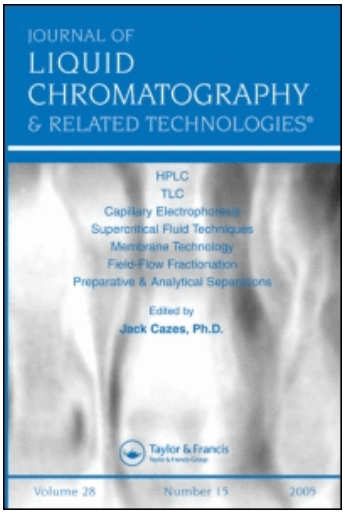

Journal of Liquid Chromatography & Related Technologies

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 Idei, M. , Mezö, I. , Vadász, Zs. , Horváth, A. , Teplán, I. and Kéri, Gy.(1992) 'Capillary Electrophoretic Analysis of Somatostatin Analog Peptides. Effect of Organic Solvents as Buffer Modifiers', Journal of Liquid Chromatography & Related Technologies, 15: 18, 3181 — 3192
To link to this Article: DOI: 10.1080/10826079208020877
URL: http://dx.doi.org/10.1080/10826079208020877

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(18), 3181-3192 (1992)

CAPILLARY ELECTROPHORETIC ANALYSIS OF SOMATOSTATIN ANALOG PEPTIDES. EFFECT OF ORGANIC SOLVENTS AS BUFFER MODIFIERS

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ABSTRACT

Capillary electrophoretic (CE) method utilizing triethylammonium phosphate/organic modifier solvents as CE buffer was developed for analysis of new proprietary Somatostatin analog peptides synthetized in our laboratory. Acetonitrile, methanol, ethanol and i-propanol were applied as organic modifiers. Applicability of the systems in the analysis of Somatostatin analog peptides was evaluated and optimum conditions of the separation were determined. The effects of the organic solvents on the migration times of the S-analog peptides, elution order and selectivity in the CE systems were investigated. The migration time, elution order and selectivity can be influenced modifying the composition of the electrophoretic buffer with organic solvents.

Applying different organic solvents as modifiers the elution order of the peptides can be changed in different ways. The effect of the alcohols on the CE migration time of the peptides is quite opposite to that of the acetonitrile. The degree of the effect of the alcohols at a given concentration depends on the nature of the alcohol: the longer the carbon chain of the alcohol the greater the increase of the CE migration time of the peptide comparing to the migration time obtained in buffer not containing organic solvents.

INTRODUCTION

Somatostatin, also named growth hormone (GH) release-inhibiting hormone (GHR-IH) is a tetradecapeptide characterized as an inhibitor of GH secretion from the anterior pituitary gland (1). It has been demonstrated that somatostatin analogs have growth inhibitory properties both in vivo and in vitro on various endocrine tumors (2-5), inhibit various other endocrine secretions (i.e. glucagon, insulin, gastrin) and several functions in a large variety of cells as well (6-8).

The wide diversity of biological effects of Somatostatin and their physiological significance led to substantial efforts to determine the main structural features responsible for the various biological functions.

As a part of these efforts several analogs have been synthetized in our laboratory in order to have an insight into the structural requirements for achieving selective biological activity of somatostatin analogs. Our aims were precisely to inhibit tumor growth accompanied with or separated from the inhibition of the release of growth hormone and to reveal their other biological properties including tyrosine kinase inhibitory activity (9-14).

For these purposes highly purified and well characterized peptides are needed. As a consequence it is essential for the laboratories working on this field to have fast, simple however reliable methods of high performance for the analysis of the synthetic somatostatin analogs.

Recently capillary electrophoresis has become a powerful method in several areas of analysis because of its high efficiency, selectivity and speed of the analysis (15-18). Similarly to the HPLC versatility of the separation process may be very important in CE, too (19). This versatility may be achieved by modifying the surface of the capillary wall, or modifying the composition of the electrophoretic buffer or by derivatization of the analyte (20, 21-29).

In accordance with the needs mentioned before our aim was to develop a CE method (utilizing buffers modified with organic solvents) suitable for analysis of S-analog peptides.

