AD-A116 806 ARIZONA UNIV TUCSON DEPT OF CHEMISTRY F/G 7/3
SEPARATION OF ALIPHATIC ALCOHOLS BY PAIRED ION LIQUID CHROMATOG--ETC(U)
1981 T GNANASAMBANDAN, H FREISER N00014-81-K-0576
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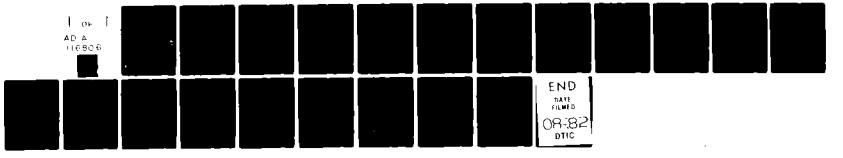

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: 4	2. GOVT ACCESSION NO.: AD-A116	3. RECIPIENT'S CATALOG NUMBER: 806
4. TITLE (and Subtitle): "Separation of Aliphatic Alcohols by Paired Ion Liquid Chromatography" (12)		5. TYPE OF REPORT & PERIOD COVERED: Technical -- Interim
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): T. Gnansambandan and H. Freiser		8. CONTRACT OR GRANT NUMBER(s): N00014-81-K-0576
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Chemistry, University of Arizona, Tucson, Arizona 85721		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS: Materials Sciences Division, Office of Naval Research, Arlington, Virginia 22217		12. REPORT DATE
		13. NUMBER OF PAGES: 18
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report): unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

to be published in Analytical Chemistry

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Reversed phase paired ion partition chromatography, dye enhanced detection of nonionic compounds, dye-association complexes

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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OFFICE OF NAVAL RESEARCH

Contract N00014-81-K-0576

TECHNICAL REPORT NO. 4

"Separation of Aliphatic Alcohols by Paired Ion Liquid Chromatography"

by

T. Gnanasambandan and H. Freiser

submitted to

Analytical Chemistry

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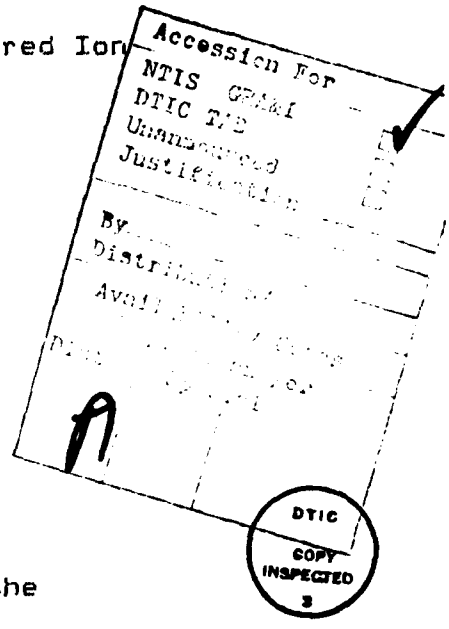


ABSTRACT

Using the cationic dye,methylene blue (as the chloride salt) to enhance the sensitivity of detection of species not possessing chromophores as a 10^{-4} M component of a methanol/water mobile phase, a series of alcohols from ethanol to t-pentanol were separated on Partisil ODS with good baseline separation at submicrogram levels using spectrophotometric detection at 651 nm. A retention model based on dye-alcohol complex formation and equilibrium partitioning of these species is advanced.

During the course of study of reversed phase paired ion partition chromatography, we observed that neutral substances such as aliphatic alcohols, esters, ketones, etc., undergo chromatographic separation in some as yet undefined association with the ion-pair.

Using the cationic dye, methylene blue (as the chloride salt) to enhance the sensitivity of detection of species not

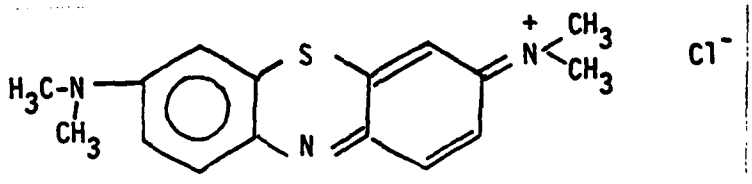
po sessing chromophores as a 10^{-4} M component of a methanol/water mobile phase, a series of alcohols from ethanol to t-Pentanol were separated on Partisil ODS with good baseline separation (Fig. 1) at submicrogram levels using spectrophotometric detection at 651nm (1).

In order to fully exploit this novel method of separation and detection of organic neutrals, it is necessary to study the characteristics of the process involved and to elucidate the underlying principles. This report describes our investigation of the phenomena.

