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CHROMATOGRAPHIC ANALYSIS OF ORGANIC COMPOUNDS ON IMPREGNATED CHEMICALLY BONDED STATIONARY PHASES. PART 1

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SUMMARY

Non-polar (RP-2, RP-8, and RP-18) and polar (NH_2, CN, and diol) chemically bonded stationary phases used in TLC have been impregnated with solutions of organic substances at different concentrations and the effect of impregnation on the mechanism of retention of alcohols, higher fatty acids, amino acids, and medicines has been investigated.

INTRODUCTION

Chemically bonded stationary phases are widely used to separate many groups of organic compounds. In recent years they have become very popular because of their stability, which leads to better selectivity and repeatability of analytical results. Despite these advantages, these phases are still subject to further improvement, with the objective of optimization of separation conditions, increasing retention and selectivity, obtaining compact spots, improving detection of the substances chromatographed, and reducing analysis time. Impregnation is one of the methods most commonly used for additional modification of chemically bonded stationary phases. It involves coating the stationary phase with a non-volatile agent, usually as a solution in a volatile solvent which is later evaporated. The impregnating substance, or mixture, remains adsorbed on this phase as a result of physical adsorption [1,2].

Impregnated chemically bonded stationary phases can be used to separate very diverse compounds which have often been separated on the unmodified phase. If, however, the impregnating agent is appropriately chosen, more compact spots can be achieved. Impregnation changes retention, expanding the separating power of a chromatographic system. It also enables the use of mobile phases of composition differing from those used

on the unmodified phase. As already mentioned, impregnation of chemically bonded stationary phases been investigated only recently and the literature on the subject is scarce. Most of the available literature describes impregnation with non-polar liquids, amino acids, and detergents [4–13]. The topicality and importance of impregnation of chemically bonded stationary phases has inspired our studies in this field; the results are presented in this paper.

Analysis was performed on non-polar (RP-2, RP-8, RP-18) and polar (NH_2, CN, diol) chemically bonded stationary phases. The studies were performed in two parts. We first focused on developing methods of impregnation with solutions of organic compounds of different concentration. The non-polar phases were impregnated with solutions of squalane and squalene, and RP-8 and RP-18 were also impregnated with sodium dodecylsulphate (SDS) and the amino acids L-lysine and L-arginine. The polar phases were impregnated with solutions of the chiral compounds L-(+)-tartaric acid and D-(+)-galactose. In the second part of our studies we determined the effect of the impregnating agent on the mechanism of retention of the substances analysed by measurement of the retention of homologous groups of compounds chromatographed on both the modified and unmodified stationary phases.

EXPERIMENTAL

Analytes and Analyte Solutions

Details of the compounds chromatographed and the solutions prepared are listed in Table I. Ten microlitres of the solutions were applied to both unmodified and impregnated stationary phases.

The Stationary Phases

Six non-polar (RP-2, RP-8, RP-18) and polar (NH_2, CN, diol) chemically bonded stationary phases routinely used in thin-layer chromatography (TLC) were studied. The characteristics are briefly summarized in Table II.

Impregnants, and Impregnation of the Stationary Phases

Glass plates coated with the stationary phases listed in Table II were cut into 10 cm × 10 cm pieces which were dried at 100°C for 10 min, and carefully weighed. The plates were then immersed for 15 min in 0.5%, 1%, and 5% solutions of the impregnating agents listed in Table III.

