Journal of Chromatography, 96 (1974) 163–170

CHROM. 7499

INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

VII. PARTITION OF PHENOLS IN SYSTEMS OF THE TYPE CYCLOHEXANE + POLAR SOLVENT–FORMAMIDE

E. SOCZEWIŃSKI and M. CISZEWSKA

Department of Inorganic and Analytical Chemistry, Institute of Basic Chemical Sciences, Medical Academy, 20-081 Lublin (Poland)

(First received January 14th, 1974; revised manuscript received April 3rd, 1974)

SUMMARY

Relationships between R_M values and the concentration of polar solvents in the mobile phase were determined for a group of halogenophenols, xylenols and several other derivatives of phenol. Three alcohols, oleic acid and chloroform were used as the polar component (S). It was found that alcohols, in spite of their autoassociation, have high extraction strengths relative to phenols, 1:1 solvation complexes being easily formed. Hindered solvation due to internal hydrogen bonding or steric shielding of the hydroxyl group resulted in less steep R_M *versus* log % S lines.

INTRODUCTION

The choice of solvents for chromatography is based on the eluotropic series[1–3] and a quantitative measure of the solvent strength is possible[1–7]. Instead of a series of solvents, mixtures of two solvents with differing polarities can be used, which enables the solvent strength to be changed in a continuous manner (see, for instance, Neher's equieluotropic series[1,8]). As practical examples of the latter method of optimization, Waldi's systems for the systematic analysis of alkaloids can be cited (paper impregnated with formamide and developed with mixtures of cyclohexane and chloroform); systems of this type were also recommended by Macek and Procházka in their standard set of solvent systems[9,10]. Variation of solvent composition, in addition to giving an appropriate range of distribution coefficients, can also improve the selectivity of separation[11], particularly when the additive interacts selectively with some of the components to be separated.

The relationships between chromatographic parameters and the composition of the mixed phase can often be described by equations derived by application of the law of mass action to the formation of molecular complexes (π-bonding, hydrogen bonding, ionization, formation of ion pairs; for references, see Part VI[12]). A difficulty in the interpretation of solvent composition effects is that the concentration of the

TABLE I

100× R_F VALUES OF PHENOLS FOR VARIOUS CONCENTRATIONS OF FIVE POLAR SOLVENTS IN CYCLOHEXANE

Polar component (S)	*Concentration of S (% v/v)*	*Phenol* *23MP*	*25MP*	*26MP*	*34MP*	*35MP*	*EU*	*IEU*	*GU*	*TY*	*3CP*	*4CP*	*4C3MP*	*24DCP*	*35DCP*	*4BP*	*4IP*
n-Hexanol	1	25	27	43	17	21	65	60	23	57	13	11	19	31	27	14	16
	2	37	41	53	30	34	66	62	26	68	21	18	28	38	44	23	26
	5	58	60	69	52	56	72	67	34	83	42	37	51	53	67	44	47
	10	73	74	77	68	71	79	75	43	–	60	56	68	66	81	62	65
	20	83	82	85	79	80	84	81	54	–	75	72	80	77	90	76	79
	50	93	92	92	90	91	91	88	67	–	87	85	92	91	–	89	91
n-Octanol	1	29	33	52	20	25	65	61	27	71	12	9	19	34	23	14	16
	2	42	45	59	32	40	68	65	30	77	22	17	33	41	39	25	29
	5	61	65	70	52	58	75	72	36	88	41	33	55	56	62	46	49
	10	75	76	79	67	72	80	77	41	93	60	51	72	69	79	63	67
	20	82	84	84	78	81	85	83	48	–	73	67	84	79	88	77	81
	50	91	92	89	87	90	91	88	63	–	88	83	93	89	–	90	92
Oleyl alcohol	1	22	24	45	16	19	65	63	23	68	8	7	13	31	16	8	10
	2	31	35	52	22	27	68	64	25	75	14	11	22	37	27	15	18
	5	47	53	59	38	43	71	67	28	82	28	23	40	50	47	29	35
	10	60	66	64	53	56	73	70	34	86	43	37	56	59	65	46	53
	20	71	75	71	65	69	77	74	39	89	58	52	71	72	80	61	69
	50	84	86	85	80	82	83	81	51	–	76	71	84	85	89	78	83
Oleic acid	1	19	23	48	12	15	65	62	25	59	4	3	7	24	6	4	5
	2	20	23	50	12	15	67	62	27	65	5	4	10	25	8	6	7
	5	23	27	53	15	19	69	64	29	71	9	7	15	30	16	10	11
	10	33	35	57	23	28	70	66	31	74	15	12	25	37	28	16	18
	20	45	47	62	39	43	73	70	33	79	26	22	40	49	46	28	33
	50	69	70	75	62	67	80	78	45	88	54	50	68	65	70	56	62
Chloroform	1	16	18	44	10	11	68	60	23	62	3	2	8	35	6	4	5
	2	18	20	47	11	13	69	61	24	64	3	2	8	36	6	4	5
	5	21	23	52	14	15	71	65	27	67	4	3	9	38	7	5	6
	10	25	28	56	17	19	77	71	33	74	6	5	12	42	10	7	8
	20	35	39	62	25	27	82	78	44	80	9	7	18	48	15	10	12
	50	56	59	70	46	49	91	88	63	89	17	15	34	57	30	20	24
	100	72	73	77	69	71	94	92	75	93	38	35	56	67	53	43	49