EXPERIMENTAL

Materials

Peptides were synthetized in our laboratory (9, 12, 30), their composition, peptide content and purity were analyzed. The peptide content of each peptide sample was between 83.4-85.1%, the chromatographic purity of each peptide was better than 97.1 %. The chemical structures of the peptides applied in this work are as follow:

S-218 D-Phe-Cys(-)-Tyr-Trp-Lys-Ala-Cys(-)-Thr-NH_2

S-220 D-Phg-Cys(-)-Tyr-Trp-Lys-Val-Cys(-)-Thr-NH_2

S-228 D-Phe-Cys(-)-Tyr-Trp-Lys-Pro-Cys(-)-Thr-NH_2

S-232 D-Phe-Cys(-)-Tyr-Trp-Lys-Cys(-)-Thr-NH_2

S-248 H-asp.-indolinyl-Cys(-)-Tyr-Trp-Lys-Val Cys(-)-Thr-NH_2

S-250 D-Phe-Cys(-)-Tyr-Trp-Lys-Leu-Cys(-)-Thr-NH_2

Chemicals

HPLC grade acetonitrile, methanol, ethanol and i-propyl alcohol was purchased from Chemolab (Budapest, Hungary). Ortho - phosphoric acid, triethyl amine were purchased from Fluka (Buchs, Switzerland). Solutions were prepared with water treated with Elgastat UHP (Elga Ltd., England).

Methods

Capillary electrophoretic analysis were performed with ISCO Model 3850 Capillary Electropherograph (Lincoln, NE, USA). Capillary: uncoated silica capillary (60 cm x 50 μm); detection: 215 nm, sensitivity 0.02, rise time 0.8 sec; voltage: 30 kV; injection volume 10 μl. Buffers: 0.25N TEAP buffer (pH=2.25) modified with different organic solvents (ACN, MeOH, EtOH and IPA) in different concentrations. Concentrations of the organic modifiers in the TEAP buffer (in v/v %): 0; 5; 10; 15; 20; 25 and 30.

Peptide samples in concentration of 1mg peptide/ml water were used in CE analysis.

Digital data collected from CE runs were stored and analyzed by ISCO ChemResearch controlling and data handling system. Data collection sampling rate was 8 sec^{-1}. Four injections were made from each peptide samples at every buffer composition.

RESULTS AND DISCUSSION

Table 1. shows the migration times of the S-analog peptides obtained with CE analysis in TEAP buffers modified with different organic solvents.

Figure 1. and 2. show the migration time of S-218 via percentage of each organic solvent, and migration times of each peptide via percentage of the MeOH, respectively. These are representative descriptions to show clearly the trends. Similar result have been obtained for each peptide and organic solvent.

Figure 3. shows that the optimal separation of the 6 peptides can be performed in 13.2:86.8 v/v% ACN/TEAP system.

Data of the Fig.1. show that the effect of the alcohols on the migration time of the peptide is quite

TABLE 1.

Migration Times of the S-analog Peptides

conc. of modifier (v/v%)	migration times (sec) PEPTIDE S-218	S-220	S-228	S-232	S-248	S-250
ACN						
0	465.9	440.5	460.7	460.0	471.7	455.7
5	395.3	377.2	389.3	406.0	400.0	391.3
10	357.6	327.7	339.5	357.3	346.4	340.7
15	339.2	317.8	324.0	335.2	329.3	326.1
20	327.0	312.0	318.5	324.0	313.1	317.1
25	317.2	304.3	308.2	308.1	309.3	308.0
30	311.2	302.2	307.9	289.7	307.2	303.2
MeOH						
5	469.5	445.0	476.0	468.0	488.1	480.0
10	487.8	468.6	494.2	479.9	513.0	507.7
15	516.1	508.0	521.3	518.0	538.1	528.1
20	556.0	548.3	547.3	560.0	568.0	548.2
25	596.2	585.3	577.1	600.0	595.0	568.1
30	650.2	634.0	614.1	644.4	627.8	591.2
EtOH						
5	488.2	452.0	471.3	480.0	504.0	492.1
10	523.2	506.3	526.0	503.8	548.2	539.5
15	551.2	576.0	600.3	582.0	618.0	603.9
20	646.3	648.5	680.0	673.1	690.2	632.1
25	733.1	720.3	760.2	763.0	769.0	811.3
30	832.6	795.4	841.9	859.6	847.2	826.2
IPA						
5	503.2	481.9	505.2	504.0	520.0	500.0
10	562.2	539.8	561.0	553.1	577.2	557.2
15	664.3	651.8	669.8	680.0	683.1	650.5
20	780.1	780.0	783.2	808.2	796.0	761.0
25	904.2	893.7	900.1	925.2	908.0	856.2
30	1078.8	1027.7	1022.1	1078.2	1027.2	863.8

CE analysis performed in TEAP buffer modified with different organic solvents (for parameters of the analysis and for buffer compositions see Methods). SD values of parallel runs (n=4) are not shown in the Table for clarity. Figure 1. shows typical SD values.