EXPERIMENTAL SECTION

Materials: The alcohols, ethanol, propanols, butanols and the amyl alcohols, used for the study were obtained from various souces, (Baker Analyzed Fisher Scientific) and were used as received. Deionized water and methanol were used for all mobile phase preparations.

Methylene blue



M.W. 373.9, Baker analyzed was used as received.

Apparatus: A modular, high performance liquid chromatograph, consisting of an Altex pump (Model 110A), a variable wave length, u.v. visible detector (Schoeffel Model Sf 770 Spectroflow), a refractive index detector (Showa Denko K.K. Shodex RI Model SE-11) and a strip chart recorder (Linear

Instruments Model 261M/M) was used. The sample injector was 10μL loop injector (Spectra physics Rotary valve injector SP-419-0410).

Mobile Phase: The mobile phases consisted of 1.0×10^{-4} M methylene blue in various mixtures of degassed methanol and water ranging from 5 to 80 volume percent methanol.

Column: A Partisil-5-ODS (Whatman) 25cm x 6.26mm, reported to be 10% carbon loaded with <95% surface hydroxyl coverage was used. Zorbax ODS (duPont) 25cm x 4.6mm column was also used.

Conditioning of Column: Before use, the column was loaded with methylene blue by passing mobile phase through the column until an absorbance equivalent to that of the original mobile phase is observed. When this plateau or background level absorbance is achieved, samples are introduced. To remove the dye, a solution of 50 volume percent chloroform in methanol is passed through the column until the absorbance falls to the level of the blank mobile phase. Alternatively, 10 vol.% dimethylsulfoxide in methanol could be used. Both of these solvent systems efficiently remove the sorbed methylene blue.

Analysis Conditions: Samples injected are of 1 vol % solutions of the alcohols in the mobile phase without dye, and are injected via the 10uL loop sample valve. For the study with the dye-free column, the sample solutions are of 25 vol % solution in mobile phase. A higher concentration is used because of the

low sensitivity of the refractive index detector. Mobile phase composition varied from 90/10 to 30/70 volume ratio water/methanol. The flow rate through the column was either 0.5mL or 1.0mL per minute.

The capacity factor,k, was calculated as (Vr-Vo)/Vo where Vr is the apparent retention time volume of the solute and Vo is the dead volume of the column. For Vo, the retention volume of water was used.

Column efficiency, expressed as the number of theoretical plates of the column was calculated using the formula

$$N=5.54\ (t_r/w_{1/2})^2$$

where t_r is the retention time of the solute and $w_{1/2}$ is the width of the base at half height. For the Zorbax column the solutes chosen were p-xylene, naphthalene and anthracene, and the mobile phase was 80/20 volume ratio methanol/water. The study was conducted on the dye-free column. The theoretical plates before and after use remained to be the same within our experimental error. The column had 5500 ± 500 plates.

RESULTS AND DISCUSSION

Paired ion partition chromatography has enjoyed a position of justified prominence in the separation of charged organic species (2-6). The application to the separation of neutral species, however, first described by us (1), was totally unanticipated. In fact, neutrals have been used as markers in paired ion chromatography to clarify the role of ion paring in separation of charged species. Knox used benzyl alcohol as a solute whose v was esstentially unaffected by ion pairing reagents and, therefore, provided a dramatic contrast to the behavior of ionic species (5).

One of the major questions to be answered in order to understand the nature of these separations is the manner in which the neutral solutes are associated with the dye in both mobile and immobile phases. For example, is a dye-alcohol complex formed or is the excess dye present in the chromatographic zone because of the local excess alcohol concentration? A comparison of the capacity factors for the alcohols obtained both in the presence and absence of the dye shows that the same sequence is observed in both cases, and further, that the two sets of capacity factors are linearly related. The linear relationship of the capacity factors(Figure 2) indicates that the chromatographic processes are similar. That they cannot be identical,however,is seen from the different values for the corresponding capacity factors as well as from the slope of the line,1.74,showing the greater separation achieved in the presence of the dye. Had the species been identical, a slope of

unity would have been exhibited.

Each of the alcohols studied exhibits a linear calibration curve(Figure 3),from whose slope along with the known value of the molar absorbance of methylene blue a stoichiometric relationship between alcohol and dye can be estimated. Values of mole ratios of alcohol:dye obtained in this manner(Table I) are puzzling on two counts. First, the indicated stoichiometry, particularly for the lower alcohols, do not seem rational. Second, these ratios vary much more widely than might be expected from successive members of a homologous series.

