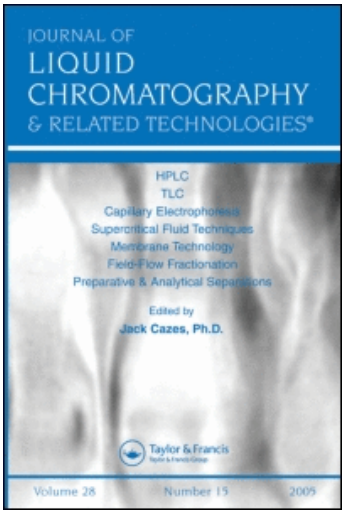

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Ion Exchange Application of Overpressured Thin-Layer Chromatography

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ION EXCHANGE APPLICATION OF OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

The application of overpressured thin-layer chromatography introduced into the field of ion exchange chromatography. The basic differences between overpressured thin-layer chromatography and classical thin-layer chromatography are discussed including the distinction between the separations performed on thin-layer plates containing silica gel and a mixture of ion exchanger material and silica gel. The basic increase of flow velocity of solvent front with the aid of a pressurized ultra-micro chamber and the effect of flow velocity on the height equivalent of the theoretical plates are also presented. For basic amino acids, the flow velocity vs plate height curves show optima at a moderately high rate of development.

INTRODUCTION

Ion exchange application of overpressured thin-layer chromatography (OPTLC) is an extension of the progress in some distinct fields of thin-layer chromatography (TLC).

Several authors have used TLC on sorbent layer consisting of ion exchanger cellulose. These studies were expanded by Berger and Blanquest (1) as well as Lepri and coworkers (2) whe used mixtures of cellulose and ion exchanger resin. Dévényi and his coworkers (3-9) reported the construction and application of mechanically stable sorbent layer consisting of strongly acidic cation exchanger. These ready-made plates are supplied by Chinoin-Nagytétény (Budapest, Hungary) as Fixion 50 X 8 and Macherey-Nagel (Duren, FRG) as Ionex 25. In his publications Dévényi discussed the wide application of Fixion 50 X 8 in the quantitative and qualitative determination of amino acids from protein and peptide hydrolysates, body fluids, etc. The separation takes only few hours, the samples from the hydrolysates can be directly applied to the plates even from 6 N hydrochloric acid solution. The method can be used as a micro determination in the sequence analysis using endopeptidase (8-9). Ion exchange TLC seems to be a suitable method both before and in conjunction with automatic amino acid analysis.

On the other hand, theoretical and practical development, using OPTLC showed, that the vapor phase over the sorbent layer may be eliminated (10) and the

flow velocity of solvent front increased (11-12). These results were accomplished using silica gel TLC and organic solvents. For practical reasons, a major part of the illustrative experiments was performed with dye-substances (11-13).

Kaiser determined the basis of high performance thin-layer chromatography (HPTLC) and stated its advantages (14); namely, the short time of development and excellent resolution. HPTLC however needs not only the special HPTLC-chamber but also extra instrumentation for application of the sample and detection of the separated substances. The primary limit of HPTLC is not the apparatus requirement but the very limited distance of development.

Guiochon (15-16) explained the factors which determine TLC and HPTLC and stated that the present conditions of HPTLC can not ensure the optimal flow velocity over a certain distance of development.

Tyihak and coworkers (11-13, 17) developed the apparatus and the methodology of OPTLC including the pressurized ultra-micro chamber (PUM-chamber) for both circular (13) and linear development (11-12, 17). These apparatus demonstrate the improvement of several hitherto unknown factors, these include:

a./ the role of vapor phase in TLC, the elimination of vapor phase over the sorbent layer and the beneficial effect of its elimination on the reproducibility and uniformity of TLC experiments (11-12),

b./ the possibility of constant velocity of solvent front during the development and over a short distance (11-12),

c./ the possibility of increasing the rate of development (11-13, 17), i.e. the significant decrease of time of development,

d./ the phenomenon, that the basic increase of flow velocity does not result in a decrease in the efficiency of TLC (17).

In this study the effect of vapor phase and solvent flow velocity on the characteristics of TLC and OPTLC using silica gel and Fixion 50 X 8 plates is compared.

APPARATUS

Set up for conventional TLC

The experiments were performed in Desaga Chambers. The solvent system used were added 1 hour prior to development in each case. Saturation was achieved by lining the developing tank with filter paper, while

the non-saturated chamber was unlined with filter paper. The covered plates (very similar to Tyihak's UM-chamber (10)) serve to show the effect of the elimination of vapor phase over the sorbent layer when covered with a glass plate.

