the range of 0.1 to 10. The quantitative transfer of lower phase is further simplified if the 13 x 100 mm tube used for equilibration is modified by pressing a depression approx. 2 mm wide and 2 mm deep in the bottom of the tube. Placing the tip of the Pasteur pipet used to transfer the lower phase in this depression aids in splitting the two phases precisely at the interface.

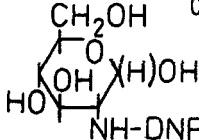
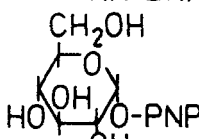
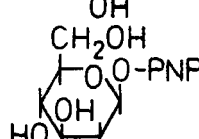
### RESULTS AND DISCUSSION

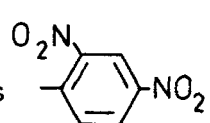
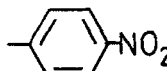
The DNP-amino alcohols are all yellow compounds with an absorbance maximum at 348 mμ. The long wavelength absorption makes them suitable for evaluation of aromatic solvents as well as technical grade solvents which might contain uv-absorbing impurities. The lack of a shift in the absorption spectrum in either 0.1 N HCl or 0.1 N NaOH indicates that the pKa of the compounds lies outside the range of 1 to 13. They are therefore nonionizing over the range of pH commonly encountered in CCC and are thus well suited for the evaluation of both buffered and neutral solvent systems. This is a major advantage over the DNP-amino acids which are suitable only for strongly acidic systems.

The PNP derivatives of a variety of sugars, which are available commercially, also serve as convenient standard solutes, particularly for the evaluation of very polar solvent systems. These are colorless compounds with an absorption maximum at 294 nm, which is still of sufficiently long wavelength to avoid interference from most uv-absorbing solvents and impurities. The structures of the α and β-PNP-glucosides are shown in Table 1.

TABLE 1

DNP-Amino Alcohols and p-Nitrophenyl Glucosides

No.	Derivative of	Structure	No. OH Groups
I	1-Amino-2-Propanol	$\text{CH}_3-\overset{\text{OH}}{\text{C}}-\text{CH}_2-\text{NH}-\text{DNP}$	1
II	1-Amino-3-Propanol	$\text{HOCH}_2-\text{CH}_2-\text{CH}_2-\text{NH}-\text{DNP}$	1
III	3-Amino-2-Methyl-1,3-Propanediol	$\text{H}_3\text{C}-\overset{\text{CH}_2\text{OH}}{\underset{\text{CH}_2-\text{OH}}{\text{C}}}-\text{NH}-\text{DNP}$	2
IV	Ethanolamine	$\text{HOCH}_2-\text{CH}_2-\text{NH}-\text{DNP}$	1
V	2-Amino-1,3-Propanediol (Serinol)	$\overset{\text{CH}_2\text{OH}}{\underset{\text{CH}_2\text{OH}}{\text{HC}}}-\text{NH}-\text{DNP}$	2
X	3-Amino-1, 2-Propanediol	$\text{HO}-\overset{\text{OH}}{\text{C}}-\text{CH}_2-\text{NH}-\text{DNP}$	2
VI	2-Amino-2-(hydroxymethyl)-1,3-Propanediol (TRIS)	$\text{HOCH}_2-\overset{\text{CH}_2\text{OH}}{\underset{\text{CH}_2\text{OH}}{\text{C}}}-\text{NH}-\text{DNP}$	3
VII	D(+)-Glucosamine		4
VIII	PNP- $\alpha$ -D-Glucopyranoside		4
IX	PNP- $\beta$ -D-Glucopyranoside		4

DNP is  , PNP is 

### Rank Order Polarity of the Solutes

The partition coefficients of the proposed standards (expressed as  $K_S = \text{concentration in organic phase}/\text{concentration in aqueous phase}$ ) were measured in a series of solvent systems ranging from 2% pentyl alcohol in heptane to 100% pentanol versus water. These results are presented in Fig. 1. The measurements serve to establish the rank order polarity of the compounds as well as to convey some indication of their partitioning behavior in solvent systems covering a wide range of polarity.

