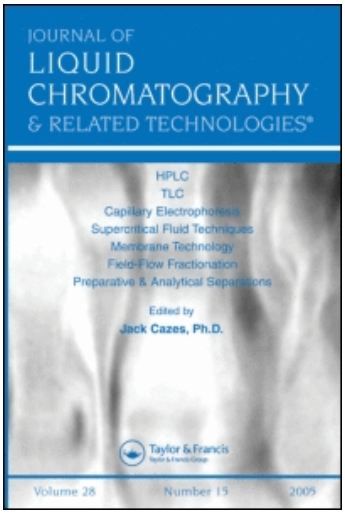

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Różyło, J. K. , Malinowska, I. and Gwis-chomicz, D.(1986) 'Chitin as Stationary Phase in Thin-Layer Chromatography-Application of Chromatographic Process Optimization Theory for Chitin Layers', Journal of Liquid Chromatography & Related Technologies, 9: 16, 3447 — 3459
To link to this Article: DOI: 10.1080/01483918608077795
URL: http://dx.doi.org/10.1080/01483918608077795

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(16), 3447-3459 (1986)

CHITIN AS STATIONARY PHASE IN THIN-LAYER CHROMATOGRAPHY—APPLICATION OF CHROMATOGRAPHIC PROCESS OPTIMIZATION THEORY FOR CHITIN LAYERS

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ABSTRACT

This paper describes initial of two basic theories of adsorption thin-layer chromatography potimization namely thermodynamic adsorption theory and theory based on mass action law, so called Snyder's theory in chromatographic systems containing chitin as stationary phase on wchich various amino acids were chromatographed. The results obtained were comparable to those obtained for analogous chromatographic systems containing silica gel as stationary phase. Comparison of applicability of these theories for well investigated silica gel and for not enough investigate chitin will permit to draw introductory conclusions about the processes carrying in the chromatographic systems containing chitin as stationary phase.

 0148-3919/86/0916-3447$3.50/0

INTRODUCTION

In connection with increasing interest about using of natural materials in various science branches a problem arises towards utilizing of some widespred in nature substances as stationary phases in chromatography. One among such substances is widespred in nature biopolymer. Its physical properties permit to its application as stationary phase in chromatography, and chemical properties i.e. structure similar to known and widely used in chromatography cellulose incline to undertaking of tests towards utilization of chitin as stationary phase in chromatography [1]. Because of established and potential possibilities of many compounds separation on chitin, a problem of a priori prediction of separation condition arises. Mixtures separation on chitin may be based on adsorption, ion exchange, ability of mobile and stationary phase to complex formation or combination of all these mechanisms. In this paper we would like to present application of chromatographic process optimization theory for amino acids chromatpgraphy in comparison to well investigated silica.

THEORY

In this paper were tested two theories of thin-layer chromatographic process optimization for the systems containing binary mobile phases.

A. Thermodynamic optimization theory

The basic eugation of this theory based on the classical thermodynamical approaches for the systems containing binary mobile phase has the following form [2,3]:

$$R_{M12} = x_1 \Delta R_{M12} + (x_1^s - x_1)(\Delta R_{M12} + A_z) + R_{M2} \qquad (1)$$

where:

R_{M12} - R_M value of given substance in binary mobile phase "1+2",

$\Delta R_{M12} = R_{M1} - R_{M2}$ - difference between R_M values for a given substance in pure solvents 1 and 2 (1 denotes more polar solvent),

x_1 - volume or molar fraction of more polar component of binary mobile phase,

$x_1^s - x_1$ - adsorption excess of component "1" of binary mobile phase, which can be determined from Everett eq. [4]

$$x_1^s - x_1 = \frac{x_1(K_{12}-1)(1-x_1)}{1+(K_{12}-1)x_1} \qquad (2)$$

where K_{12} is distribution function of components of the mobile phase. For ideal or conformal regular mixtures [5]

$$-\log K_{12} = \Delta R_{M12} \qquad (3)$$

A_z - parameter described intermolecular interactions between chromatographed substance and mobile phase components.