free additive is included in the solvation constant; hence autoassociation of the additive should be taken into account[13,14]. As Littlewood and Willmott noted[13], the autoassociation of a polar additive (solvent) may have different effects on solutes with different molecular structures. Thus, the concentration of an alkanol in the stationary phase has a much greater effect on the retention of volatile alkanols (class AB) than on solutes of class B (notation of hydrogen bonding ability after Pimentel and McClellan[15]), which Littlewood and Willmott[13] explained by the differences in the molecular solvation mechanism: molecules of the lower alkanols can be incorporated into the association chain at any position, while electron donor solutes can only form hydrogen bonds with the terminal molecules of the solvent. Another explanation is that proton donor solutes can be hydrogen bonded to the lone electron pairs that remain on each oxygen atom after the formation of the association chain[16] (*cf.*, Part VI[12], formula on p. 156). Alkanols are thus analogous to solvents of class B (*cf.*, Part V[17], Figs. 6a, 6b). Analogous effects have also been observed in liquid–liquid chromatography[17] using aqueous systems. In the present study, further phenols have been investigated using formamide as the stationary phase and solutions of *n*-hexanol, *n*-octanol, oleyl alcohol, oleic acid (associated solvents) and chloroform (class A) as the developing solvent.

EXPERIMENTAL

Whatman No. 41 paper strips were impregnated with 20% (v/v) acetone solutions of formamide and developed with cyclohexane solutions of *n*-hexanol, *n*-octanol and oleyl alcohol, oleic acid and chloroform of various concentrations. The chromatograms were detected by immersion in bis-diazotized benzidine reagent[18] after spraying with a saturated solution of sodium hydrogen carbonate. The R_F values are given in Table I.

The phenols are denoted here and in the figures by the following abbreviations: P = phenol; C = chloro; B = bromo; I = iodo; M = methyl; D = di; EU = eugenol; IEU = isoeugenol; TY = thymol; GU = guaiacol (*e.g.*, 4C3MP = 4-chloro-3-methylphenol).

RESULTS AND DISCUSSION

The experimental results are presented as R_M *versus* log % S plots, the scale being convenient for practical reasons (see the comments in Part VI[12]); owing to the molar volumes of the polar solvents being comparable with or higher than that of cyclohexane, the resulting deformations are insignificant[14]. On the other hand, larger deviations from the theoretical R_M *versus* composition curves could be caused by variations in the solvation constants with composition of the medium, autoassociation of solvents of class AB (alcohols and oleic acid), and varying solubility of formamide in the less polar phase; in order to reduce the last effect, solutions of the associated solvents of concentration not exceeding 50% (v/v) were used.

