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INVESTIGATIONS ON THE MECHANISM AND SELECTIVITY OF CHROMATOGRAPHY ON THIN LAYERS OF POLYAMIDE

II. COMPARISON OF POLYAMIDE AND CELLULOSE IMPREGNATED WITH FORMAMIDE USING MODERATELY POLAR DEVELOPING SOLVENTS

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SUMMARY

A number of phenols was chromatographed on thin layers of polyamide and on formamide-impregnated paper using proton-donor and electron-donor solvents diluted with cyclohexane. The experimental results represented as R_M *versus* log X_s relationships, where X_s is the mole fraction of the polar solvent, confirm analogies between formamide and polyamide systems due to interactions of phenols with the carbonyl groups in the stationary phase. The significant role of solvation in the mobile phase found for both systems is typical of a liquid–liquid partition mechanism; some differences in chromatographic behaviour are interpreted as the result of the solubility of formamide in more polar mobile phases and selective sorption of the polar component by polyamide.

INTRODUCTION

Polyamide gels should have chemical properties comparable with those of formamide, which is frequently employed as a stationary phase in liquid–liquid partition chromatography.

Comparison of the chromatographic results obtained for thin layers of polyamide and for paper impregnated with formamide could be expected to elucidate some aspects of sorption by polyamide, especially for series of binary solvent mixtures composed of a polar solvent and a non-polar diluent, as solvent composition effects in these systems can be interpreted in terms of the solvation equilibria involved[1-4]. The peptide-like grouping present both in formamide and polyamide suggests that the mechanism of sorption by the stationary phase might be expected to be similar in both instances. For instance, Martin and Husband[5] compared the R_F values of phenols obtained for polyamide-impregnated paper with those reported by Macák and Kubeš[6], who used paper impregnated with formamide, and found that the chromatographic

results were similar in both systems. Further data that confirm the analogy between the two stationary phases have been reported by Graham[7] and Graham and Daly[8,9], who used cellulose impregnated with simple amides, including formamide. In their work, however, the number of phenols and solvents that could be compared was limited owing to the specific properties of formamide, especially its miscibility with more polar liquids, which precluded their use as developing solvents. Therefore, more polar solutes, such as di- and trihydroxybenzenes and nitrophenols, could not be separated in formamide systems owing to the low R_F values. In this respect, polyamide can be considered as a complementary stationary phase, because in polyamide systems the use of polar solvents or their mixtures is possible and even necessary in order to ensure the formation of a gel-like sorbent with a high affinity for polar solutes. Comparison of both types of chromatographic systems can therefore best be performed by using mixed solvents with different polarities.

EXPERIMENTAL

Thin layers of polyamide (Woelm, Eschwege, G.F.R.) were prepared on glass plates using a Desaga-type spreader and activated as described in Part I[3]. The plates were developed in glass tanks by the ascending technique using binary solvent mixtures, the diluting solvent being cyclohexane; the polar components were of classes A and B in the classification of Pimentel and McClellan[10]. Paper strips (Whatman No. 4, 9 × 20.5 cm) were impregnated with 20% (v/v) acetone solutions of formamide and contained ca. 0.5 ml of formamide per gram of dry paper. After spotting the phenols, the strips were transferred into glass tanks (7 × 9 × 24 cm) for descending development, saturated with the vapour of the binary developing solvents. In both series of experiments, the spots were detected with bis-diazotized benzidine reagent after spraying with a saturated aqueous solution of sodium hydrogen carbonate[11].

RESULTS AND DISCUSSION

The results are presented in Figs. 1–10 as R_M versus log X_s plots, where X_s is the mole fraction of polar solvent, the phenols being denoted by abbreviations, as indicated in Table I. Tables II and III show the actual R_F values obtained.