This parameter can be calculated by various methods. In one among these methods A_z values can be calculated directly from the chromatographic data. More detailled information about this method are given in our previous papers [6-9].

B. Snyder's theory

A second very popular optimization theory is the theory based on mass action law. Fundamental equation of this theory describing capacity coefficient values of the substance chromatographed in multicomponent mobile phase though capacity coefficients of this substance in monocomponent mobile phase has the following form [10,11]

$$k' = \sum_{i=1}^{n} \frac{x_i}{k_i'} \qquad (4)$$

where x_i is volume or molar fraction of i-th component in n-component mobile phase, and k_i' is k' value of the sub-

stance chromatographed in pure "i".solvent. For the systems containing binary mobile phase eq. 4 assumes following form:

$$k' = \frac{x_1}{k_1} + \frac{x_2}{k_2} \qquad (5)$$

Using relationship between R_M and log k' $R_M = \log k'$ [12], we can use equation 5 using k' values obtained directly from R_M measurements by TLC method. Equation 5 assumes then a following from:

$$10^{-R_{M12}} = x_1 \, 10^{-R_{M1}} + x_2 \, 10^{-R_{M2}} \qquad (6)$$

METHODS AND RESULTS

Our investigations were carried out by TLC method. Chitin used in the experiments was produced from krill in Department of Invertebrates technology Marine Fisching Institute Gdynia, Poland using acid-basic method. Glass plates were covered with chitin suspension and then dired in air. Comparative investigations were carried out on TLC plates produced by MERCK and covered with silica gel 60 H. Amino acids were used in the form of 1% solutions in water-methanol mixtures. As mobile phases were used binary mixtures of solvents.

Table 1.
Amino acids and binary mobile phases used in investigations.

amino acids	binary mobile phases
glicine valine leucine alanine phenylalanine serine threonine histidine	ethanol-methanol n-propanol-methanol acetone-methanol acetone-ethanol

RESULTS

As mentioned above the main purpose of this work is testing of popular chromatographic process optimization theories for chromatographic systems containing chitin as stationary phase. In order to this R_M values for investigated chromatographed substances were calculated with help of the equations 1 and 6. The results obtained were compared to those obtained for analogic chromatographic systems containing well investigated silica gel as stationary phase. This comparison has determination of tested theories applicability in view in relation to chitin and also on the basis of comparison between theoretical and experimental relations in the systems investigated obtaining introductory information about the processes carrying out during chromatograms development in the chromatographic systems containing chitin as stationary phase.

The results obtained are pr sented on four figures in the form relationship between experimental R_M values $/R_{Me}/$ and theoretical values $/R_{Mc}/$ calculated from equation 1 and 6 for all chromatographic systems investigated. From graphical relationships $R_{Me}=f(R_{Mc})$ it can be seen, that these relationaships are linear for both tested theories and for both compared stationary phases. In the case of ideal agreement between exoerimental and theoretical values relationship $R_{Me}= f(R_{Mc})$ are represented by straight line passing though origin and having a slope "a" equal to unity. Because of deviation from ideal relations a graphical documentation is completed by the table in which $R_{Me} = aR_{Mc} + b$ straight lines parameters are listed (Table 2)

For the mobile phase systems containing succeeding homologous alcohols serie units / ethanol-methanol and n-propanol-methanol/ a good agreement experimental and theoretical data is observed which appears in $R_{Me}=f(R_{Mc})$ dependences proceeding. These dependences are as mentioned already linear. Straight line parameters oscillate around unity for slope "a" value and arround zero for "b" para-