The values of the ratios can be understood, however, if it is assumed that they result from complex-forming reactions that do not go to completion and which, in the time scale of the experiment, are rapidly reversible. Inasmuch as the shapes of the chromatographic curves indicate a fairly high column efficiency and hence,absence of slow kinetic effects, it seems reasonable to assume that the dye-alcohol interaction may be characterized as a fairly rapidly established equilibrium system.

The following equations serve to represent the phenomena on the basis of the equilibrium assumption:

$$D^+,Cl^-(i) \rightleftharpoons D,Cl \longrightarrow D^+ + Cl^- \qquad (1)$$

where D represents the methylene blue cation

$$D^+,Cl^-(i) + A \overset{K}{\rightleftharpoons} DA^+ + Cl^- \qquad (2)$$

where A represents the alcohol

In the absence of alcohol, the equilibrium described in eqn.1 is

far to the left. In fact, no appreciable amount of dye remains in the mobile phase until the column is "saturated" with the dye.

The distribution of the dye between the immobile and mobile phases refpresents a partitioning equilibrium far in favor of the immobile phase. The ratio of the dye concentrations in the two phases is virtually constant over the range from $1x10^{-6}$ M to $2x10^{-4}$ M (mobile phase concentrations). It is interesting to observe that the immobilized dye at the higher concentration would represent a thickness of approximately 0.02 monolayers if the distribution were interpreted as adsorption. Thus, our results would indicate that the distribution is more akin to liquid-liquid partition chromatography. It should be noted that, in the polar mobile phase, at the low dye concentrations employed, it may be assumed that virtually total dissociation of the ion pair occurs.

As indicated by eqn.2, the alcohol will react with immobilized dye, possibly in the immobilized phase, to form a complex species of greater column affinity than itself(as shown by the higher k′ values of the complex)in a rapid equilibrium fashion, which can be expressed by

$$K = \frac{[DA]}{[D,Cl]_i [A]} \qquad (3)$$

In eqn.3, inasmuch as the concentration of the dye in the mobile phase remains essentially constant, the value of K is seen to be proportional to the reciprocal of the mole ratios of

alcohol:dye summarized in Table 1. Another measure of K is incorporated in the value of k',a composite of the formation constant,K, of the complex and its true distribution ratio. The plausibility of this relationship may be seen from Figure 4 where the values of (DA)/(A)(the reciprocal mole ratio) for the various alcohols are seen to vary linearly with the corresponding values of the capacity factor.

These results resemble those observed by Schill in paired ion solvent extraction (2,7) in which auxilliary solvent components were found to enhance extractability of ion pairs. Schill attributed this phenomena to adduct formation in a formulation quite similar to our equations (2) and (3). Of course, in those instances the neutral adducting agents were used at macro concentrations. More recently, Johansson(8) applied thise same principle to enhance the retention volumes of hydrophilic ion pairs. Our work would seem the first to demonstrate that such beahvior can be observed when the ion pair, i.e., the dye, if present at concentration levels far in excess of the neutral species.

Obviously, the methanol in the mobile phase plays a role in the overall equilibrium aside from its effect on the polarity of the mobile phase. In fact,this can also be described by eqn.2, since this alcohol competes with the analyte alcohols. Since the methanol is present in much higher concentration, the K characterizing the dye:methanol complex is far smaller than that for the others, or the phenomenon would not be observed. Also, as expected from this interaction, the methanol level in

the mobile phase dramatically affects both the amount of dye immobilized on the column (Figure 5) and the chromatographic characteristrics of the other alcohols. As the level of mthanol increases, both the k′ values (Table 2) and the sensitivity of detection decrease.

CONCLUSIONS

Reversed phase paired ion partition chromatography employing a highly absorbing dye as one of the ions, not only offers obvious advantages for determination of aliphatic anions adn cations, but has provided a novel, highly sensitive method for alcohols. Calibration curves for samples in the submicrogram range are linear (rel. std. dev. <2%). Values of capacity factors are found to be reproducible to better than 1%. The technique is simple, reliable, rapid, and also applicable to other families of neutral compounds, such as trhe carboxylic acids, esters, ketones, etc. The column, once conditioned, can be used continuously for several weeks. Moreover, the regenerated column retains its original characteristics. In addition to column efficiency, the observed k′ values and sensitivities remain quantitiatively the same.

The behavior of the alcohols on the dye-containing column can be attributed to the formation of a dye-alchohol complex which has a higher k′ than does the alcohol itself. Formation of the complex occurs in a rapidly reversible equilibrium fashion characterized by equilibrium constants whose increase with the distribution ratios of the alcohols indicate that the

same structural influences are at work in both processes.