Set up for OPTLC

The sorbent layer is sandwiched between a plastic sheet and a foil which is tightly pressed onto the thin-layer by a gas pressure up to 300 kPa (about 3 atm). This gas pressure exists in the space between the membrane ant the upper support block. The upper support block is also the holder of the pressure inlet, pressure gauge, inlet for developing solvent system and O-ring holding the membrane. The upper and lower support blocks are the frame of the whole set up and held together by several screw-like tools. The dimensions of the membrane are 200 x 200 mm; those of the support blocks are 230 x 230 mm.

EXPERIMENTS

Basic amino acids were separated on silica gel plates using propanol-ammonia, and chloroform-methanol-water as mobil phases. The R_f vs front distance data are given in Table 1. in the cases of:

Table 1.

The dependence of R_f values of basic amino acids from the running distance of front. Silica gel sorbent layer and n. propanol - ammonia (1:1) were used.

front distance (mm)	R_f x 100								
	non-saturated chamber			saturated chamber			PUM-chamber		
	Arg	His	Lys	Arg	His	Lys	Arg	His	Lys
160	37	62	75	34	59	72	35	60	74
145	34	56	69	32	53	72	34	55	71
130	30	46	62	27	43	59	30	46	62
115	26	36	55	23	33	52	26	36	55
100	21	31	48	17	28	41	20	31	48
85	19	28	47	15	24	41	19	27	47
70	17	24	46	14	20	41	17	23	41
55	13	18	40	11	16	38	13	18	40
40	13	17	38	10	15	35	13	17	38

saturated chamber, non-covered sorbent layer (S - NC)
non-saturated chamber, non-covered layer (NS - NC).

The front distance vs time diagrams show the situation in the cases of S-NC, NS-NC as well as S-C (saturated chamber, covered sorbent layer) and NS-C (non-saturated chamber, covered sorbent layer) developments with dichloro-methane and chlorform-methanol-water mobile phases. In addition to the chromatography in a conventional chamber (TLC), the experiments in the PUM-chamber are also shown using three different flow velocities. In a very similar arrangement, the same amino acids were also separated on Fixion 50 X 8 chromatoplates, using the saturated and non-saturated chambers as well as covered and non-covered sorbent; the front distance vs time diagrams are also presented (Figs. 1 and 2).

All plates were 200 mm x 200 mm in size.

TLC aluminium sheets silica gel 60 F_{254}, precoated were used (E. Merck, Darmstadt, FRG) and Fixion 50 X 8 chromatoplates (Chinoin-Nagyteteny, Budapest, Hungary). The solvent systems for development of silica gel plates were:

a./ n-propanol-25 % ammonia (1:1),

b./ dichloromethane,

c./ chloroform-methanol-water (7:5:1),
the solvents for the Fixion 50 X 8 plates were:
d./ sodium citrate aqueous solutioon, pH = 4.3,
containing 1.2 normal sodium ion,
e./ sodium citrate aqueous solution, pH = 3.26,
containing 0.2 normal sodium ion.

The R_f values of amino acids investigated in the a./. c./, d./ and e./ solvent systems are given in Tables 1, 2, 3 and 4.

DISCUSSION

Fig. 1 shows the distance reached by the solvent front in silica gel precoated sheets in the case of S-NC, S-C, NS-C and NS-NC systems, using the conventional TLC. Fig. 1 also presents the results of experiments in PUM-chamber using different flow velocities. The strikingly common characteristics of the conventional TLC runs are, the further the solvent front, the slower its movement. At the same time, when OPTLC was used, a plot of front vs time gave nearly straight lines. The experiments in saturated and non-saturated chambers but with non-covered plates differ from each other significantly, while the two curves taken dur-

ing TLC of S-C and NS-C are essentially the same (Fig. 1). These curves demonstrate the basic role of vapor phase in the developing characteristics of conventional TLC. Also the fundamental role of the vapor phase is indicated since significant deviations were found with respect to the R_f values of amino acids (Tables 1 and 2).

Fig. 2 shows the solvent front distance vs time diagrams for Fixion 50 X 8 chromatoplates. For Fixion, the presence or absence of vapor phase plays only a minor role as both the running characteristics and the R_f values are practically independent from the saturation of the chamber (Fig. 2, Tables 3 and 4).