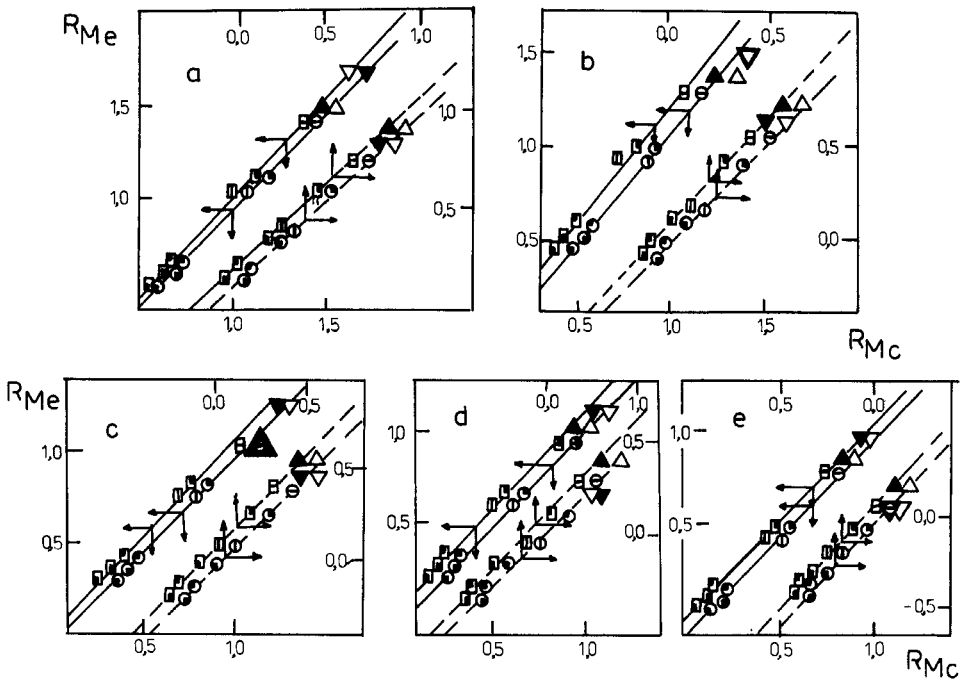

Fig. 1.

Relationschip between R_M values obtained by experimental data (R_{Me}) and obtained by theoretical way (R_{Mc}) on the basis equation 1 and 6 on SiO_2 (lines) and on Chitin layers (dashed lines).

chromatographed substances	eq.1	eq.6
glicine	◐	▣
alanine	◐	▣
waline	◕	▣
leucine	◕	▣
phenylalanine	◕	▣
threonine	◕	▣
seryne	△	▲
histidine	▽	▼

Mobile phase: ethanol-methanol. Volume fraction on more polar component of mobie phase: a. $x_1 = 0.1$, b. $x_1 = 0.3$, c. $x_1 = 0.5$, d. $x_1 = 0.7$, e. $x_1 = 0.9$.