Credit. This research was supported by a grant from the office of Naval Research.

Literature cited.

(1) Gnanasambandan T., Freiser, H., Anal. Chem., 1981, 53, 909-911.

(2) Schill, G., in "Advances in Ion Exhange and Solvent Extraction; Marinsky, J.A., Marcus, Y., Eds.;Marcel Dekker: New York, 1974, Vol. 6, p. 1.

(3) Horvath, C.,. Melander, J. Chromatogr. Sci. 15, 393, 1977.

(4) Bidlingmeyer, B.A., J. Chromatogr., 18, 525, 1980.

(5) Knox, J., Hartwick, R., J. chromatogr., 204 (1981), 3-21.

(6) DiNunzio, J., Freiser, H., Talanta, 1979, 26, 587-589.

(7) Modin, R., Acta Pharm. Suec., 1971, 8 509.

(8) Johansson, ?., J. of Liquid Chromatography 4(8) 1435-1457 (1981).

Table 1. Alcohol: Dye Ratios in Chromatographic Peaks On Partisil ODS-2

ROH	ROH/DYE Mole Ratio	k'
Ethanol	15	.8
i-Propanol	5.0	1.8
n-Propanol	3.0	2.2
t-Butanol	2.0	3.8
2-Butanol	1.5	5.1
n-Butanol	1.0	5.7
T-Amyl alcohol	0.5	10.8

Table 2. Variation of Capacity Factors on Comparison of Mobile Phase Zorbax ODS Column

Mobile Phase Composition	Capacity Factors					
H20/MEOH	EtOH	i-PrOH	n-PrOH	t-BuOH	2-BuOH	n-BuOH
90/10	1.0	1.71	2.1	3.8	5.0	6.4
80/20	0.9	1.6	1.9	3.5	4.4	5.8
70/30	-	-	-	1.4	2.0	2.23
60/40	-	-	-	0.9	1.6	2.0

Legend of Figures

Experimental conditons for Figures 1-4:

Mobile phase: Methanol/H_2O 5/95 V/V

10^{-4} M Methylene blue.

Flow rate: 1.0mL per min.

Detection wavelength: 651nm

Figure 1: Separation of Alphatic Alcohols.

Samples: 1. Methanol 2. Ethanol 3. isopropanol
4. n-propanol 5. t-Butanol 6. 2-Butanol
7. n-Butanol 8. t-amyl alcohol.

Sample size: 10μl, containing 80 μg of each alcohol.

Figure 2. Comparison of Capacity Factors of Alcohols in Presence and Absence of Dye. Alcohol numbers correspond to that of Figure 1. Equation of line Y = 1.74X0.99. cor. coeff. = 0.997

Figure 3. Calibration Curves of Various Alcohols.

Figure 4. Dependence of Conbcentration Ratio of Dye: Alcohol on Capacity Factors.

Figure 5. Dependence of the Amount of Dye on the Column on Methanol Concentration.

