STUDY ON HPLC OF AROMATIC AMINO ACIDS AND OLIGO PEPTIDES USING MICROSPHERICAL CARBON PACKINGS

(HPLC of Amino Acids and Oligo Peptides on Carbon Column)

Tiansheng Su,[1] Xianchuan Chen and Tsuneo Okuyama*
Department of Chemistry, Faculty of Sciece
Tokyo Metropolitan University, Setagaya-ku, Tokyo, Japan 158
(Tel : 813-717-0111 cxt. 3426 ; Fax : 813-725-8101)

ABSTRACT

A microspherical carbon (MSC) as HPLC packings has been tested and its chromartographic propertier for the separation of aromatic amino acids and some oligo peptides have been studied in this paper. Theapparent characterization o packings were described by scanning electron microggraph and physical parameters. The mixture of aramatic amino acids can be separated satisfacterily on MSC column (7.8 mm I. D. × 50 mm) using acetonitrile eluate of the linear gradient, while the non-aromatic amino acids were not retained under the same conditions. The good results for the separation of some oligo peptides bearing aromatic structure can be obtained, while the other type of oligo peptides were not yet retained on MSC column unless the sample possess rather strong hydrophobicity. The high selective retention is a significant characteristic of MSC packing, and the chromatographic method described is useful for the separation of biochemical substances especially as some oligo peptides from the hydrolysis products of proteins or from the drinks. The separation machanism and exiting problem for MSC packings were also discussed preliminarily.

INTRODUCTION

It is generally believed that the carbonaceous material possesses the good chemical stability, the range wide pH adaptability and the valuable adsorption characteristics. The porous carbon beads as gas chromatographic packins have been used widely due to its excellent separation performance. Since the appearace of the reversed phase chromatography, the porous or non-porous microparticle carbon havn been tentatived to use in HPLC as column packings, because one might assume that carbon would be similar to reversed phase silical packings in the chromatographic properties. In the past some years, the many different carbonaceous packings for HPLC purposes have been introduced on the preparation methods, the structure characterization and the chromatographic performances (1-10). But judging from its development, this kind of packings have not yet been made rapid progress, perhaps it is difficult to prepare a microspherical carbon with the suitable particle size distribution, the effective pore range and the pure surface.

As a starting point, it might be useful to study the carbonaceous packing material for the separation of biochemical substances especially as some oligo peptides. In this

1. Present address : Institute of Chemistry, Academia Sinica, Beijing, China 100080
*Present address : Department of Dental Biothechnology Bioengineering, Kanagawa Dental College.

paper, a microspherical carbon (MSC) as HPLC packings has been tested, and its chromatographic behaviors for the separation of the aromatic amino acids and some oligo peptides with these kinds of amino acid residues have been studied.

MATERIALS AND METHODS

Apparatus : The instruments used were Yokogowa LC 100 system ; NEC pc-9801vm personal computer (Japan).

Reagents : Acetonitrile (chromatographic grade) was obtained from Kanto Chemical Co. Ltd. (Japan).

Samples : Amino acids and acetyl derivative of amino acids were obtained from Wako Pure Chemical Industries Ltd. Synthetic oligo peptides were obtained from Nutritional Biochemicals Corporation (USA).

Column : The separation column of microspherical carbon packings (7.8 mm I. D.× 50 mm) was offered by Toa Nenryo K. K (Japan).

Chromatographic Procedure : Some aromatic type of amino acids were separated on MSC column with acetonitrile eluate of the linear gradient from 1 % (containing 0.07 % TFA) to 60% (containing 0.1 % TFA) in 20 minutes at a flow rate of 1 ml/min. Under the same conditions, some peptides were separated. All the experiment were conducted at the room temperature and effluent were monitored at 210 nm.

RESULTS AND DISCUSSIONS

Apparent Characterization of MSC Packings

In general, the physical structure of column packings is the main factor effecting chromatographic dynamical behaviors. It is necessary for HPLC packings that the good rigidity, the suitable particle size and distribution, and the effective surface area and structural shape. The physical parameters of MSC packings used were shown in Table 1. Fig. 1a and 1b show the scanning electron micrograph of packings. From the apparent shape, we can see that the carbonaceous packings used is the complete microspherical products which would be favourable for packing process and improving chromatographic performance. The particle size distribution of packings has been shown in Fig. 2, where 2–7 μm of particles are over 90% which will be also available for HPLC, though its distribution is rater broad. The particle rigidity confirmed by the experiment was sufficient for HPLC application. From the scanning electron micrograph (Fig. 1b), we can observe that the formation of wrinkles and holes on the microspherical surface are quite clear, that also illustrate the parkings having porous structure.