For the polyamide systems investigated, the R_M versus log X_s relationships were linear in most instances. On the other hand, for formamide systems, the lines were usually curved, the slope increasing with increasing concentration of the polar solvent. These curvilinear relationships probably arise owing to the fact that the R_F values of the solutes were generally higher for formamide systems, so that cyclohexane cannot be considered as an "inert" diluent for many solutes and contributed to some extent to the extraction strength of the mobile phase ($R_F > 0$ for the formamide–cyclohexane system; compare the analogous situation for aqueous systems in ref. 1, Fig. 9B7, and ref. 12, Fig. 8). This interpretation is substantiated by the curvilinear relationship for 2,6-dimethylphenol shown in Fig. 1 (highest curve) and the linear relationship for most of the strongly sorbed phenols shown in Figs. 1 and 6. Steeper relationships at high X_s values may also be due to the increased miscibility of the two liquid phases.

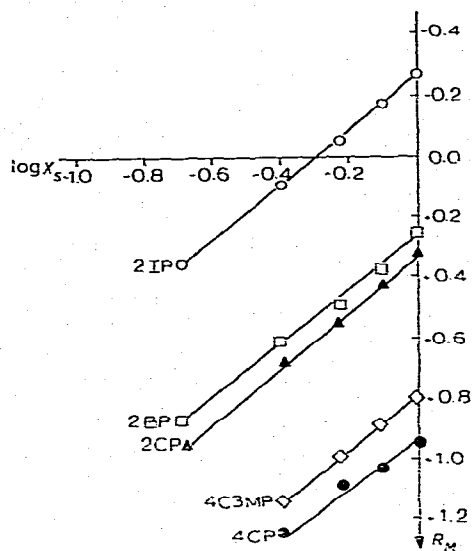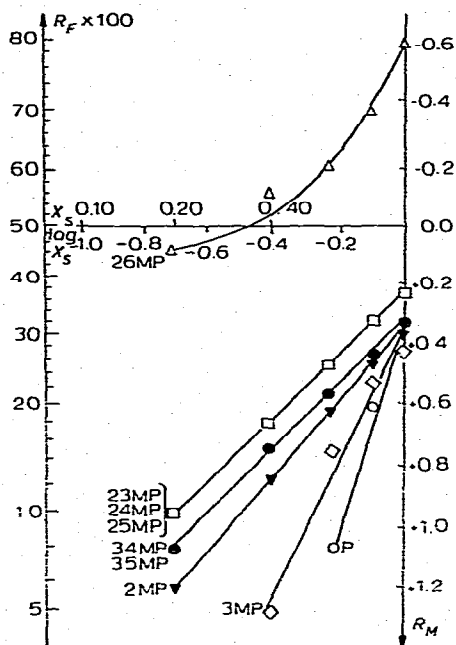The methyl derivatives of phenol behave in an analogous manner in both

Fig. 1. R_M *versus* log X_s relationships for phenols for polyamide systems. Polar solvent (S): chloroform. Compare Fig. 6.

Fig. 2. R_M *versus* log X_s relationships for phenols for polyamide systems. Polar solvent (S): chloroform. Compare Fig. 7.
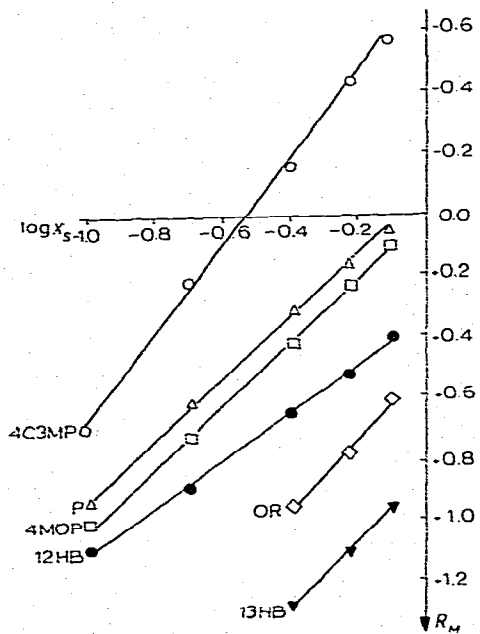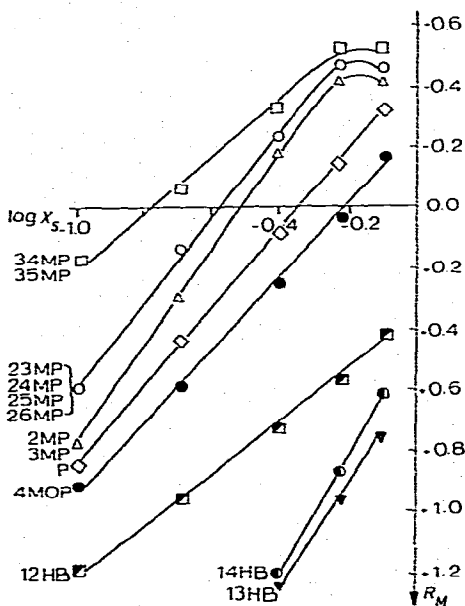