Table I

The analytes, and the solutions prepared

Analyte	Analyte solvent	Concentration
Lauric acid	Ethanol	0.5% (*w*/*v*)
Myristic acid	Ethanol	0.5% (*w*/*v*)
Palmitic acid	Ethanol	0.5% (*w*/*v*)
Stearic acid	Ethanol	0.5% (*w*/*v*)
Arachidic acid	Ethanol	0.5% (*w*/*v*)
Lauryl alcohol	Chloroform	0.5% (*w*/*v*)
Myristyl alcohol	Chloroform	0.5% (*w*/*v*)
Palmityl alcohol	Chloroform	0.5% (*w*/*v*)
Stearyl alcohol	Chloroform	0.5% (*w*/*v*)
Arachidyl alcohol	Chloroform	0.5% (*w*/*v*)
Atenolol	Ethanol	0.01 M
Propranolol	Ethanol	0.01 M
Metaprolol	Chloroform	0.01 M
L-Arginine	Ethanol–water, 7:3 (*v*/*v*)	0.5% (*w*/*v*)
L-Lysine	Ethanol–water, 7:3 (*v*/*v*)	0.5% (*w*/*v*)
L-Threonine	Ethanol–water, 7:3 (*v*/*v*)	0.5% (*w*/*v*)
L-Methionine	Ethanol–water, 7:3 (*v*/*v*)	0.5% (*w*/*v*)
L-Serine	Methanol–water, 6:4 (*v*/*v*)	0.5% (*w*/*v*)
2-L-Phenylalanine	Methanol–water, 6:4 (*v*/*v*)	0.5% (*w*/*v*)

Chromatography

Chromatography was performed in classic Stahl-type chromatographic chambers (Camag, Muttenz, Switzerland) containing 10 mL mobile phase. The mobile phases used are listed in Table IV. Chromatograms were developed to a distance of 8 cm then dried at ambient temperature for 24 h. After drying detection was performed with a 0.2% solution of ninhydrin in acetone or, for higher fatty acids and alcohols, in iodine vapour. R_F values were then measured.

RESULTS AND DISCUSSION

In the first part of these studies the impregnation coefficient, *i*, was calculated by use of the equation:

$$i = (b - a)/a$$

where *a* is the mass of the plate coated with unmodified stationary phase

Table II

Characteristics of the phases investigated [14,15]

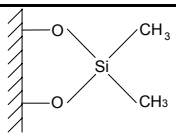
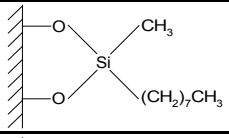
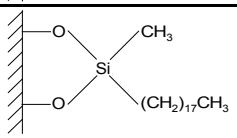
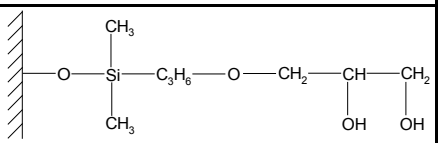
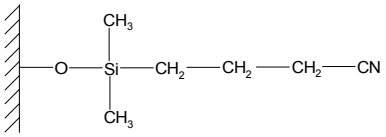
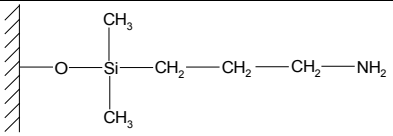
No.	Stationary phase	Chemically bonded ligand	Mode	Producer	Cat. #
1	Methyl F_{254S}		TLC	Merck	1.05747
2	Octyl F_{254S}		TLC	Merck	1.15388
3	Octadecyl F_{254S}		TLC	Merck	1.15389
4	Diol F_{254S}		HPTLC	Merck	1.12668
5	3-Cyanopropyl F_{254S}		HPTLC	Merck	1.12571
6	3-Aminopropyl		HPTLC	Merck	1.12572

Table III

The impregnants, and the solutions prepared

Impregnant	Solvent
Squalane	*n*-Hexane
Squalene	Acetone
L-Lysine	Methanol–water, 6:4 (*v*/*v*)
L-Arginine	Methanol–water, 6:4 (*v*/*v*)
Sodium dodecylsulphate (SDS)	Methanol–water, 6:4 (*v*/*v*)
L-(+)-Tartaric acid	Ethanol
D-(+)-Galactose	Water–ethanol, 8:2 (*v*/*v*)