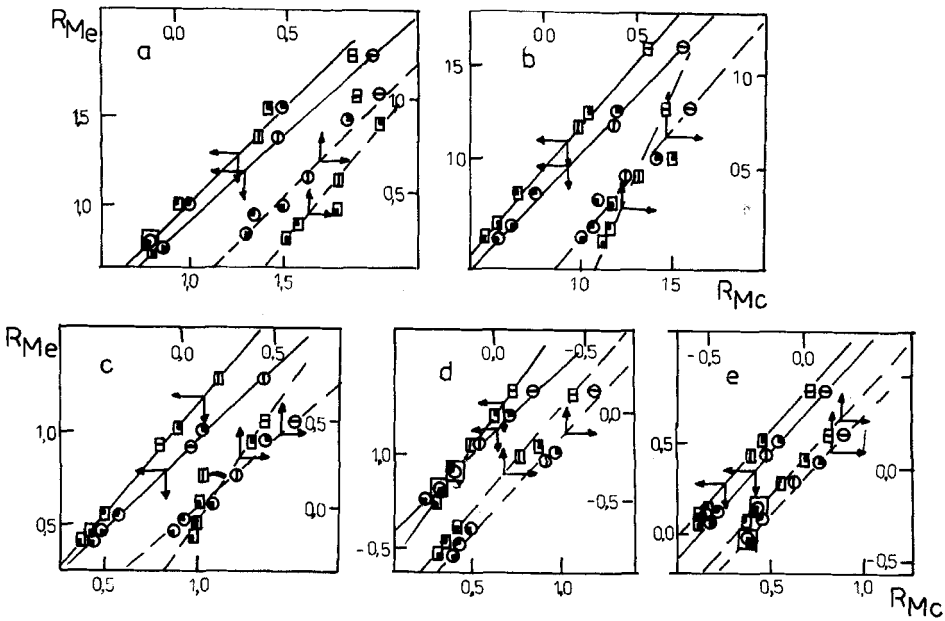

Fig. 2.
Relationschip $R_{Me} = f(R_{Mc})$. Mobile phase: n-propanol - - methanol. For symbols see fig. 1.

meter i.e. for parameter defining a straight line schift in respect to origin of co-ordinate axes. Both equations are most valid for ethanol-methanol mixture either on SiO_2 or on chitin (fig.1, table 2a) . This fact can explained by this that mobile phase consists of components having silimilar polarity, chemical structure and molecule size (succeeding units of homologous serie). For mixtures containing solvents of more differentiated polarity (e.g. - n-propanol -methanol) $R_{Me} = f(R_{Mc})$ dependences proceeding is also more differentiated in respect either to both optimization theories or to stationary phase used. From fig. 2 and from data given in table 2b results, that differences between values calculated from equation 1 and 6 are greater

Table 2.
Parameters a and b in given chromatographic systems.

x_1	Stationary phase							
	silica gel				chitin			
	eq.1				eq. 6			
	a	b	a	b	a	b	a	b
	mobile phase: ethanol-methanol							
0.1	1.00	0.02	1.00	0.00	1.00	0.00	1.00	-0.04
0.3	1.02	-0.15	0.82	0.38	1.00	0.00	1.00	-0.02
0.5	1.00	0.00	0.82	0.00	1.00	0.02	0.80	-0.06
0.7	1.00	0.00	1.00	-0.30	1.00	0.08	0.90	0.00
0.9	1.11	0.02	1.11	0.00	1.00	0.02	1.00	-0.02
	mobile phase: n-propanol-methanol							
0.1	0.90	0.10	0.88	0.16	1.00	0.00	1.40	0.30
0.3	1.00	0.00	1.14	0.00	1.30	0.80	2.14	0.26
0.5	1.00	0.00	1.25	0.00	1.00	0.02	1.53	0.09
0.7	1.12	0.02	1.14	0.01	1.01	0.02	1.23	-0.02
0.9	1.06	0.07	1.00	0.00	1.00	0.10	0.89	-0.06
	mobile phase: acetone-methanol							
0.1	0.92	0.20	1.00	0.14	1.17	-0.05	2.30	-1.16
0.3	1.00	0.00	1.50	0.26	1.27	-0.20	2.22	-0.51
0.5	1.00	0.00	1.00	0.20	1.27	0.08	2.50	0.00
0.7	1.00	-0.02	1.07	0.19	1.00	0.04	1.70	-0.11
0.9	1.20	0.08	1.20	0.02	2.50	0.02	1.90	-0.02
	mobile phase: acetone-ethanol							
0.1	0.95	0.08	1.15	-0.16	1.05	-0.03	1.70	-0.05
0.3	1.10	-0.13	1.10	-0.05	1.15	-0.01	1.15	0.45
0.5	1.10	0.01	1.20	0.06	1.15	0.21	1.20	-0.03
0.7	1.10	-0.10	1.10	0.04	1.00	-0.01	1.20	-0.02
0.9	1.05	-0.07	1.05	0.00	1.10	-0.04	1.30	-0.02

especially then chitin is used as stationary phase. Significant differences are observed also in case of acetone--alcohol mobile phase type. In spite that retionship $R_{Me} = f(R_{Mc})$ is as before linear significantly greater deviations of theoretical and experimental values and significant scetter of individual of individual points in relation to straight line is observed. Similarly as in the case of n-propanol-methanol mobile phase the differences are greater for the chromatographic sytems containing chitin as stationary phase. This is illustrated graphically on fig. 3 and 4 and also in table 2 (c and d). Optimization theory testing independently on comparison of experimental and theoretical values obtained on the basis of these theories by comparison of parameters typical for these theories may be helpful in definition of the phenomena taking plan during chromatographic process. Thermodynamic optimization theory include A_z parameter describing interactions between mobile phase components and chromatographed substances.