Fig. 3. R_M *versus* log X_s relationships for phenols for polyamide systems. Polar solvent (S): diethyl ether. Compare Fig. 8.

Fig. 4. R_M *versus* log X_s relationships for phenols for polyamide systems. Polar solvent (S): di-*n*-butyl ether. Compare Fig. 9.
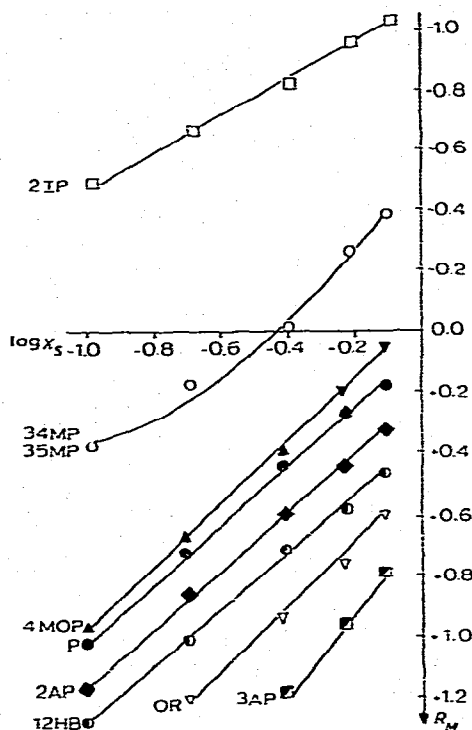
Fig. 5. R_M versus log X, relationships for phenols for polyamide systems. Polar solvent (S): diiso-amyl ether. Compare Fig. 10.
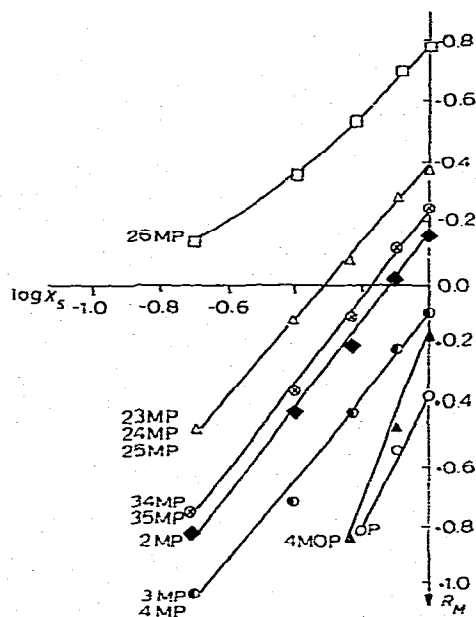
Fig. 6. R_M versus log X, relationships for phenols for formamide systems. Polar solvent (S): chloro-form.

systems: the R_F values increase in the order phenol < cresols < xylenols and within groups of isomeric phenols the isomers with a methyl group in the *ortho*-position have higher values. The slopes of most alkylphenols are close to unity, in accordance with those expected when 1:1 solvation complexes are assumed.