Table IV

The mobile phases used

Components	Quantitative proportions (*v*/*v*)	Analytes	Stationary phase
Methanol–water	9.25:0.75	Higher fatty acids and alcohols	RP-8, RP-18
Methanol–water	8:2	Higher fatty alcohols	RP-2
Methanol–water	9:1	Higher fatty acids	RP-2
Acetonitrile–methanol	9:1	Atenolol, propanolol	RP-8, RP-18
Acetonitrile–methanol	6:4	Metaprolol	RP-8, RP-18
Methanol–water	5:5	Amino acids	RP-8, RP-18
Acetone–methanol–water–buffer, pH 9	2:2:4:2	Amino acids	NH_2, CN, diol

and *b* the mass of the same plate coated with impregnated stationary phase. In the second step of our studies we examined the effect of impregnation on the separation of organic compounds. The results obtained are listed in Tables V–XI and Figs 1–4.

The regular dependence of the impregnation coefficient, *i*, on the concentration of the impregnating agent solution, and the effect on *i* of both the type of chemically bonded ligand and the impregnating agent used are apparent from the results. The results also indicate that impregnation affects retention, as measured by the retardation R_F.

The results in Table V show that for the same concentration of squalane in *n*-hexane *i* increases in the order RP-8 < RP-2 < RP-18 whereas if squalene is used as impregnating agent the order is RP-2 < RP-8 < RP-18. Comparison of the results obtained after impregnation with squalane and squalene shows that *i* is larger for squalene, probably because the double

Table V

Impregnation factors obtained after impregnation of RP-2, RP-8, and RP-18 plates with 0.5, 1, or 5% solutions of squalane in *n*-hexane or squalene in acetone

Concentration of impregnating agent (%)	Impregnation factor					
	Squalane			Squalene		
	RP-2	RP-8	RP-18	RP-2	RP-8	RP-18
0.5	1.61×10^{-4}	1.50×10^{-4}	1.98×10^{-4}	3.69×10^{-4}	5.88×10^{-4}	5.81×10^{-4}
1	4.73×10^{-4}	3.57×10^{-4}	5.75×10^{-4}	5.13×10^{-4}	5.21×10^{-4}	7.79×10^{-4}
5	8.98×10^{-4}	1.91×10^{-3}	1.54×10^{-3}	9.26×10^{-4}	1.02×10^{-3}	2.67×10^{-3}

bonds of squalene interact more readily with the silanol groups on the surface of the silica matrix**.**

It is apparent from analysis of the results presented in Table VI and Figs 1–3 that for use of amino acids as impregnating agents values of *i* are larger for RP-8 than for RP-18. The opposite is observed for impregnation with sodium dodecylsulphate. Impregnation with SDS results in the highest *i* values on RP-18, probably because the amino acids penetrate the chemically bonded C_8 and C_{18} ligands less effectively than sodium dodecylsulphate, because of the structural similarity of SDS and the octadecyl ligands. SDS has a long carbon chain with no attached side groups which could impede penetration among the octyl and octadecyl chains, the so-called 'brushes' of the stationary phase.

Table VI

Impregnation factors obtained after impregnation of RP-8 and RP-18 plates with 0.5, 1, or 5% solutions of L-lysine, L-arginine, or sodium dodecylsulphate (SDS) in methanol–water, 60:40 (*v*/*v*)

Concentration of impregnating agent (%)	Impregnation factor					
	L-Lysine		L-Arginine		SDS	
	RP-8	RP-18	RP-8	RP-18	RP-8	RP-18
0.5	4.73×10^{-4}	7.18×10^{-5}	8.60×10^{-4}	9.76×10^{-4}	5.32×10^{-4}	1.04×10^{-3}
1	8.52×10^{-4}	6.75×10^{-4}	2.45×10^{-3}	1.59×10^{-3}	1.46×10^{-3}	1.77×10^{-3}
5	3.21×10^{-3}	2.63×10^{-4}	5.29×10^{-3}	5.14×10^{-3}	5.13×10^{-3}	5.40×10^{-3}