Figure 1

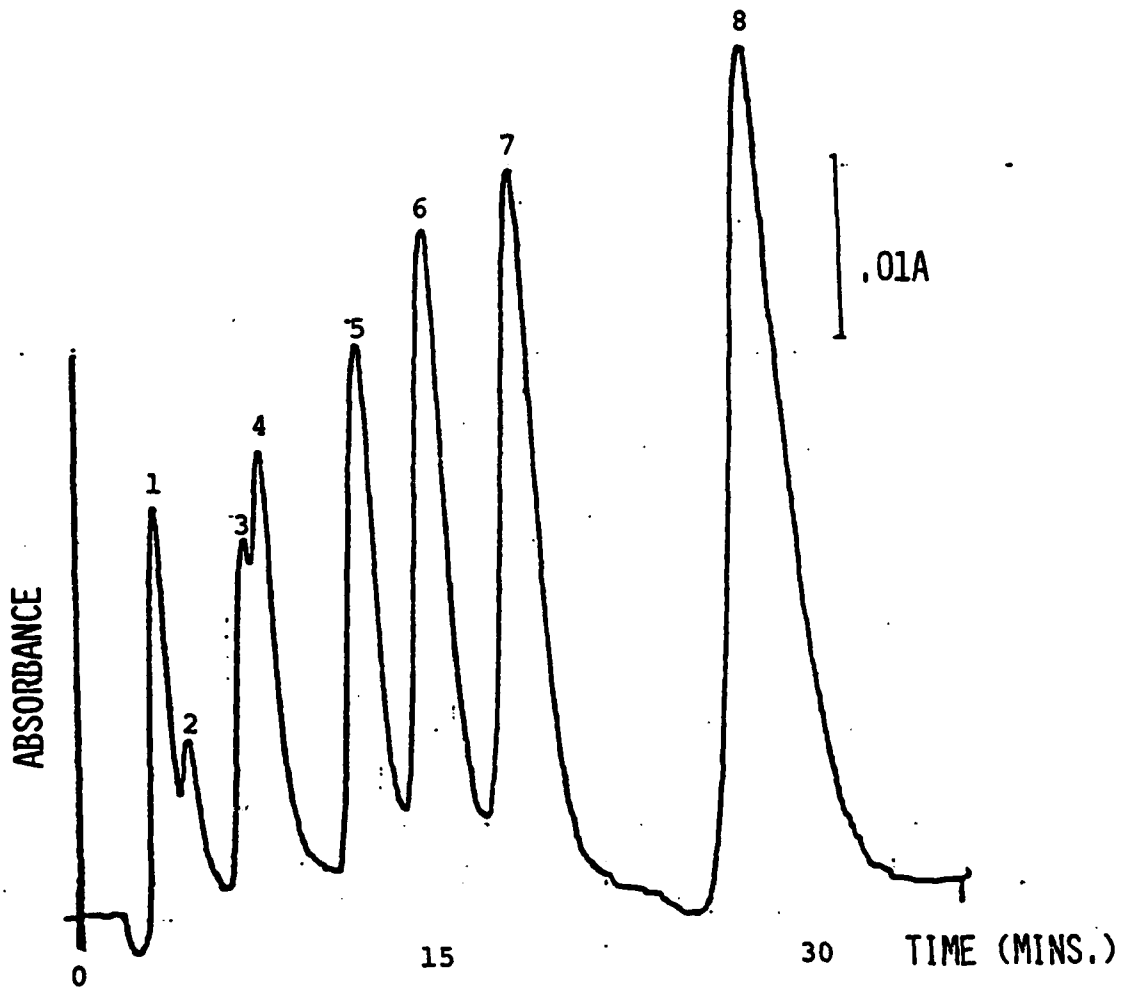


Figure 2