The R_M versus log X_s relationships for halogenophenols in polyamide and formamide systems are presented in Figs. 2 and 7. In both systems only the *ortho*-derivatives had moderate R_F values, their sequence in both instances being 2-chloro-phenol < 2-bromophenol < 2-iodophenol, in accordance with the results obtained by Bark and Graham[13] for polyamide-impregnated paper. The sorption thus decreases with increase in the steric shielding of the polar group by the halogen atom, which can form an internal hydrogen bond with the hydroxyl group. *Para*-isomers of the halogeno-phenols are sorbed much more strongly by both formamide and polyamide (cf., 4-chlorophenol and 4-chloro-3-methylphenol, the R_F values of which are similar to those of dihydroxybenzenes), which Bark and Graham[13] attributed to stronger acidic properties of halogenophenols and to possible interactions between the halogen atoms and the imino groups of the polymer. For formamide systems, the R_M versus log X_s relationships are mostly curvilinear and steeper at higher concentrations of chloro-form.

Fig. 7. R_M *versus* log X_s relationships for phenols for formamide systems. Polar solvent (S): chloroform.
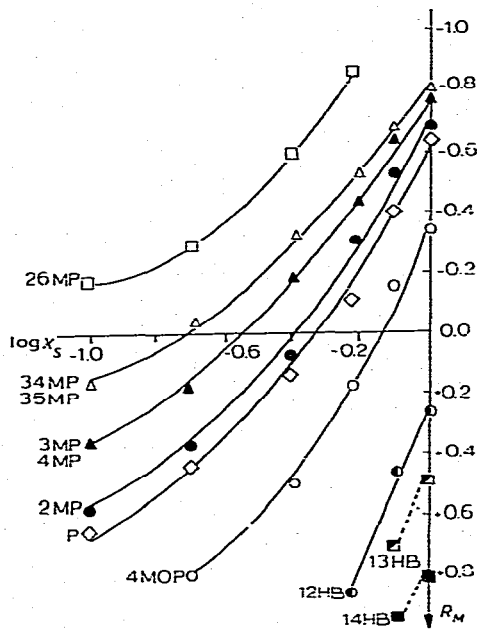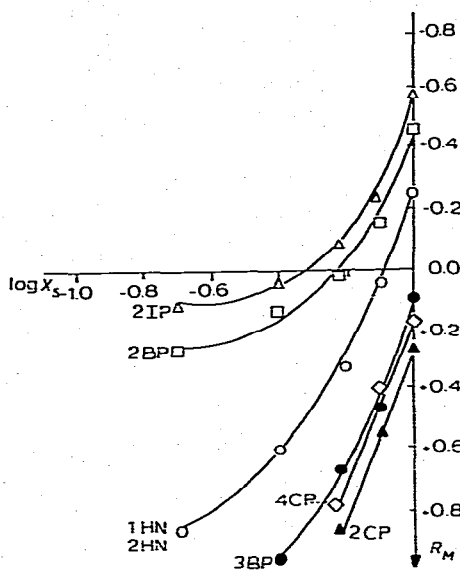
Fig. 8. R_M *versus* log X_s relationships for phenols for formamide systems. Polar solvent (S): diethyl ether.
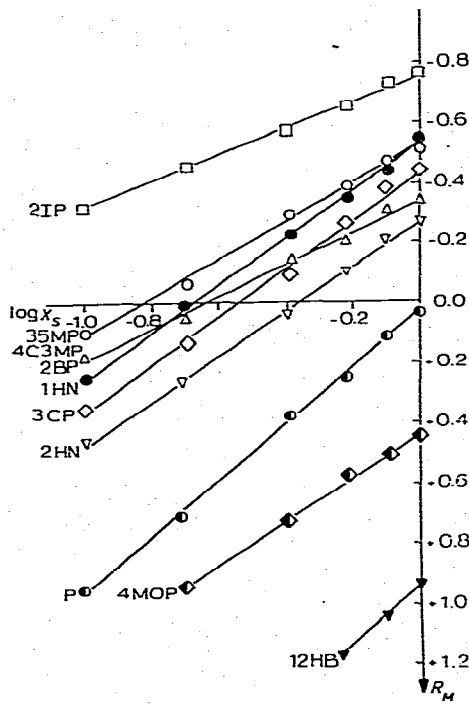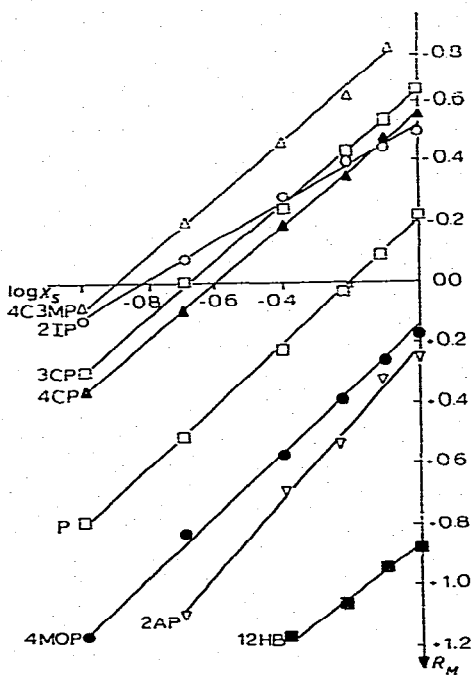