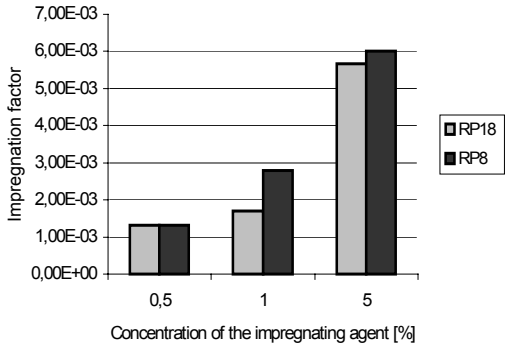


Fig. 1

Comparison of impregnation factors after impregnation of RP-8 and RP-18 with L-arginine solutions of different concentration.

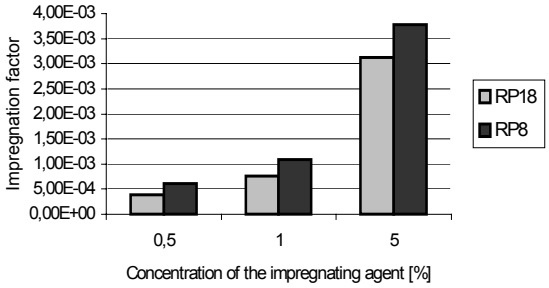


Fig. 2

Comparison of impregnation factors after impregnation of RP-8 and RP-18 with L-lysine solutions of different concentration

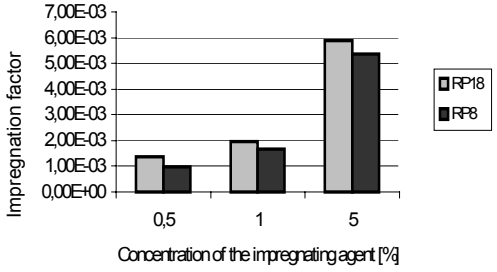


Fig. 3

Comparison of impregnation factors after impregnation of RP-8 and RP-18 with sodium dodecylsulphate solutions of different concentration

Careful analysis of the data presented in Table VII reveals that for NH_2, CN, and diol phases values of the impregnation coefficients for the same concentrations of impregnating solution are different. After impregnation with L-(+)-tartaric acid the order of i is CN < diol < NH_2 and after impregnation with D-(+)-galactose the order is NH_2 < CN < diol. For all these polar phases the largest value of i was observed after impregnation with L-(+)-tartaric acid. It should, however, be stressed that molecular volumes of the impregnating agents are very different and this will undoubtedly affect the results.

The results listed in Table VIII indicate that impregnation of RP-2, RP-8, and RP-18 affects retention of the higher fatty acids and alcohols. The retardation, R_F, usually decreases with increasing concentration of the

Table VII

Impregnation factors obtained after impregnation of NH_2, CN, and diol plates with 0.5, 1, or 5% solutions of L-(+)-tartaric acid in ethyl alcohol or D-(+)-galactose in 4:1 (*v*/*v*) water–alcohol

Concentration of impregnating agent (%)	Impregnation factor					
	L-(+)-Tartaric acid			D-(+)-Galactose		
	NH_2	CN	diol	NH_2	CN	diol
0.5	1.52×10^{-3}	3.56×10^{-4}	4.94×10^{-4}	2.96×10^{-4}	3.96×10^{-4}	3.74×10^{-4}
1	2.00×10^{-3}	3.76×10^{-4}	5.09×10^{-4}	3.95×10^{-4}	4.95×10^{-4}	4.42×10^{-4}
5	2.43×10^{-3}	5.56×10^{-4}	8.43×10^{-4}	5.03×10^{-4}	5.52×10^{-4}	5.34×10^{-4}

Table VIII

R_F, I_g, and ΔR_F values of higher fatty acids and alcohols after chromatography on unmodified RP-18 and on the same phase impregnated with 0.5, 1, and 5% solutions of squalane in *n*-hexane