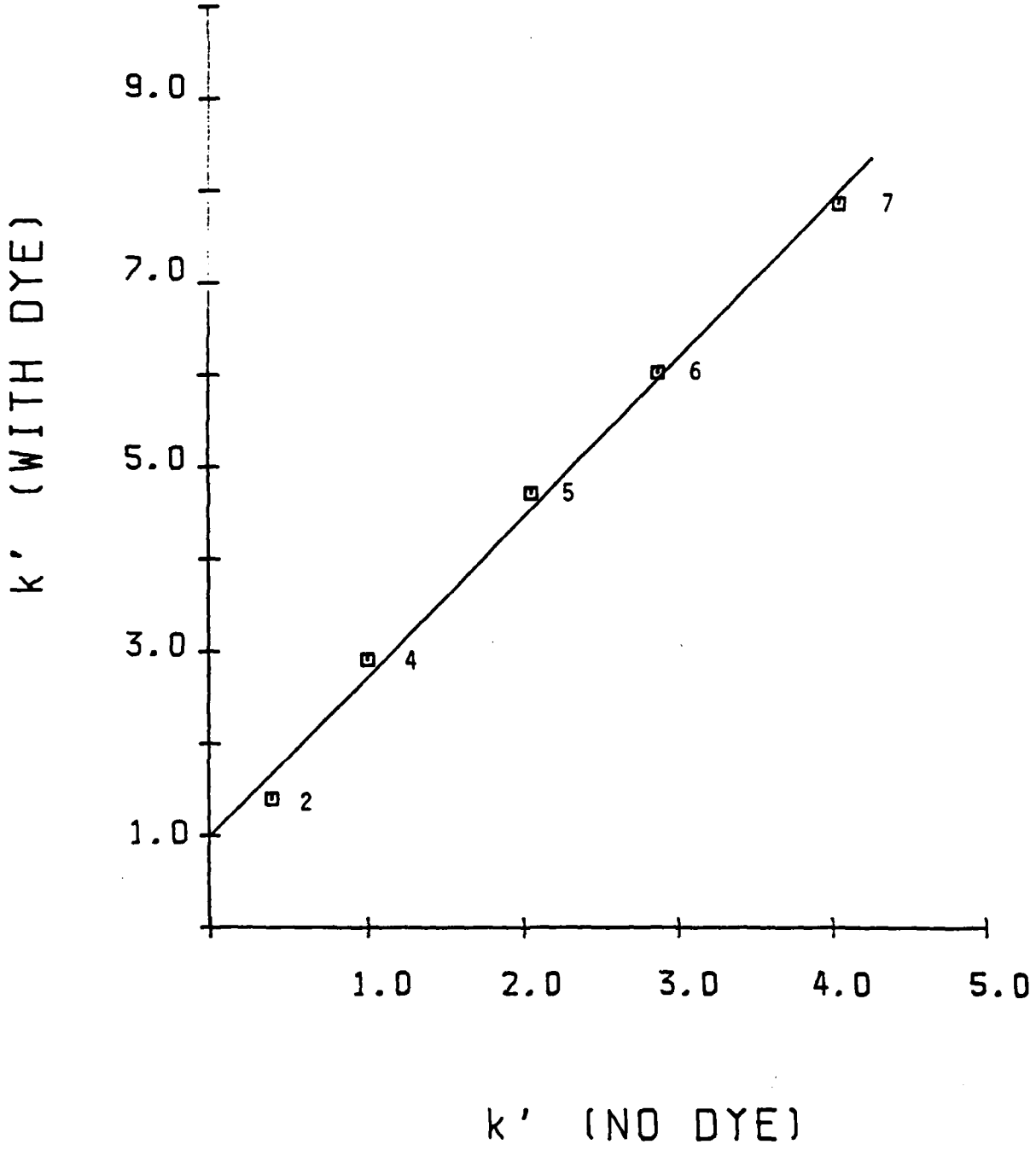
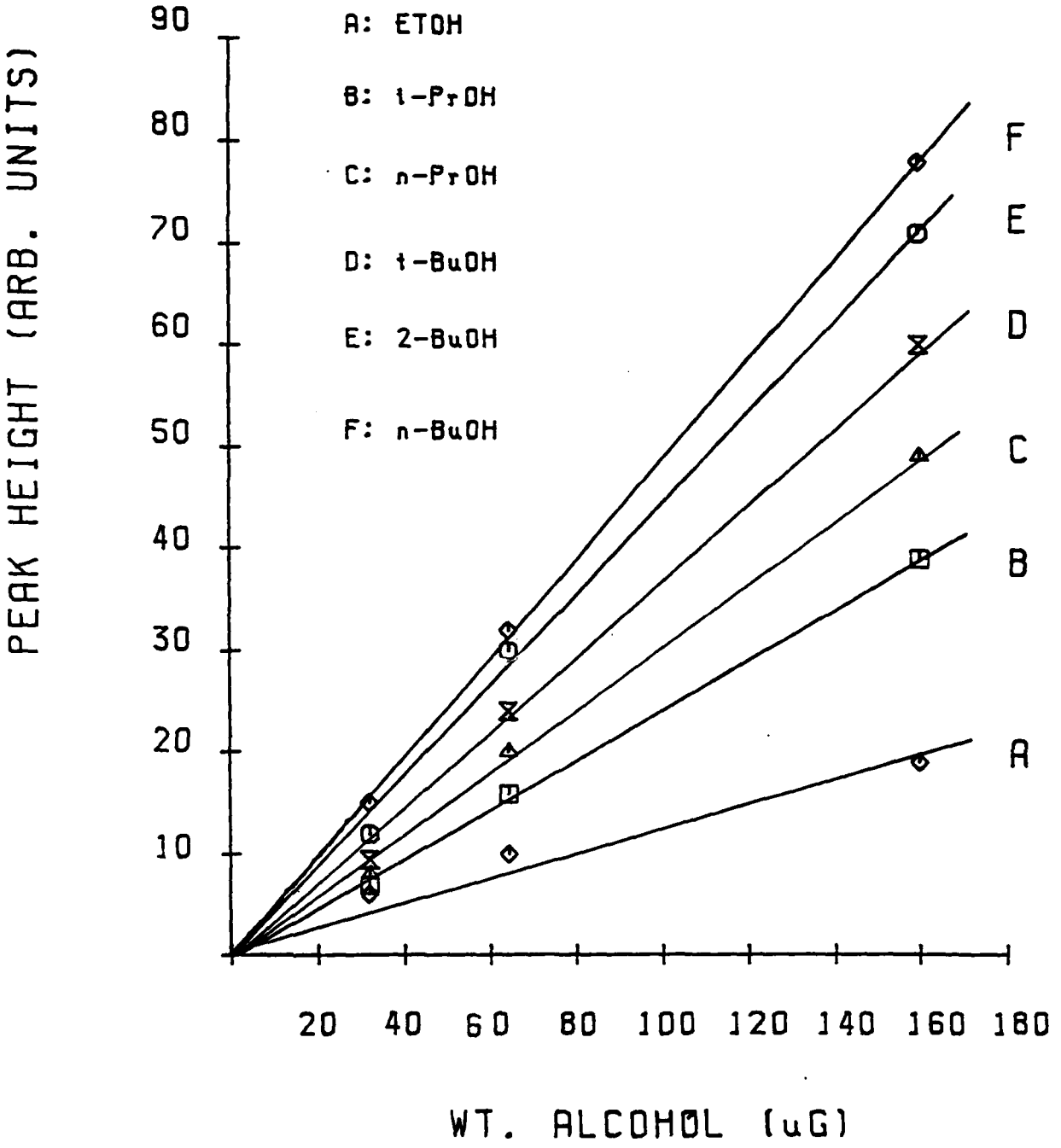