In Figs. 1–5, the results obtained for halogenophenols are presented. The sequence observed for all solvent systems is essentially the same, the R_F values increasing in the order *p*-chlorophenol < *m*-chlorophenol < *p*-bromophenol < *p*-iodophenol < *p*-chloro-*m*-cresol < 3,5-dichlorophenol.

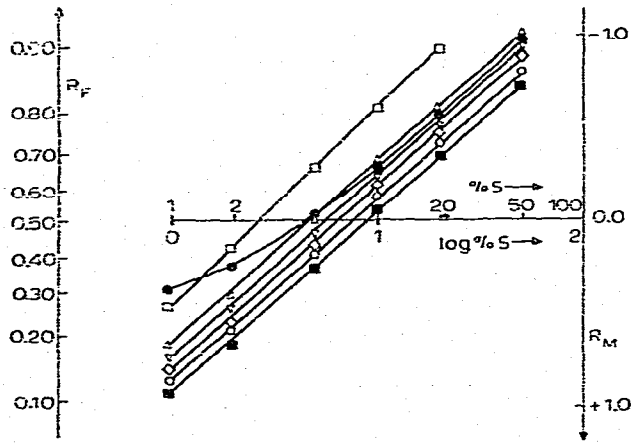
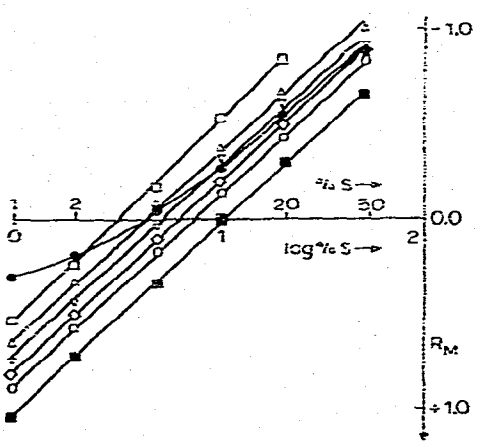


Fig. 1. R_M *versus* log "„ S relationships for halogenophenols in systems of the type cyclohexane polar solvent–formamide. Polar solvent (S) *n*-hexanol. ●, 24DCP; , 35DCP; , 4C3MP; , 4IP; , 4BP; , 3CP; ■, 4CP.

In systems with the alcohols (Figs. 1–3), the R_M *versus* log "„ S relationships for these phenols are straight lines with a slope of approximately unity, typical for solutions of electron donor solvents[17] and 1:1 solvation complexes. Thus, the autoassociation of alcohols, in accordance with previous reports, does not seem to reduce their solvent strength seriously.

2,4-Dichlorophenol behaves in a more individual manner, owing to its tendency to form an internal hydrogen bond between the hydroxyl group and the *ortho*-chlorine atom, which decreases its solubility in the formamide phase (in comparison with its 3,4-isomer), but also weakens its interaction with the alcohol molecules in the mobile phase. Therefore the line of 2,4-dichlorophenol is flatter and its curvature at low concentrations of S indicates a contribution of extraction by cyclohexane



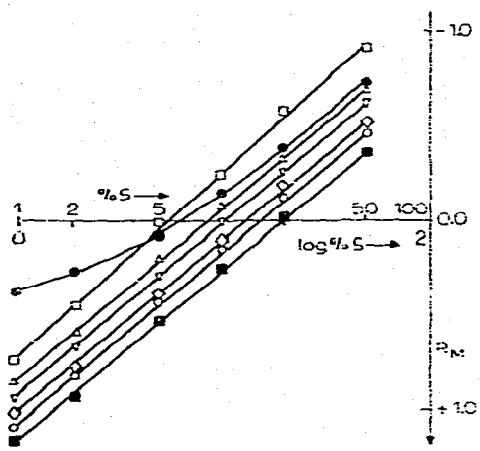
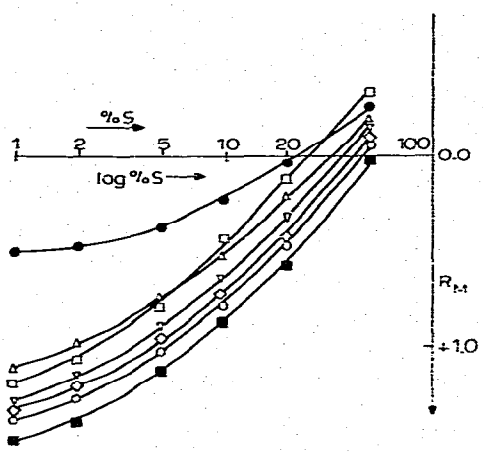


Fig. 2. As in Fig. 1; S *n*-octanol.