Fig. 3 shows the developing process of thin-layer chromatography in conventional- and in PUM-chamber. The series demonstrate a complete development in PUM-chamber while in the case of conventional TLC the solvent front moved much slower. In both cases, Fixion 50 X 8 plates were used, and the developing solution was citrate buffer at pH = 4.3 (d./).

Fig. 4 shows the plate height vs flow velocity of solvent front when basic amino acids are separated in PUM-chamber, on Fixion 50 X 8 plates and developed

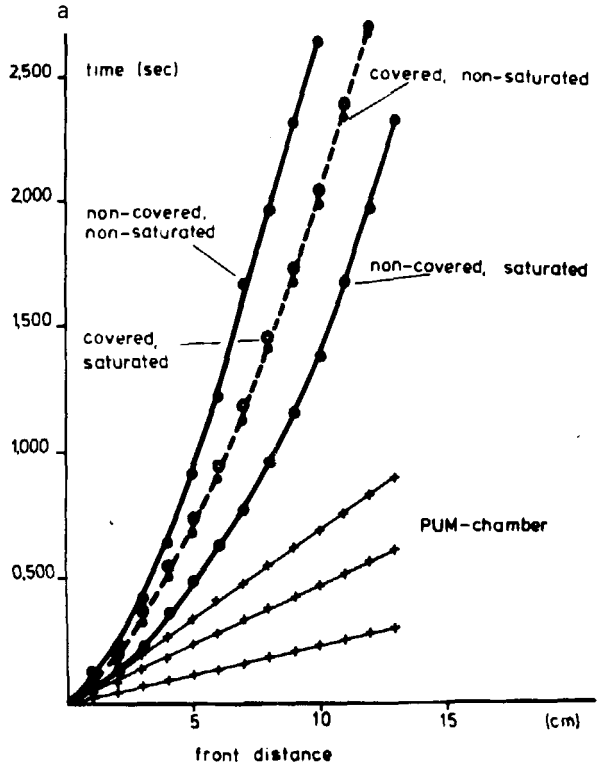

Figure 1. Diagrams are demonstrated when a volatile organic solvent (dichloromethane) was the running system (a.). The front distance vs. time of development data show difference in the case of NC-S and NC-NS chambers in solvent system chloroform-methanol-water (7:5:1) too, although the curves come close to each other (b.). Experiments were made on silica gel pre-coated layers.

in buffer d./. Each curve shows a definite maximum value, if the effect of flow velocity on the plate height is considered. Regarding the form of the curves, the ascending and the descending portions of the curves have to be considered separately. The descending part

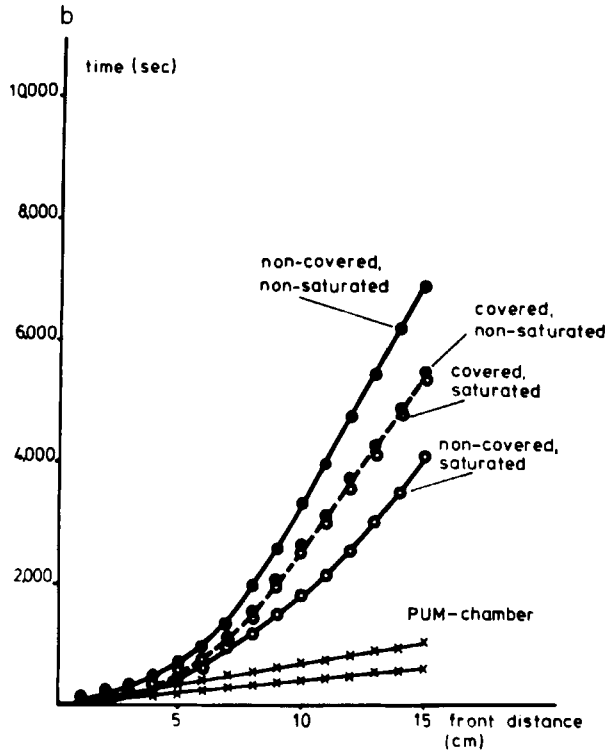

Figure 1B

shows the phenomenon, that the increase of the flow rate is possible up to a certain height of solvent front. This means an optimal flow rate of about 0.1 mm/sec in the case of the above mentioned chromatographic characteristics. At the same time, the quick ascent of the velocity vs plate height curves may be the consequence of a double effect, namely:

Table 2.