Table 1 Physical Parameters of Microspherical Carbon Packings

Particle size	1–10 μm
Specific surface area	380 m^2/g
Apparent density	0.57 g/ml
Pore volumn	1.00 ml/g
Pore renge	30–3,000,000 Å

The pore size of packings and the pore structure shape play an important role for adsorption chromatography because they will provide the effective surface area, and they could also affect the mass transfer rate and the permeability. From a judgement upon physical parameters, the porosity of packings is lower, that shows the numbers of effective pore could not adequate because of having quite wide range of the pore size

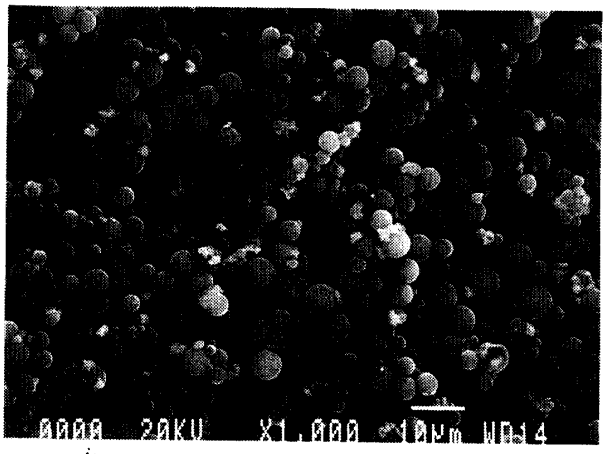

Fig. 1a

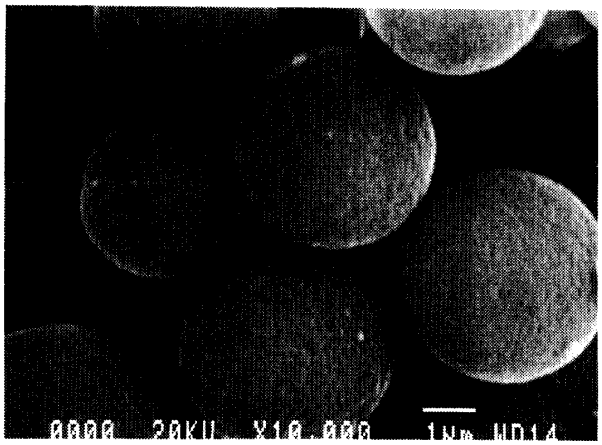

Fig. 1b

Fig. 1 Scanning electron micrograph of microspherical carbon packings

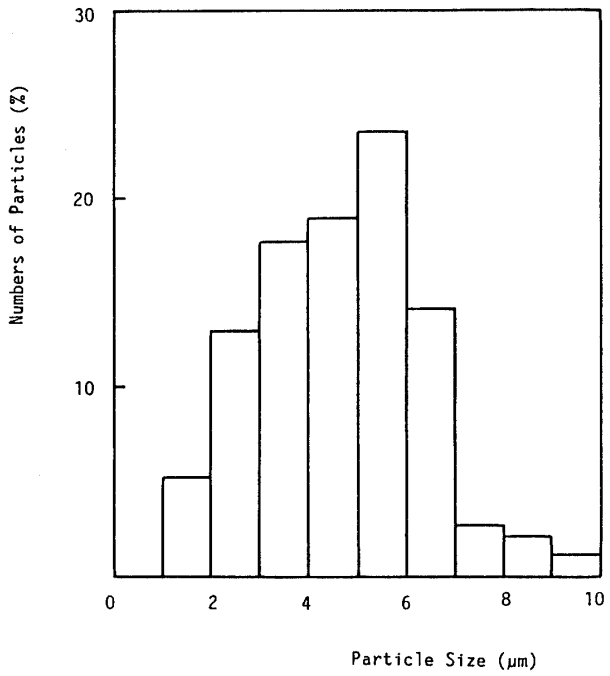

Fig. 2 The particle size distribution of microspherical carbon packings

distribution, and the proportion of micropores could be higher due to ownning larger specific surface area.

Chromatographic Behaviors of MSC packings

(1) HPLC of amino acids

Table 2 shows the hydrophobicity parameters of amino acids and their capacity radio on MSC column. It is obvious that MSC packings possesses high selective adsorption for the aromatic amino acids. while other type of amino acids were not retained under the same conditions, no matter how the sample hydrophobity. The separation of mixture of amino acids on MSC column have been shown in Fig. 3 and 4. From these results, we can see that the good selectivity and resolution for the amino acids bearing aromatic ring were a significant characteristics of MSC packings, that wold be useful for the particular separetion if this kind of compounds were needed to obtain from their mixture.