The plots of  $\log K_S$  vs  $\log \% \text{ pentanol}$  are quite linear, particularly in the more polar solvent systems, with the exception of DNP-TRIS (VI) which curves badly below  $K_S$  of about 0.5. Compound V (DNP-serinol) is quite linear over the 500 fold range of  $K_S$  from 0.04 to 20. Unfortunately, the starting material (serinol) for compound V is relatively expensive compared to the cost of the other amino alcohols. However, the less costly compound X, an isomer of V, was recently synthesized and our preliminary experience shows that it exhibits behavior almost identical to that of V.

The slopes of the lines for individual DNP-amino alcohols in Fig. 1 increases with the number of hydroxyl groups in the compound as summarized in Table 2. The plots are of the form

$$\log K_S = a(\log \% \text{ Pentanol}) + b$$

which has been reported by Soczewiński to be characteristic of partition chromatographic systems which undergo chemical interaction, such as hydrogen bonding.<sup>7,8</sup>

### Classification of Solvent Polarity

Several indicators of organic solvent polarity are commonly used such as dielectric constant or rank order in an eluotropic

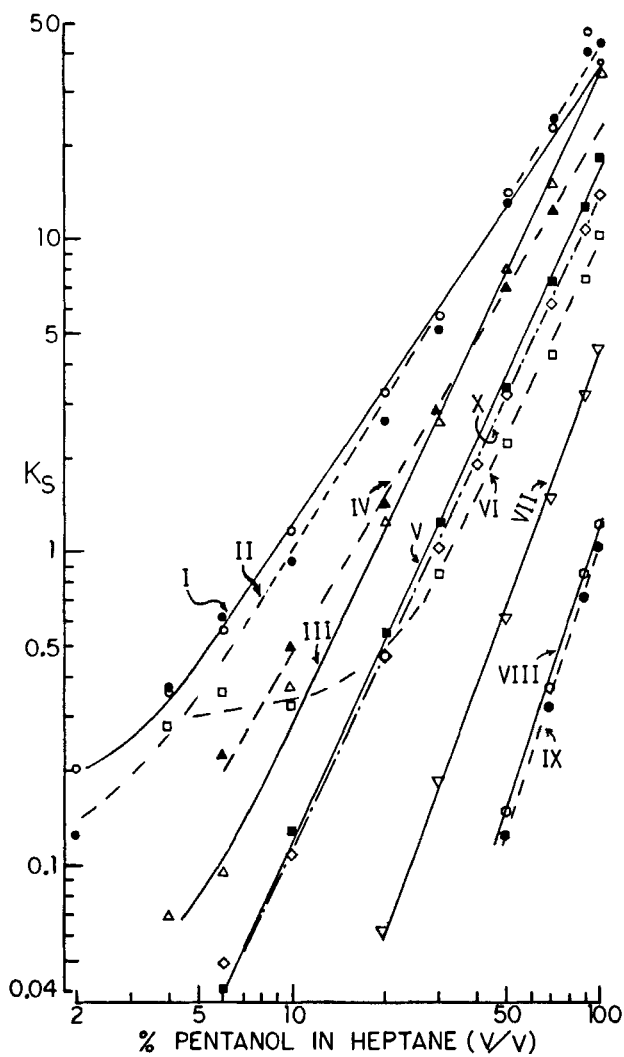


FIGURE 1. Partition coefficients for DNP-amino alcohols and PNP-glucosides. Structures are given in Table 1.