Fig. 9. R_M *versus* log X_s relationships for phenols for formamide systems. Polar solvent (S): di-*n*-butyl ether.

Fig. 10. R_M *versus* log X_s relationships for phenols for formamide systems. Polar solvent (S): di-isoamyl ether.

TABLE I

ABSOLUTE VALUES OF SLOPES OF R_M vs. LOG X_s RELATIONSHIPS FOR PHENOLS

Mobile phase: solvents of classes B and AB diluted with cyclohexane. Et = Ethyl; Bu = butyl; i-Am = isoamyl.

Solute	Abbreviation	Polyamide systems				Formamide systems			
		$CHCl_3$	Et_2O	Bu_2O	$i\text{-}Am_2O$	$CHCl_3$	Et_2O	Bu_2O	$i\text{-}Am_2O$
Phenol	P	5.0	1.2	1.0	0.8	2.0	...	1.0	0.9
2-Cresol	2MP	1.2	1.4	–	–	1.3	–	–	–
3-Cresol	3MP	2.6	1.4	...	–	1.3	–	–	–
4-Cresol	4MP	–	1.4	–	–	1.3	–	–	–
2,3-Xylenol	23MP	1.0	1.3	–		1.2	–		
2,4-Xylenol	24MP	1.0	1.3		...	1.2			
2,5-Xylenol	25MP	1.0	1.3		...	1.2			
2,6-Xylenol	26MP	–	1.3		–	0.9			–
3,4-Xylenol	34MP	1.1	0.9	–	0.6	1.3			0.8
3,5-Xylenol	35MP	1.1	0.9		0.6	1.3			0.6
Pyrocatechol	12HB		0.9	0.8	0.9		2.4	0.9	1.1
Resorcinol	13HB		1.6	1.1			2.3	–	
Hydroquinone	14HB				–	–	2.0		
4-Methoxyphenol	4MOP	1.1	1.0	1.2	1.1	3.0		1.0	0.8
Orcinol (3,5-di-hydroxytoluene)	OR		1.0	1.2	1.0				
4-Nitrophenol	4NP		–	0.7	1.0				
2-Aminophenol	2AP		1.0	0.7	1.0				–
3-Aminophenol	3AP		1.0	0.7	1.7				–
1-Naphthol	1HN	1.2	0.6				0.9		0.8
2-Naphthol	2HN	1.2	0.7				1.1		0.8
2-Chlorophenol	2CP	1.0				2.6			–
3-Chlorophenol	3CP		1.2	1.2		2.6		0.9	0.8
4-Chlorophenol	4CP	0.7						0.9	
2,6-Dichlorophenol	26CP	1.0							
4-Chloro-m-cresol	4C3MP	1.3		1.8				1.0	0.7
2-Bromophenol	2BP	0.9	0.9	...					
4-Bromophenol	4BP		–				2.2		0.7
2-Iodophenol	2IP	0.9		0.6	0.6			0.7	0.4

In further experiments. the chromatograms were developed with mixtures of cyclohexane and diethyl ether (Fig. 3). The results obtained for alkylphenols and some dihydroxy compounds are similar to those obtained for acetone systems[3]. especially the sequence and slopes of the R_M versus log X_s lines. the R_F values generally being lower.