R_f values of amino acids. Running distance was 160 mm.

sorbent	silica gel					
solvent system	n. propanol-ammonia (1:1)			chloroform-methanol-water (7:5:1)		
-chamber	S-NC	NS-NC	PUM-	S-NC	NS-NC	PUM-
amino acid	R_f x 100					
Arg	34	37	35	00	00	00
Lys	59	62	60	02	06	04
His	72	75	74	08	12	10
Phe	82	88	86	36	47	40
Tyr	69	72	70	22	27	25
DOPA	64	70	68	12	16	15
Met	77	79	78	27	35	30
Val	66	69	67	22	29	27
Ala	65	67	66	11	15	13
Gly	66	66	66	05	08	05
Leu	73	80	74	33	41	35
Ile	71	76	75	31	39	34
Thr	58	64	60	07	14	08
Ser	50	62	57	05	08	06
Pro	53	63	59	15	20	17
HyPro	51	61	57	09	14	13
Trp	70	74	71	29	41	35
5-OH-Trp	71	72	71	17	22	20
Glu	54	64	60	04	09	06
Asp	49	62	55	03	06	04

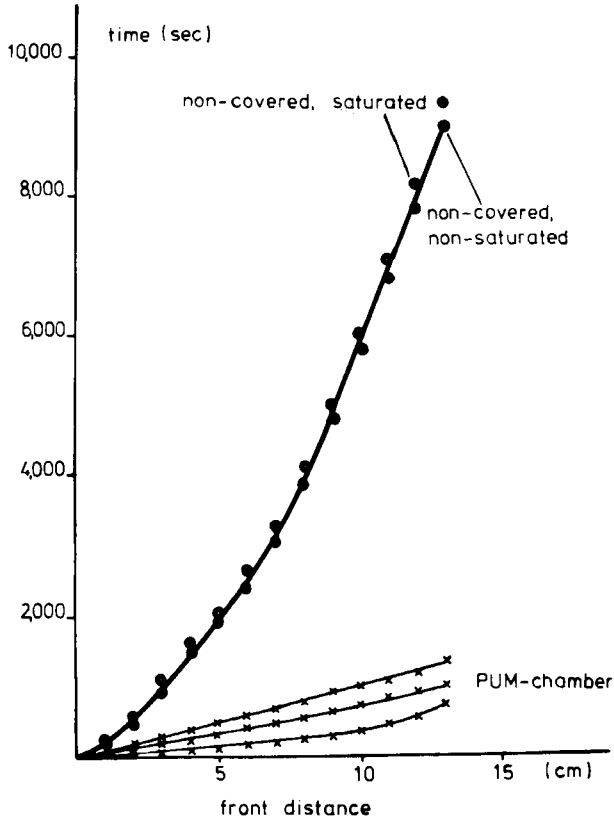

Figure 2. Flxion 50 X 8 chromatoplates were used in aqueous buffer and the front distance vs. time diagrams practically gave the same results in NC-S and NC-NS chambers.

1./ the increase of plate height is the result of the local nonequilibrium in consequence of the increase of flow velocity,

2./ a supplementary factor may be a slight overflow of the developing solvent, that is the pres-

Table 3.

The dependence of R_f of basic amino acids from the running distance of solvent front. Fixion 50 X 8 plates and citrate buffer at pH = 4.3 were used.

front distance (mm)	R_f x 100								
	non-saturated chamber			saturated chamber			PUM-chamber		
	Arg	His	Lys	Arg	His	Lys	Arg	His	Lys
160	16	25	41	16	23	40	16	25	41
145	16	25	41	16	23	40	16	25	41
130	16	25	41	16	23	40	16	25	41
115	16	25	41	16	23	40	16	25	41
100	16	25	41	16	22	40	16	25	41
85	16	25	41	16	23	40	16	25	41
70	16	26	42	16	23	41	16	25	42
55	18	27	43	17	24	41	17	26	42
40	18	27	43	17	25	41	18	26	42

Table 4.