Because this parameter is however so far cloosing parameter determined with help retention data value dependents not only on mentioned above interactions taking place in mobile phase, but also on interactions between surface and other components of stationary phase. Validity of such argumentation may be confirmed by the fact, that A_z parameter values are different for the chromatographic systems containing different staticnary phases [13] . Table 3 lists A_z values for amino acids chromatographed on chitin and SiO_2 using mobile phase investigated.

From analysis of the data listed in table 3 some regularities common for SiO_2 and chitin can be observed. For the mobile phase containing components of similar structure ethanol--methanol, n-propanol-methanol A_z values are smaller than for the mobile phases containing components differing in molecular shape and chemical structure e.g. acetone - - ethanol, acetone - methanol . Thus smallest A_z values are observed for these systems which contain two succeding

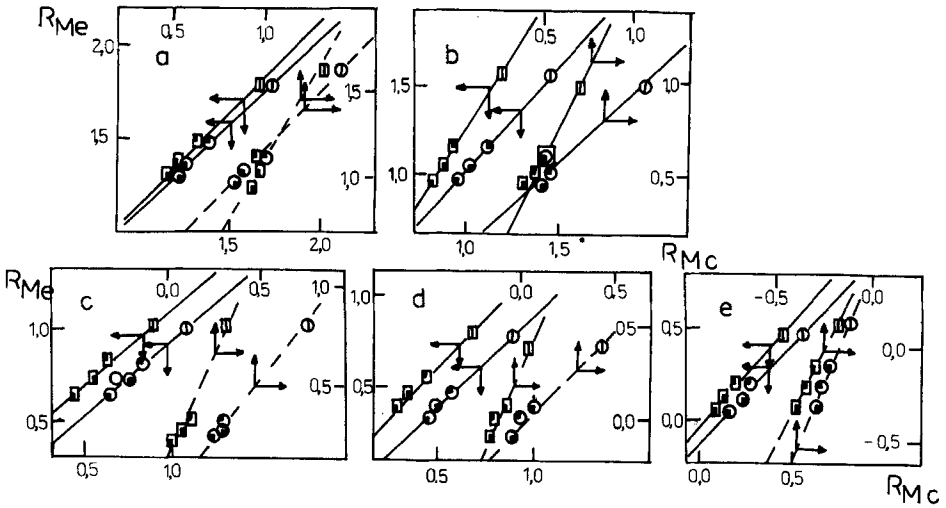

Fig. 3.
Relationschip $R_{Me} = f(R_{Mc})$. Mobile phase: acetone - - ethanol. For symbols see fig. 1.