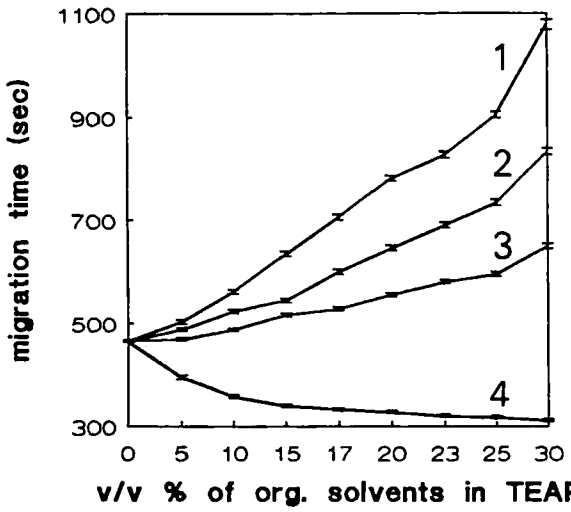

Figure 1.: CE migration times of the peptid S-218 via the precentage of the organic solvents in the TEAP buffer. For experimental parameters see Methods. Curves: 1: IPA; 2: EtOH; 3: MeOH; 4: ACN.

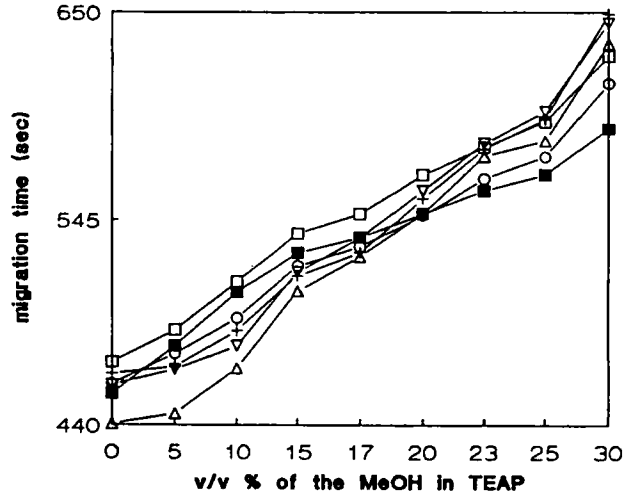

Figure 2.: Migration times of the peptides obtained by CE via percentage of the MEOH. For experimental parameters see Methods. Symbols: S-218: + ; S-220: Δ ; S-228: O ; S-232: ∇ ; S-248: □ ; S-250: ■ .

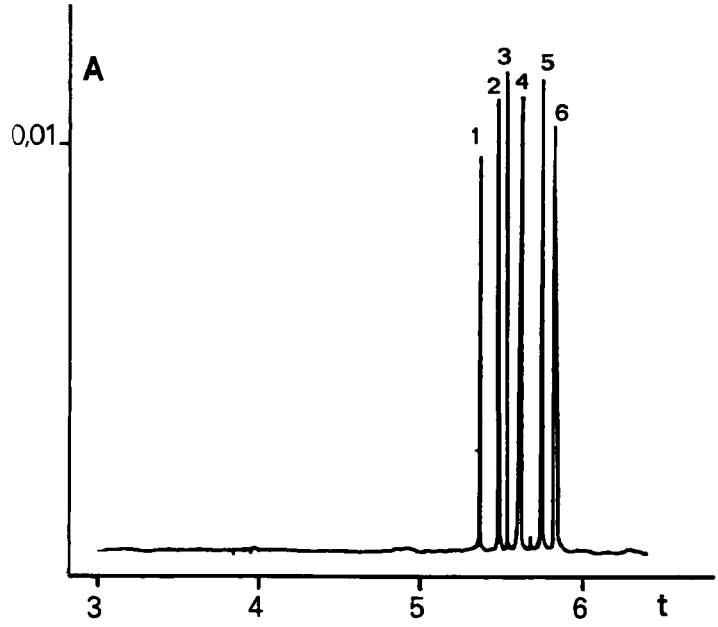

Figure 3.: Electropherogram of the mixture of the six S-analog peptides. Buffer composition: 13.2 ACN:86.8 TEAP (in v/v %); concentration of the peptides: 1 mg/ml of each in water. For other parameters see Methods. Abscissa: migration time in minutes; ordinate: absorbance at 215 nm.
Peak numbers: 1: S-220; 2: S-228; 3: S-250; 4: S-248; 5: S-232; 6: S-218.

different from that of the ACN. Meanwhile the addition of any of the alcohols increases the migration time of the peptide on a concentration dependent way, the addition of ACN concentration dependently decreases the migration time of the same peptide.