Figure 3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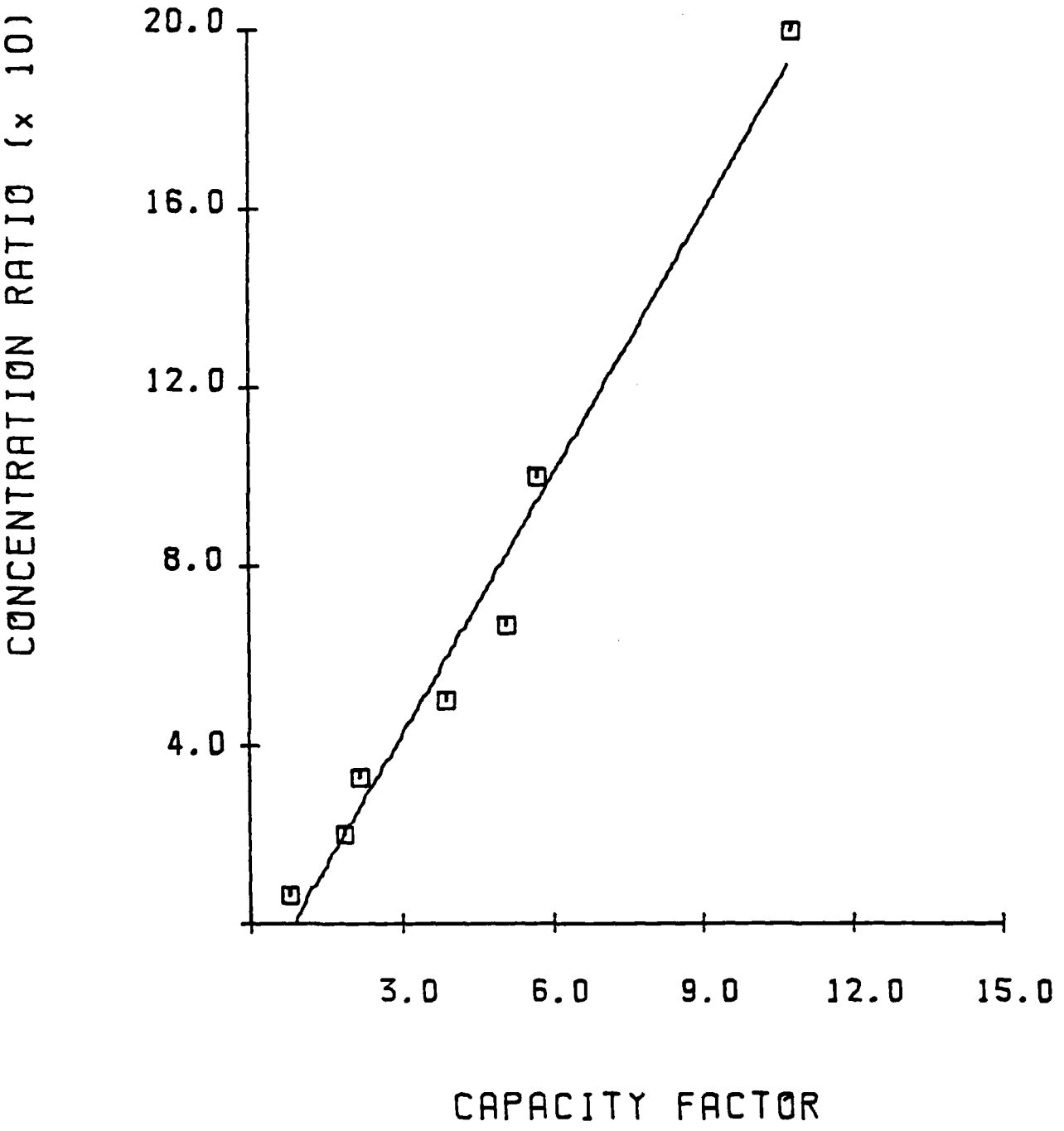
B: t-PrOH
C: n-PrOH
D: t-BuOH
E: 2-BuOH
F: n-BuOH
PEAK HEIGHT (ARB. UNITS)
90
80
70
60
50
40
30
20
10
20 40 60 80 100 120 140 160 180
WT. ALCOHOL (uG)
F
E
D
C
B
A