The use of higher ethers (di-n-butyl ether and diisoamyl ether) as developing solvents resulted, for polyamide systems, in tailing of the spots for many of the phenols so that the diagrams (Figs. 4 and 5) represent only those solutes which gave satisfactory spots. The diffuseness of spots for less polar solvents is probably caused by a decreased diffusion rate due to the weaker swelling of polyamide. On the other hand. for formamide systems. well defined spots and linear R_M versus log X_s relationships were obtained for most solutes (Figs. 9 and 10); only for diethyl ether (Fig. 8) do the lines of less polar phenols have the shape of a hockey stick. which is characteristic for cases when the solutes are extracted to a marked extent by the diluting solvent[1,3,12].

The results demonstrated that for solvent systems of moderate polarity. form-

TABLE II

$R_f \times 100$ VALUES FOR VARIOUS MOLE FRACTIONS (x) OF THE ACTIVE SOLVENT FOR POLYAMIDE SYSTEMS

Solute	Chloroform					Diethyl ether					Di-n-butyl ether					Diisoamyl ether				
	0.2	0.4	0.6	0.8	1.0	0.1	0.2	0.4	0.6	0.8	0.1	0.2	0.4	0.6	0.8	0.1	0.2	0.4	0.6	0.8
Phenol			8	20	31	13	27	45	58	67	10	20	33	41	47	9	16	26	35	40
o-Cresol	6	19	19	26	30	15	34	60	73	73										
m-Cresol		5	15	23	28	15	34	60	73	73										
p-Cresol						12	24	56	68	71	Tailing					Tailing				
2,3-Xylenol	10	18	26	33	37	20	42	63	75	75										
2,4-Xylenol	10	18	26	33	37	20	42	63	75	75										
2,5-Xylenol	10	18	26	33	37	20	42	63	75	75										
2,6-Xylenol	45	56	61	70	80	20	42	63	75	75										
3,4-Xylenol	8	16	22	27	32	40	53	68	77	77						30	40	50	65	71
3,5-Xylenol	8	16	22	27	32	40	53	68	77	77						30	40	50	65	71
Pyrocatechol						6	10	16	21	28	7	12	19	23	28	5	9	16	21	25
Resorcinol								5	10	15			5	7	10		2	4	6	9
Hydroquinone								6	12	20									2	4
p-Methoxyphenol	2	6	10	15	20	11	21	35	49	59	9	16	28	36	39	10	18	29	39	46
Orcinol (3,5-di-hydroxytoluene)																				
p-Nitrophenol						2	6	12	17	22		10	10	14	19		6	10	15	20
o-Aminophenol						3	8	12	17	25	8	12	5	7	10			6	8	12
m-Aminophenol							2	6	12	19		6	17	21	25	6	12	20	26	33
1-Naphthol	4	7	10	13	18	25	35	45	52	56			9	12	14			6	10	14
2-Naphthol	3	6	8	11	15	15	23	32	42	48	Tailing					Tailing				
o-Chlorophenol	10	18	23	28	32	10	22	40	50	62	20	32	57	70	79					
m-Chlorophenol																				
p-Chlorophenol		6	8	9	10															
2,6-Dichlorophenol	20	30	40	55	68						17	37	59	74	78					
p-Chloro-m-cresol		7	9	12	14	15	17	40	48	55										
o-Bromophenol	12	20	24	30	35															
o-Iodophenol	31	45	53	60	65						75	80	86	90	93	76	82	87	90	93