Fig. 3. As in Fig. 1; S oleyl alcohol.



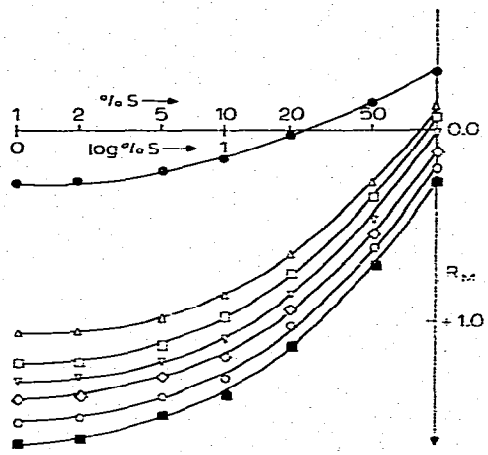


Fig. 4. As in Fig. 1; S = oleic acid.

Fig. 5. As in Fig. 1; S = chloroform.

This effect is even more pronounced for oleic acid (Fig. 4) and chloroform (Fig. 5). The consequence of this individual behaviour of 2,4-dichlorophenol is that the selectivity of separation from the remaining solutes is a function of solvent composition.

Chloro-, bromo- and iodophenol have similar pK_a values (9.45, 9.34 and 9.20, respectively) so that differences in their R_M values are mostly due to the size of the halogen substituent[19,20]. The introduction of a methyl group in the *meta*-position (4CP → 4C3MP) causes a marked increase in the R_F value due to the decreased solubility in the formamide phase ($\Delta R_M \approx -0.3$ unit).

The extraction strengths of oleic acid and chloroform are significantly lower than in the case of alcohols, the R_M *versus* log % S lines having a "hockey-stick" shape, typical of a gradual shift in solvation equilibrium, the slopes in the region of the highest percentages of the polar component being greater than unity, which could be due either to the increased solubility of formamide or to a deformation caused by variations in the solvation constants and the use of parameters expressed in terms of concentrations in moles per litre[21].

In Figs. 6–10, the R_M *versus* log % S relationships for several isomeric xylenols and some other phenols are represented. The sequence of all of the xylenols is the same in all solvent systems investigated, the R_F values increasing in the order 3,4- < 3,5- < 2,3- < 2,5- < 2,6.

Also in this case the *ortho*-effect is apparent, especially for 2,6-xylenol, shown not only by increased R_F values but also by less steep R_M *versus* composition lines. Also for these solutes the alcohols are better extractants, the lines being higher and steeper than for oleic acid and chloroform. At higher concentrations of the active component, the selectivity of the systems is reduced: apparently, effects that decrease the solubility of the solutes in the formamide phase also decrease the solubility in alcohol, with a consequent partial counterbalancing of the two effects.

Three of the remaining phenols—eugenol, isoeugenol and guaiacol—can form internal hydrogen bonds with resulting parallel, flatter R_M *versus* composition lines; guaiacol, owing to its lower molecular volume, is much more soluble in the form-