R_f values of amino acids. Running distance was 160 mm.

sorbent	Fixion 50 X 8 chromatoplates			
running system	citrate buffer at pH = 3.26 (e./)		citrate buffer at pH = 4.3 (d./)	
-chamber	NS-NC	PUM-	NS-NC	PUM-
amino acids	R_f x 100			
Arg	01	01	16	16
Lys	05	05	41	41
His	03	03	25	25
Phe	11	11	35	35
Tyr	10	10	40	40
DOPA	16	16	52	52
Met	27	27	62	62
Val	40	40	70	71
Ala	46	45	76	80
Gly	50	50	74	77
Leu	17	17	58	59
Ile	23	23	61	61
Thr	65	66	83	83
Ser	61	61	80	80
Pro	44	45	64	64
Trp	03	03	14	14
5-OH-Trp	04	04	19	19
Asp	85	85	88	91
Glu	81	82	78	78

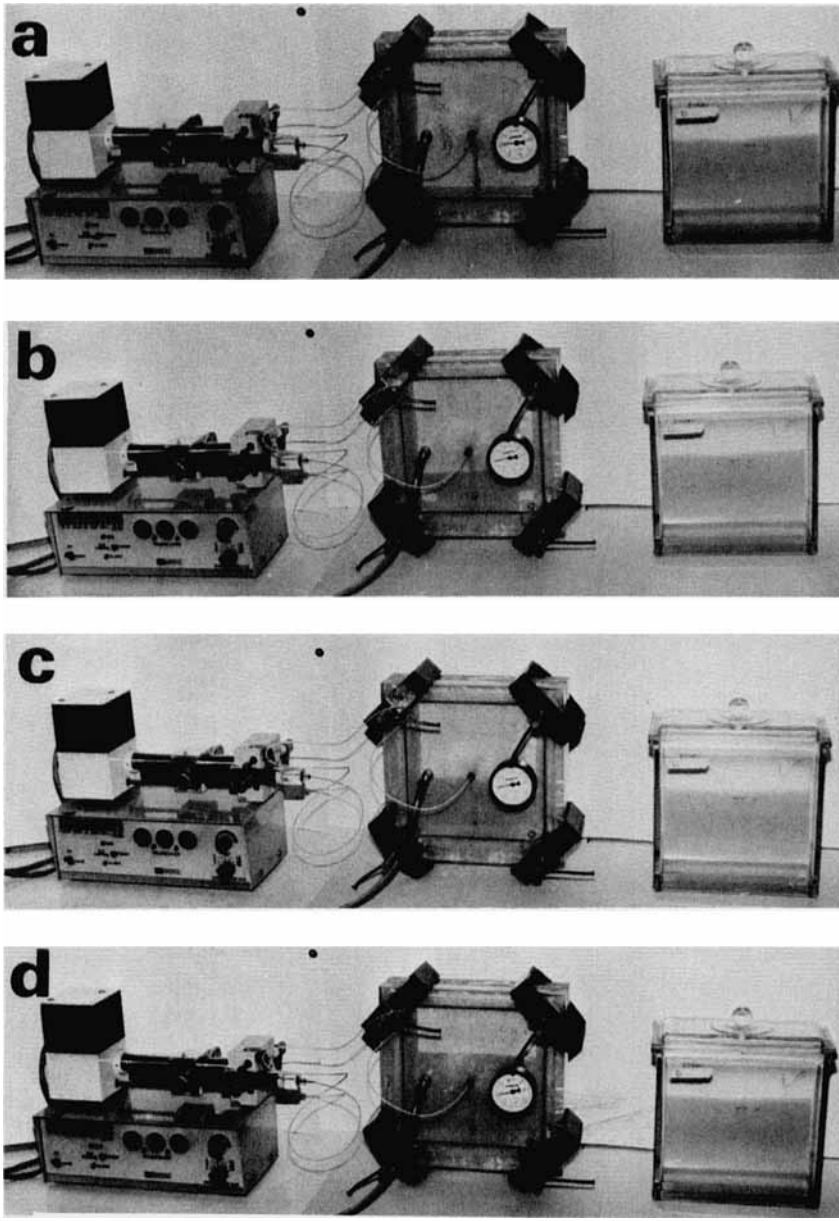

Figure 3(a-d)