Compound	R_F				I_g				ΔR_F			
	0%	0.5%	1%	5%	0%	0.5%	1%	5%	0%	0.5%	1%	5%
Lauric acid	0.66	0.65	0.65	0.54	0.55	0.46	0.50	0.69	0.08	0.07	0.09	0.12
Myristic acid	0.58	0.58	0.56	0.42	0.55	0.55	0.46	0.79	0.08	0.03	0.06	0.11
Palmitic acid	0.50	0.55	0.50	0.31	0.60	0.66	0.55	0.93	0.11	0.13	0.11	0.11
Stearic acid	0.39	0.42	0.39	0.19	0.66	0.60	0.50	1.17	0.09	0.14	0.11	0.07
Arachidic acid	0.30	0.28	0.28	0.10	0.75	0.55	0.50	1.92	0.11	0.12	0.11	0.13
Lauryl alcohol	0.62	0.62	0.61	0.45	0.50	0.46	0.43	0.86	0.12	0.13	0.11	0.11
Myristyl alcohol	0.51	0.50	0.50	0.33	0.60	0.50	0.46	0.90	0.07	0.05	0.09	0.16
Palmityl alcohol	0.39	0.37	0.39	0.23	0.66	0.66	0.50	0.93	0.06	0.06	0.05	0.03
Stearyl alcohol	0.32	0.32	0.30	0.13	0.66	0.66	0.60	1.49				
Arachidyl alcohol	0.26	0.26	0.25	0.05	0.70	0.66	0.60	1.48				

impregnant solution. The analogous effect was usually observed for the these analytes and the geometrical index I_g calculated by use of the equation:

$$I_g = k/d$$

where k is the length of the spot and d is its width. The results in Table VIII suggest that separation of the higher fatty acids and alcohols is possible on the impregnated phases and sometimes better than on the unmodified phases.

Typical results for medicines chromatographed on RP-8, both unmodified and impregnated with amino acids, given in Table IX, reveal that

separation of the β-blocking drugs is possible on phases impregnated with L-lysine but not on the unmodified phases. It should, however, be remarked that spots with diffuse tails were obtained on plates impregnated with these amino acids.

Table IX

R_F and I_g values determined for drugs on unmodified RP-8 and on the same phase impregnated with L-lysine or L-arginine

Drug	R_F				I_g			
	0%	0.5%	1%	5%	0%	0.5%	1%	5%
L-lysine								
Atenolol	0.01	0.27	0.25	0.27	1.50	2.63	0.47	3.23
Propranolol	0	0.51	0.47	0.59	–	4.29	3.04	2.64
Metaprolol	0	0.77	0.76	0.77	–	1.21	1.81	1.11
L-arginine								
Atenolol	0.51	0.62	0.66	0.86	1.52	0.64	1.69	2.86
Propranolol	0.55	0.63	0.70	0.82	0.48	0.37	1.18	2.57
Metaprolol	0.55	0.65	0.70	0.84	0.82	1.05	1.79	2.29

Results obtained for amino acids chromatographed on unmodified RP-8 and on RP-8 impregnated with sodium dodecylsulphate are listed in Table X. Careful examination of the results reveals that the higher the concentration of the impregnant the lower the value of R_F. Low values of the geometrical index and compact spots were, moreover, obtained for most of the amino acids.

Table X

R_F and I_g values obtained for amino acids on unmodified RP-8 and on the same phase impregnated with sodium dodecylsulphate (SDS)

Amino acid	R_F				I_g			
	0%	0.5%	1%	5%	0%	0.5%	1%	5%
L-Arginine	0.05	0.05	0.05	0.10	3.50	2.80	2.40	2.46
L-Lysine	–	0.14	0.12	0.11	–	2.11	1.94	5.13
L-Threonine	0.91	0.91	0.76	0.69	1.58	1.42	1.53	2.53
L-Methionine	0.74	0.65	0.63	0.60	1.77	1.33	1.48	2.05
L-Serine	0.91	0.81	0.78	0.66	1.00	1.26	1.52	1.25
2-L-Phenylalanine	0.62	0.54	0.51	0.48	1.04	1.07	1.04	1.66