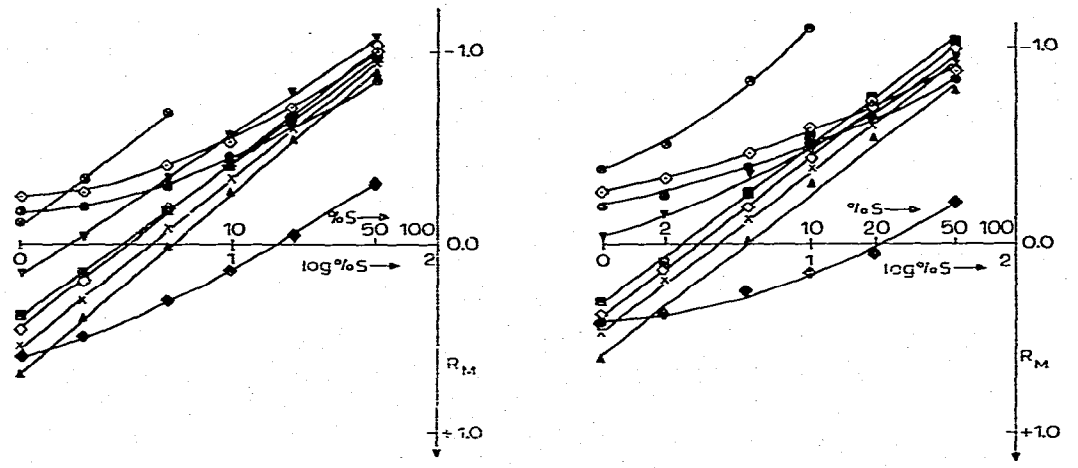


Fig. 6. R_M *versus* log % S relationships for xylenols, eugenol, isoeugenol, guaiacol and thymol. Polar solvent (S) = *n*-hexanol. ◇, EU; ●, IEU; ○, TY; ▼, 26MP; ■, 25MP; □, 23MP; ×, 35MP; ◆, GU; ▲, 34MP.

Fig. 7. As in Fig. 6; S = *n*-octanol.

amide phase. Thymol, on the other hand, is less soluble in formamide owing to steric shielding of the hydroxyl group by two *vicinal* alkyl groups, which, however, do not prevent its solvation by alcohol molecules, so that the R_M *versus* composition lines for thymol are similar to those for the xylenols. As in Figs. 1–5, the limiting slopes obtained for higher concentrations of the polar solvent (S) are close to unity or higher. A theoretical curve with its asymptotes is compared with the experimental relationships in Fig. 10 (dashed line): the lines are approximately parallel (except at higher concentrations of chloroform) which indicates that 50% solvation (except for 2,6-dimethylphenol) occurs at *ca.* 10% (v/v) concentration of chloroform in the mobile

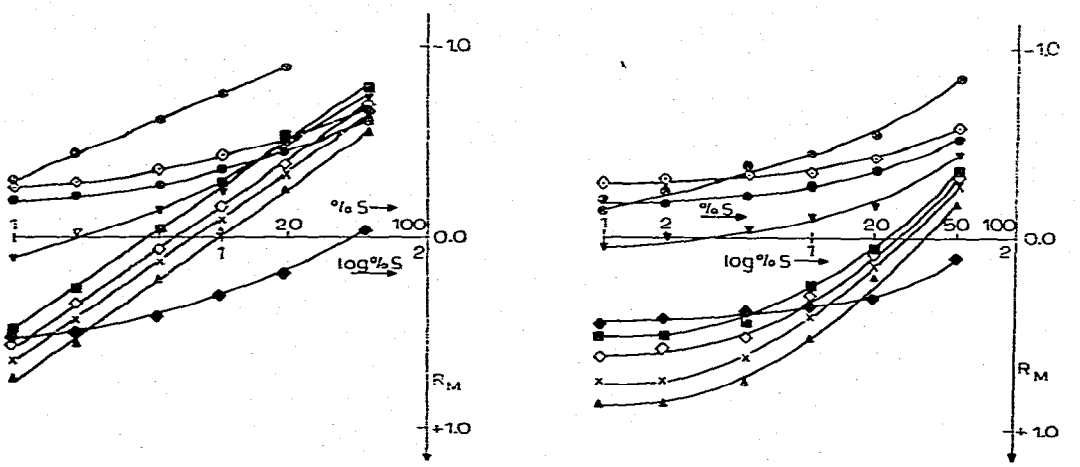


Fig. 8. As in Fig. 6; S = oleyl alcohol.

Fig. 9. As in Fig. 6; S = oleic acid.