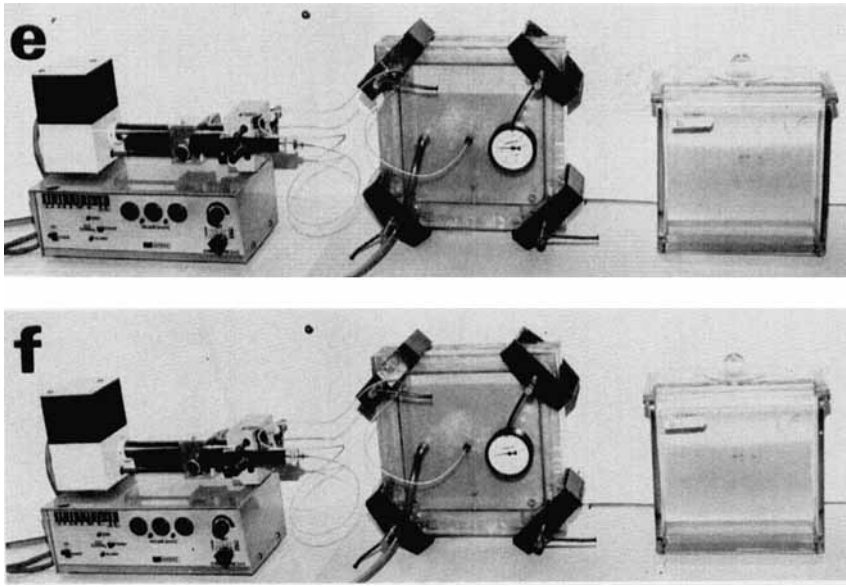

Figure 3. The processes of development are comparatively illustrated in conventional TLC- and in PUM-chambers (Figs. 3/a, 3/b, 3/c, 3/d, 3/e and 3/f). The start at the right beginning (3/a) and more stages of development are shown in PUM-chamber. The syringe-type pump (left), the PUM-chamber (middle) and the conventional TLC-chamber (right) are shown on each picture. Fixion 50 X 8 plates developed in sodium citrate buffer, pH = 4.2 were used.

sure on the foil is not sufficient to compensate for the pressure effect of the solvent supplied by the pump.

If only the first point is true, the optimal flow velocity can be stated by the above detailed way, and it is characteristic for the case of PUM-chamber, TLC

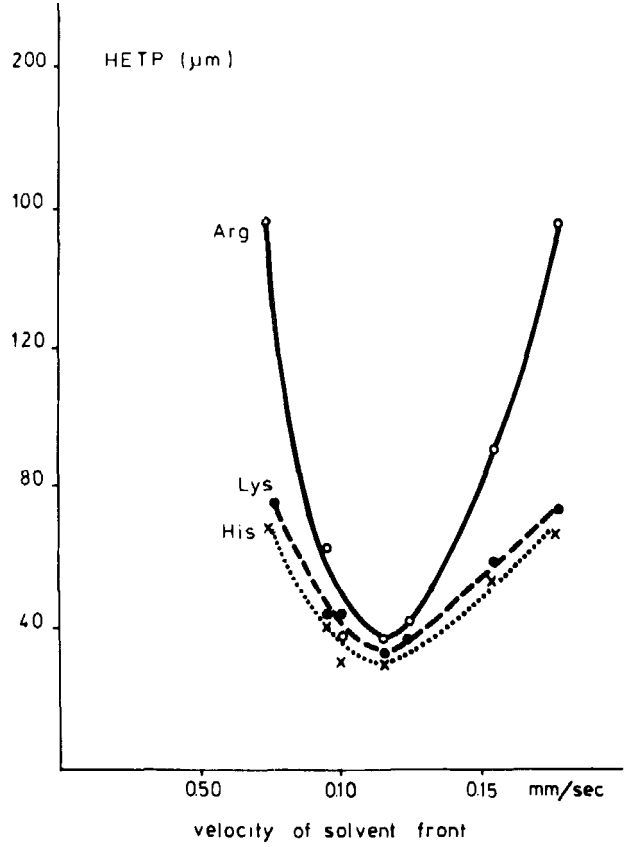

Figure 4. In case of Fixion 50 X 8 plates and PUM-chamber, the plate height vs. flow velocity curves show optima at a moderately high value of flow velocity of solvent front.

and OPTLC, when amino acids are separated on Fixion 50 X 8 chromatoplates. If the second point is fulfilled, the application of higher external pressure on the membrane will make possible smaller plate heights, i.e. better efficiencies and separations. To realize higher

external pressure (about 10 atm) on the membrane the basic reconstruction of the apparatus will be necessary, e.g. the change of plexy glass to steel, etc. At the same time, these experiments suggest the possibility of both reaching very low plate height in the OPTLC experiments and the further decrease of the time necessary for the development.

The above results in the area of separation of amino acids by OPTLC indicate that

a./ decrease of time of development, and

b./ the finding of optimal flow velocity

have been accomplished. These results allow the separation of amino acids in 10 - 18 minutes with good resolution instead of 100 - 400 minutes required by conventional TLC.

An additional point worth mentioning here is that the vapor phase does not play as major a role in ion exchange chromatography as it does when TLC silica gel layers are used.

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