The results obtained for amino acids on polar phases show that impregnation of these phases also affects retention. Comparison of the results in Table XI and Fig. 4 reveals that it is possible to obtain well-shaped and

Table XI

R_F and I_g values obtained for amino acids on unmodified NH_2 and on the same phase impregnated with 0.5%, 1%, or 5% L-(+)- tartaric acid in ethyl alcohol or D-(+)-galactose in ethanol–water

Drug	R_F				I_g			
	0%	0.5%	1%	5%	0%	0.5%	1%	5%
L-(+)- Tartaric acid								
L-Lysine	0	0.65	0.56	0.48	–	1.12	1.80	1.33
L-Threonine	0	0.72	0.67	0.58	–	1.42	1.14	1.12
L-Methionine	0	0.73	0.68	0.63	–	1.55	1.50	1.83
L-Serine	0	0.69	0.58	0.51	–	1.55	1.60	1.33
2-L-Phenylalanine	0	0.70	0.67	0.66	–	1.55	1.50	1.60
D-(+)-Galactose								
L-Lysine	0	0.82	0.75	0.71	–	1.66	1.57	1.66
L-Threonine	0	0.47	0.38	0.33	–	1.57	1.75	1.83
L-Methionine	0	0.66	0.60	0.59	–	1.43	1.83	1.75
L-Serine	0	0.62	0.52	0.45	–	1.42	1.66	2.00
2-L-Phenylalanine	0	0.69	0.64	0.54	–	1.83	1.14	1.83

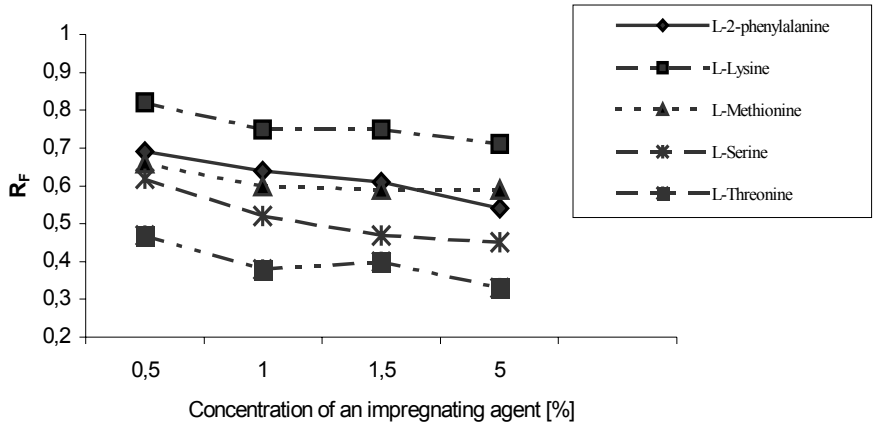


Fig. 4

Relationship between amino acid R_F values and the concentration of D-(+)-galactose in ethanol used to impregnate NH_2 plates

compact spots on the impregnated stationary phases and, even more important, to chromatograph the amino acids which do not migrate on the unmodified phases.

In summary, particular attention should be paid to the advantages of impregnation. Application of this simple method of physical modification of chemically bonded stationary phases enables improvement of chromatographic separation. This may be very important in the analysis of many chemical substances, e.g. those of biological or pharmacological interest.

CONCLUSION

For all the stationary phases studied the impregnation coefficient, *i*, increases regularly with increasing concentration of impregnating agent solution. Its value depends on the structure of the ligand chemically bonded to the silica gel matrix and the type of agent used for impregnation. Impregnation of stationary phases affects R_F values of the test substances analysed. The values of R_F and I_g obtained for many of the test substances confirmed that chromatographic separations impossible on unmodified phases could often be achieved on the impregnated phases.

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