(2) HPLC of Some Oligo Peptides

Table 3 shows the retention parame ters of some oligo peptides on MSC column. The chromatographic behaviors of oligo peptides on MSC packings are similar to the amino acids, where the solute retained were only the peptides bearing aromatic structure. Among the oligo peptides without aromatic ring, the retention behaviors of Leu-Leu seems different, the reason could be this solute having strong hydrophobicity. If the starting concentration of eluate is increased to over 3%, the capacity radio of Leu-Leu will be also close to zero. Fig. 5 shows the separation of some oligo peptides containing impurity of amino acids on MSC column. These results indicate that MSC packings possesses rather high selectivity and resolution for the aromatic oligo peptides. This significant characteristics of packing has provided a favourable condition for the separation of hydrolysis

products of products of proteins which is a important rsearch subject in the biochemical field. Fig. 6 shows the separation of mixture of oligo peptides in the hydrolysis products of trypsin, which illustrate the feasibility of this method using MSC packings. Aspartame (Asp-Phe-Me), a important additives of food i. e. artificial sweetenes, as the derivative of aromatic oligo peptides possesses the good elution behaviors as well on MSC column, Fig. 7 show the separation of aspartame in Pol sweet and Cake light respectively, which indicate that the method will be also suitable for the analysis of the commercial low calorie drinks.

Above preliminary results dealt mainly with two separation pooblem, the aromatic amino acids and oligo peptides bearing aromatic structure. Perhaps these good performances of the carbonaceous packings could open up a new way for its application in the protein chemistry and the food chemistry, especially of the separation of aromatic oligo peptides.

The selectivity of MSC packings towards the separation of the other solute are also the aromatic compounds including some isomers. These characteristics of selective retention seems to be depended mainly on the effect of the charge transfer i. e. the interaction of conjugated π-bonds between the sample and the packings because the separation machanism can not be explained satisfacterily by the interaction of hydrophobicity. Judging from the stationary phase, it is probable that the carbonaceous packings bears a more or less aromatic type of structure which could be advantageous to adsorption and desorption for the solute containing similar structure. On the other hand, it is also clear that the retention order of aromatic amino acids on MSC packings are consistent with their conjugative effect. Although MSC as the reversed phase packings was used in HPLC, its chromatographic behaviors shows a hydrophilic selectivity towars polar solutes. These properties results from the polar functional groups oxygen-containing at the carbon surface. These functional groups probably are C=O, C-OH, C-O-C, COOH etc. which could provide

Table 2 Hybrophobicicity Parameters of Amino Acids and Their Capacity radio on MSC Packings

Amino acids	Hydrophobicity parameters	Capacity radio
tryptophane	2.31	7.60
phenylalanine	2.24	3.56
leucine	1.99	0
isoleucine	1.99	0
tyrosine	1.70	3.92
valine	1.46	0
cystine	1.11	0
methionine	1.08	0
proline	1.01	0
alanine	0.53	0
lysine	0.52	0
glycine	0.00	0
aspartic acid	−0.02	0
glutamic acid	−0.07	0
histidine	−0.23	0
threonine	−0.26	0
serine	−0.56	0
arginine	—	0
N-Ac-Trp	—	13.61
N-Ac-Tyr	—	7.76

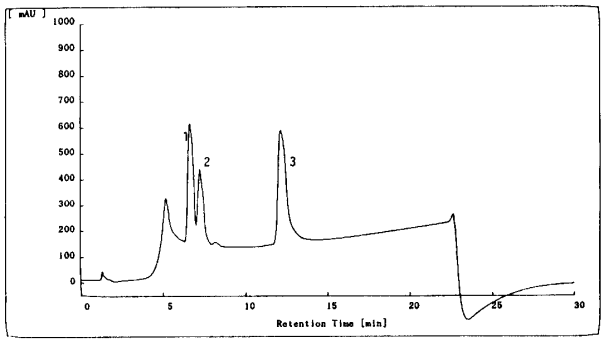

Fig. 3 The separation of aromatic amino acids on MSC column
1. phenylalanine
2. tyrosine
3. tryptophan

the good selectivity, and could also lead to decrease the column efficiency due to their non-homogeneous distribution at the packings surface. Generally, it is difficult to eliminate these groups from the carbon surface, even if to be reducted by high-temperature. So it is important to improve the distribtion state of the surface groups in the preparation process.

The experimental results show that the columne efficiency of MSC packings is lower as compared with the reversed phase silica. The chief reason of this drawback can be explained by as following.

(1) Judging from chemical structure of packings, the distribution of the functional groups containing on the carbon surface are not homogeneous, which as nonspecific adsorption site will lead to the non-equili brium state in the desorption process. Accoding to the thermodynamical theory the specific retetion volume (Vg) in adsorption chromatography can be shown by following equation,

$$\ln Vg = -\Delta H/RT + S/R.$$

If a comparison is made between the adsorption surface of the homogeneity and the non-homogeneity, the adsorption heat effect of the latter will be larger and the entropy decrease towards the non-homogeneous surface is less which will lead to reduce the chromatographic capacity. In the adsorption or the desorption, the energy change is the kinetic equilibrium process. From kinetic heat effect,

$$\Delta G = \int \left(\frac{d\Delta H}{dt}\right) dt$$

Thus, the tail formation towards the packings bearing non-homogeneous surface will be inevitable outcome in the chromatographic process.