Figure 4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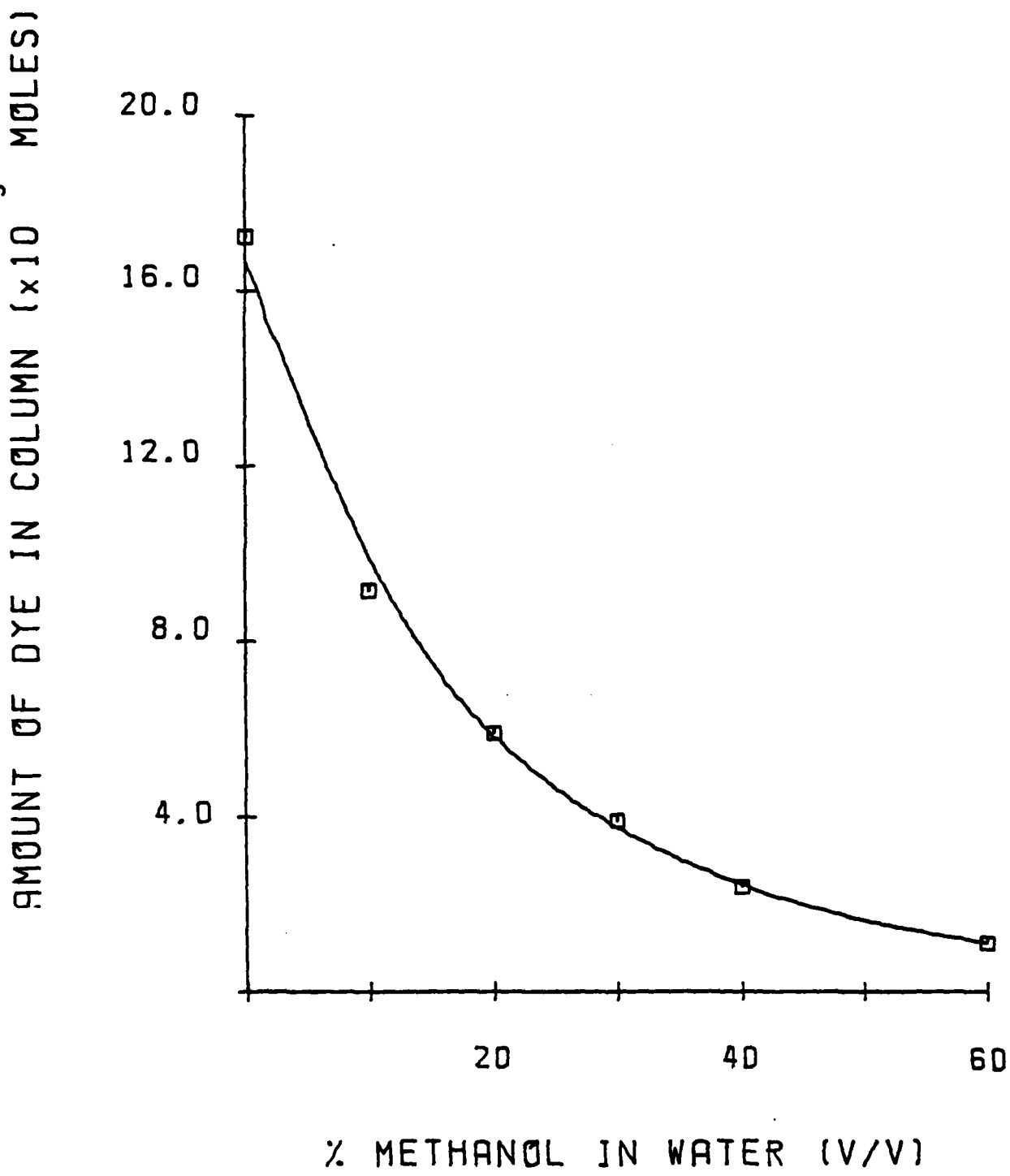
4.0
3.0
6.0
9.0
12.0
15.0
CAPACITY FACTOR

Figure 5


AMOUNT OF DYE IN COLUMN (x10 5 MOLES)
20.0
16.0
12.0
8.0
4.0
20
40
60
% METHANOL IN WATER (V/V)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8-8