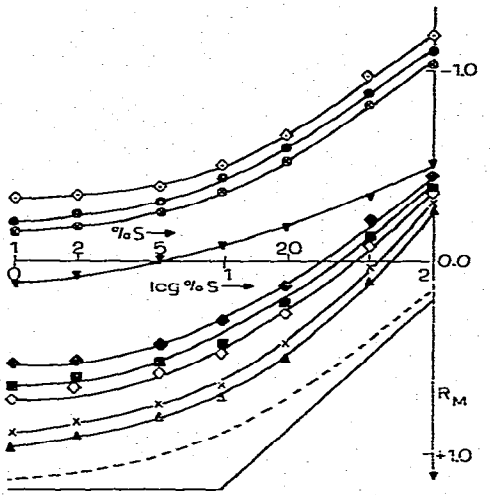


Fig. 10. As in Fig. 6; S = chloroform. Dashed line represents a theoretical R_M *versus* composition curve, its asymptotes crossing at a composition corresponding to 50% solvation of the solute.

phase, if the chromatographic process depends on idealized liquid–liquid partition only.

The experimental results demonstrate that solutions of alkanols, in spite of their autoassociation, are good extractants of phenols, the 1:1 solvation complex predominating down to 1% solutions in cyclohexane (unless there is a parallel depression of adsorption on the formamide surface: the conclusion should be confirmed by static experiments, *cf.* Part VI[12]. Oleic acid is a less effective extractant of phenols. Hindered solvation of the hydroxyl group (intermolecular hydrogen bond, steric shielding) is reflected by less steep R_M *versus* composition lines, which is equivalent to a shift to higher values of the concentration that corresponds to 50% solvation (Fig. 10).

REFERENCES

1 E. Stahl (Editor), *Dünnschicht-Chromatographie*, Springer, Berlin, 1967.
2 E. Hecker, *Verteilungsverfahren in Laboratorium*, Verlag Chemie, Weinheim, 1954.
3 K. Randerath, *Thin-Layer Chromatography*, Verlag Chemie, Weinheim, 1964.
4 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
5 L. Rohrschneider, *Z. Anal. Chem.*, 170 (1959) 256.
6 A. B. Littlewood, *J. Gas Chromatogr.*, 1, No. 11, (1963) 16.
7 E. Soczewiński, W. Maciejewicz and M. Bieganowska, *Separ. Sci.*, 5 (1970) 365.
8 R. Neher, in G. B. Marini-Bettòlo (Editor), *Thin-Layer Chromatography*, Elsevier, Amsterdam, 1964.
9 K. Macek and Z. Procházka, in I. M. Hais and K. Macek (Editors), *Handbuch der Papierchromatographie*, Fischer, Jena, 1963, pp. 124–137.
10 K. Macek (Editor), *Pharmaceutical Applications of Thin-Layer and Paper Chromatography*, Elsevier, Amsterdam, 1972.
11 B. L. Karger, *Anal. Chem.*, 39 (July 1967) 24A.
12 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 96 (1974) 155.
13 A. B. Littlewood and F. W. Willmott, *Anal. Chem.*, 38 (1966) 1031.
14 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.

15 G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond*, Freeman, San Francisco, 1960.
16 A. V. Iogansen and G. A. Kurkchi, in *Fiziko-Khimicheskoe Primenenie Gazovoi Khromatografii*, Khimiya, Moscow, 1973, Ch. III, pp. 122–186.
17 E. Soczewiński and G. Matysik, *Int. Symp. VI Chromatogr. Electrophor.*, Presses Académiques Européennes, Brussels, 1971, p. 203.
18 B. Åkermark, H. Erdtman and C. A. Wachtmeister, *Acta Chim. Scand.*, 13 (1959) 1855.
19 L. S. Bark and R. J. T. Graham, *J. Chromatogr.*, 25 (1966) 357.
20 I. E. Bush, *Methods Biochem. Anal.*, 13 (1965) 357.
21 C. Eon and B. L. Karger, *J. Chromatogr. Sci.*, 10 (1972) 140.