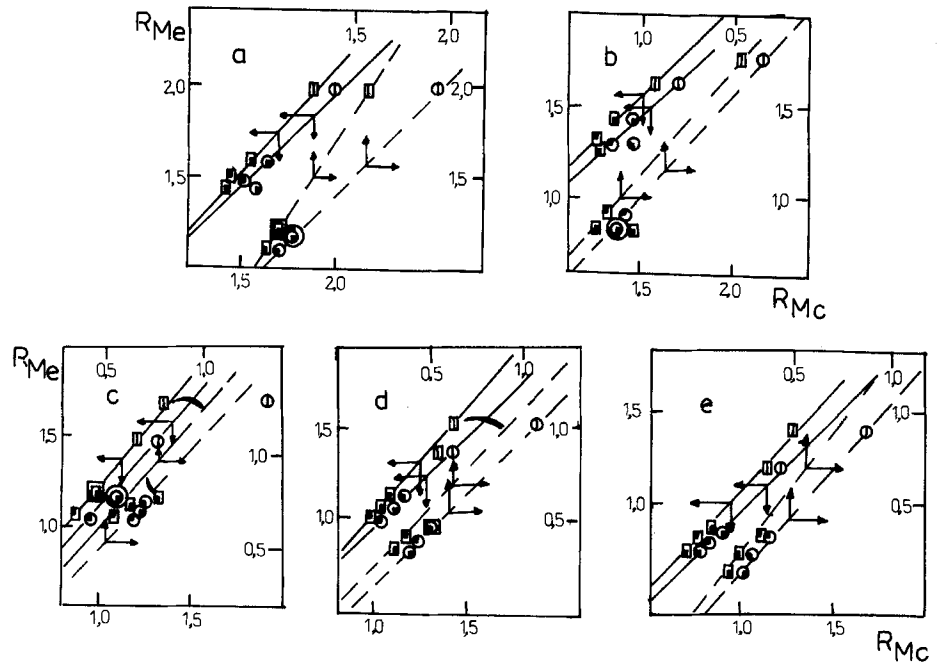

Fig. 4.
Relationship $R_{Me} = f(R_{Mc})$. Mobile phase: acetone - - methanol. For symbols see fig. 1.

homologous serie units ethanol-methanol . This is due by greater differences in mobile phase components molecules dimensions and weaker interactions between OH groups of n-propanol and chromatographic systems components and hence smaller elution power.

For the chromatographic systems containing mobile phase of acetone-alcohol type, A_z values increase with increas of elution power of more active mobile phase component. Thus A_z values for acetone-ethanol system are smaller than for acetone-methanol system. Analysis of the data presented above indicates A_z values increase with increase of elution power difference for individual mobile phase components independently on stationary phase type. Data listed in table 3 suggest on exsistence of correlation between A_z value and structure of amino acid chromatographed. A_z values decrease with increase of nonpolar hydrocarbon chain lenght in amino acid molecule. This is related to reduction of carboxyl group specific interactions by aliphatic chain. This problem is ilustrated for example of glycine, alanine, valine and leucine. Introduction of aromatic ring into amino acid molecule reduce A_z value (for example alanine and phenylalanine) and changes the relation between A_z values determined for SiO_2 and chitin. Effect of OH group on A_z values be observed e.g for alanine and serine. Serine containing OH graup in molecule (incontary to alanine) has A_z values significantly greater than alanine. Threonine has of turn smaller A_z values in comparison to its proceding homologue serine. For this example we can observe an effect of aliphatic chain lenght in amino acid molecule on A_z values with changes A_z values of chromatographed substance molecular structure for two stationary phase i.e. silica gel and chitin are analogical.

Differences between experimental and theoretical R_M values calculated from eq. 1 and 6 can be explained by nonideality of the chromatographic system investigated. Both theories examined assume the lack of specific intermolecular interactions in chromatographic systems and

Table 3.

A_z values for chromatographed substances in given chromatographic systems on SiO_2 and Chitin layers.

Substances	ethanol-methanol		n-propanol-methanol	
	SiO_2	chitin	SiO_2	chitin
glicine	0.830	0.848	1.071	1.334
alanine	0.907	0.723	0.963	1.240
valine	0.673	0.581	0.735	0.911
leucine	0.678	0.517	0.761	0.827
phenylalanine	0.381	0.432	0.848	0.726
threonine	0.845	0.725	0.976	1.121
seryne	0.869	0.864	-	-
histidine	0.728	0.874	-	-
	acetone-methanol		acetone - ethanol	
alanine	1.494	1.572	0.916	1.265
valine	1.410	1.399	0.890	1.081
leucine	1.320	1.377	0.750	1.054
phenalalanine	1.285	1.156	1.134	0.738

energetical homogeneity of the adsorbent surface, whereas in the system investigated by us because of type of the substances separated (amino acids) active solvents capable to hydrogen bond formision were used as mobile phases. From earlier investigations it is known, that SiO_2 has a heterogenous surface [14] and chitin because of presence on its surface of various functional graups represents also an energetically heterogenous stationary phase. In spite of these inaccuracies however both considerated theory can used succesively for preliminary determination of amino acids separation conditions on chitin layer.

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