The degree of the increase of the migration time caused by the alcohol at a given concentration is dependent on the nature of the alcohol applied. The longer the carbon chain of the alcohol the greater the increase of the migration time caused.

Table II.
Viscosity of the CE buffers (cP)

org. solvent	concentration in v/v %		
	10	20	30
ACN	0.934	0.758	0.595
MeOH	1.080	1.220	1.441
EtOH	1.323	1.825	2.182
IPA	1.990	2.500	2.824

Table III.
Ionic strength of the CE buffers

	conc. of the organic solvent in the TEAP (in v/v %)						
	0	5	10	15	20	25	30
ionic str.	0.4985	0.4736	0.4487	0.4237	0.3988	0.3738	0.3489

The net mobility of the analyte is the sum of the electrophoretic (u_{ep}) and electroosmotic (u_{eo}) mobilities:

$$u_{net} = u_{ep} + u_{eo}$$

$$u_{ep} = e / 3x10^7 \, Z \, \eta \sqrt{C}$$

$$u_{eo} = D \, \zeta_{eo} / 4\pi \, \eta$$

where e: surface charge density of the analyte; Z: charge of the buffer ion; C: concentration of the buffer ion; η: viscosity of the buffer; D: dielectric constant of the buffer; ζ_{eo}: Zeta potential of the capillary wall (20).

Change in the viscosity and/or the ionic strength of the buffer results in change of the electrophoretic and electroosmotic mobilities.

The alcohols increase, while the ACN decrease the viscosity of the buffer (31, 35, Table II.). This change in

the viscosity could explain the opposite effect of the alcohols and the ACN on the net mobility and therefore on the migration time of the peptides. ACN dilutes the ion concentration and decreases the viscosity. Both changes result in increase of the net mobility. The alcohols applied here increase the viscosity but decrease the ionic strength (31, 35, see Table II. and III.).

These changes alter the mobility into opposite directions. However the product of the two parameters ($\eta \times \sqrt{C}$) increase and therefore both the electrophoretic and electroosmotic mobilities decrease (31).

Similarly, these differences can explain why the different alcohols influence the migration time in different degree. The higher the viscosity of the alcohol applied the greater the increase in the migration time caused at a given concentration.

However as Figure 2. shows not only the migration times but even the elution order of the peptides can be changed by changing the concentration of the organic solvent. Simple increase or decrease of the viscosity and the ionic strength only could alter proportionaly the migration times of each peptides but could not change their order of elution, could not change their migration times relative to each other.

There is an aggreement between the effects of the organic modifiers and their dipole momentums (which indicates their ability to form associates). The ACN (having a much more higher dipole momentum than the water has) can decrease the migration times while the alcohols (having dipole momentum lower than that of the water) can increase the migration times. (The sequence of the solvents in decreasing order of their dipole momentums: ACN > water > MeOH > EtOH > IPA) It can not be ruled out that the modifiers can interact with the capillary wall and/or can form associates in the buffer.

These may result in either change of the electroosmotic flow influenced by the net charge of the capillary surface and the viscosity of the buffer or change in the

hydrodynamic parameter (Stokes radius) of the analyte (32-34).

In conclusion the results demonstrate that capillary electrophoresis is a fast and easily applicable method in separation of S-analog peptides. The six peptides investigated here can be separated within 6 minutes.

Modifying the composition of the mobil phase we are able to influence the migration time, elution order and selectivity in the CE system.

ACKNOWLEDGEMENTS

This work has been supported financially by the Hungarian Academy of Sciences, Grant No. OTKA 2618.

LIST OF ABBREVIATIONS

CE: capillary electrophoresis; ACN: acetonitrile; MeOH: methanol; EtOH: ethanol; IPA: 2-propyl alcohol; TEAP: triethyl-ammonium phosphate; S-analoge : somatostatin analog; asp.: aspartil.

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Received: June 16, 1992
Accepted: June 29, 1992