TABLE III

$R_f \times 100$ VALUES FOR VARIOUS MOLE FRACTIONS (X) OF THE ACTIVE SOLVENT FOR FORMAMIDE SYSTEMS

Solute	Chloroform					Diethyl ether						Di-n-butyl ether						Diisoamyl ether					
	0.2	0.4	0.6	0.8	1.0	0.1	0.2	0.4	0.6	0.8	1.0	0.1	0.2	0.4	0.6	0.8	1.0	0.1	0.2	0.4	0.6	0.8	1.0
Phenol	–	–	14	21	31	18	26	42	55	71	81	14	23	32	46	54	62	10	16	30	36	43	49
o-Cresol	13	27	39	51	58	22	31	44	66	73	82												
m-Cresol	9	16	28	37	45	31	40	60	76	82	85												
p-Cresol	9	16	28	37	45	31	40	60	76	82	85												
2,3-Xylenol	25	44	54	65	70	53	62	73	84	89	90												
2,4-Xylenol	25	44	54	65	70	53	62	73	84	89	90												
2,5-Xylenol	25	44	54	65	70	53	62	73	84	89	90												
2,6-Xylenol	57	70	78	83	86	58	65	80	88	90													
3,4-Xylenol	15	31	44	55	63	40	52	68	73	83	86							45	54	65	71	75	77
3,5-Xylenol	15	31	44	55	63	40	52	68	73	83	86							45	54	65	71	75	77
Pyrocatechol									12	26	35		2	6	8		11				6	8	10
Resorcinol										17	24												
Hydroquinone										10	13												
p-Methoxyphenol			13	25	40		14	24	40	58	68	6	13	21	29	35	40		10	16	21	24	26
Orcinol (3,5-di-hydroxytoluene)									5	20	40												
p-Nitrophenol						23	39	55	73	80	80			17	22	32	36	5	7	12	18	23	26
o-Aminophenol										88	84							36	50	62	68	73	77
1-Naphthol	12	20	32	47	63	41	59	72	83	88	88	34	50	62	73	77	82	26	36	49	56	61	64
2-Naphthol	12	20	32	47	63	25	41	59	76	82	84	30	44	60	70	75	78						
o-Chlorophenol	–	–	12	22	34																		
m-Chlorophenol			15	29	40	56	70	82	90			45	61	74	81	86		31	41	55	64	70	73
p-Chlorophenol			15	29	40																		
2,6-Dichlorophenol	43	54	62	74	79																		
p-Chloro-m-cresol	10	16	24	41	60	44	60	74	82	86	86	45	61	74	81	86		45	54	65	71	75	77
o-Bromophenol	35	42	49	58	74	–	–	–	–	–	–							40	48	56	60	66	68
p-Bromophenol	–	10	13	25	45																		
p-Iodophenol	44	47	55	63	79							42	54	65	71	73	76	66	73	78	82	84	85

amide and polyamide have analogous sorption properties and that in both instances solvation effects in the mobile phase are the governing process, as in other liquid–liquid partition systems[1]. Differences between polyamide and formamide systems are mostly due to interactions between the two phases. In formamide systems, the two liquid phases are mutually saturated, but it is probably the solubility of formamide in the less polar mobile phase that plays the more decisive role, especially at high concentrations of the polar component in the mobile phase, which is then, in fact, a ternary solvent. It should be pointed out that the mole fraction of formamide, owing to its low molar volume, is significantly greater than its solubility expressed as a volume percentage. On the other hand, polyamide is insoluble in the mobile phase: however, the solubility of the solvents in polyamide is an important factor, and the properties of the gel are a complex function of the qualitative and quantitative composition of the solvent. Furthermore, the gel cannot be considered to be homogeneous. For less polar solvents, adsorption of solutes on the interface between the two phases should be taken into account.

The results indicate that there is only a limited range of mixed solvents that can be used for both formamide and polyamide systems, which are otherwise complementary as far as the polarity of the developing solvents is concerned. Both systems have much in common: however, there are also important differences that can introduce dissimilarities in the behaviour of solutes chromatographed in polyamide and formamide systems.

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