TABLE 2

Slopes for  $\log K_S$  vs  $\log \% \text{ Pentanol}$  Plots in Fig. 1

<u>Compounds</u>	<u>OH Groups</u>	<u>Slopes</u>
I, II, IV	1	1.47, 1.62, 1.68
III, V, X	2	2.11, 2.17, 2.11
VII	4	2.71
VIII, IX	4	2.98, 3.07

series. However, these measures refer to relative strengths of the pure, dry solvent. In CCC the solvent is saturated with the other phase, usually water and the other phase is saturated with solvent. In ternary systems the phase compositions are further modified by the presence of the third mutually miscible solvent. It is therefore useful to have a simple means of ranking the polarity of mutually saturated solvent systems. This can be done, at least for their behavior toward hydroxylic solutes, by measuring the partition coefficient of any one of the proposed standards in the series of solvent systems. However, since it is easiest to measure partition coefficients in the vicinity of unity, it is better to employ a series of standard solutes and to switch to a more polar standard as the solvent polarity increases. For instance, compounds I or II, V or X and VII all exhibit good linear behavior and would cover the range of polarity from 5% to 100% pentanol in overlapping stages defined by partition coefficients ranging from 0.2 to 5. Preliminary investigation of the progressively more polar glycosides, o-nitrophenyl- $\beta$ -D-galactopyranoside and p-nitrophenyl- $\beta$ -D-lactopyranoside suggests that they may be useful to extend the scale of polarity

TABLE 3

CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O Systems Employed in CCC<sup>9,10</sup>

<u>Mixture</u>	<u>Composition, (v/v/v)</u>		<u>K<sub>s</sub></u>	
	CHCl <sub>3</sub> /MeOH/H <sub>2</sub> O		<u>VII</u>	<u>VIII</u>
	<u>intiger ratio</u>	<u>ratio %</u>		
a	13:7:8	46:25:29	0.10	0.06
b	4:4:3	36:36:28	0.29	0.14
c	5:9:7	24:43:33	0.32	0.16
d	13:7:4	54:29:17	0.34	0.18
e	5:6:4	33:40:27	0.39	0.23
f	5:5:3	39:39:22	0.44	0.25
g	43:37:20	43:37:20	0.47	0.29
h	10:12:7	34:41:25	0.50	0.32
i	7:13:8	25:46:29	0.54	0.34
j	50:57:30	36:42:22	0.54	0.35
k	5:10:6	24:48:28	0.57	0.36

beyond 100% pentanol/H<sub>2</sub>O. The DNP-amino alcohols and even DNP-amino alkanes are insufficiently soluble in systems containing less than 2% pentanol in heptane for use as indicators of the polarity of lipophilic solvent systems.

#### Application to the CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O System

Eleven different mixtures of CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O have been employed in CCC for the separation of a variety of natural products.<sup>9,10</sup> These are listed in Table 3. Their compositions

are indicated in conventional terms as smallest whole number volume ratios and also in terms of volume percent. Neither notation suggests the rank order polarity of these systems. A good indication of their relative polarity could be obtained from the ternary equilibrium diagram but that appears not to have been published in sufficient detail to convey this information.<sup>11,12</sup> Measurement of the partition coefficients,  $K_S$ , of either compound VII or VIII allowed the systems to be listed in the rank order polarity shown in Table 3. It is clear that the organic phase of system 2 is least polar and that of system k is most polar. It is also apparent that systems c and d are likely to behave identically in chromatography and that they differ only slightly from systems b and e. Likewise, systems f, g and h and i are very similar. System j is also very similar to the most polar system k. These initial classifications have been confirmed by comparing the proximity of these compositions to tie lines on the  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  equilibrium diagram which we have recently constructed.

### Conclusion

A series of nonionizing DNP-amino alcohols covering a wide range of polarity can be conveniently used to establish the rank order polarity of solvent systems employed in CCC by measuring the partition coefficients of one or more of the compounds in the series of solvent systems. Very polar solvent systems may also be classified by measuring the partitioning behavior of PNP-glycosides.

### ACKNOWLEDGEMENT

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