(2) It is also necessary to consider from the physical structure of packings. The diffusion factor (γ_s) of solute in chromatographic process is as following,

$$\gamma_s = Ds/Dm$$

where Ds and Dm are diffusion coefficient in the stationary phase and the moving phase

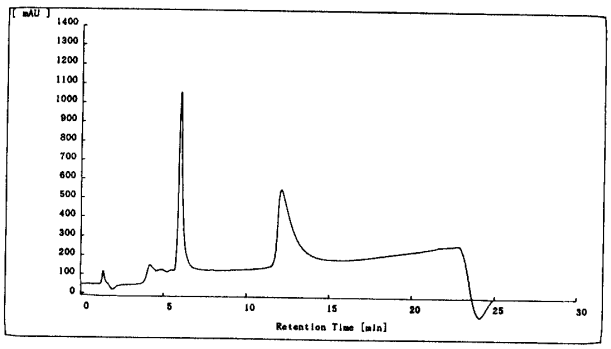

Fig. 4 The separation of tyrosine and acetyl-tyrosine on MSC column

Table. 3 Capacity Radio of Some Oligo Peptides on MSC Column

Peptides	Capacity radio
Leu-Gly	0.23
Glu-Leu	0.38
His-His	0.26
Leu-Leu	3.30
Ala-Phe	5.31
Gly-Trp	8,69
Gly-Gly-Ala	0.23
Leu-Gly-Gly	0.23
Asp-Phe-Me	5.15

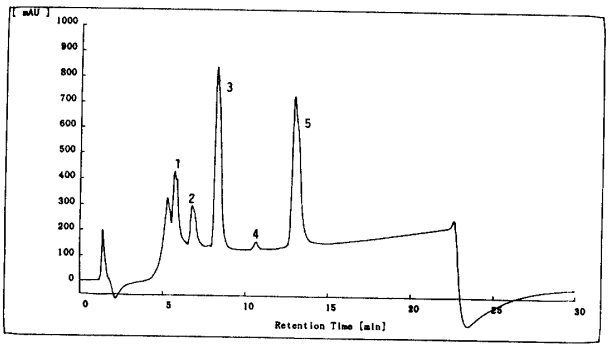

Fig. 5 The separation of some oligo peptides on MSC column

1. L-leuyl-L-leucine
2. phenylalanine
3. DL-alanyl-DL-phenylalanine
4. tryptophan
5. glycyl-L-tryptophan

respectivly· The diffusion factor, as a measurement of the obstruction effect of the solute mass transfer, dependes mainly the tortuosity factor of packings which means the effect of the elongation of the average length due to the micropore tortuosity inside te particle of packings. Owing to rather high proportion of micropore in MSC packings, the tortuosity factor is larger, that means the larger obstruction effect for the mass transfer will be encountered when the solute diffuse into the porous material.

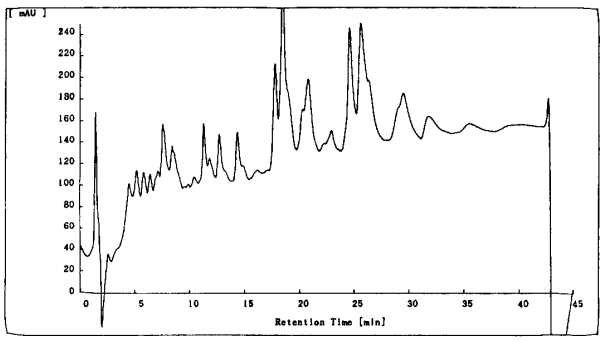

Fig. 6 The separation of hydrolysis products of trypsin on MSC column

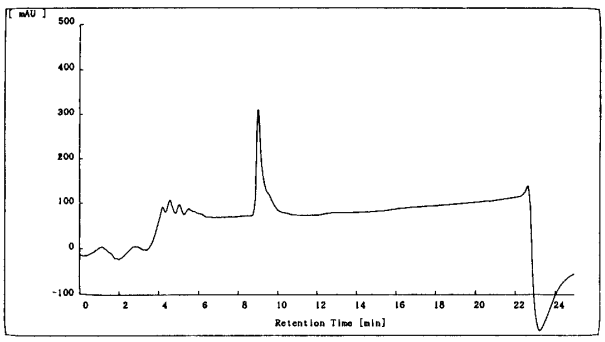

Fig. 7a The separation of aspartame in Pol Sweet on MSC column

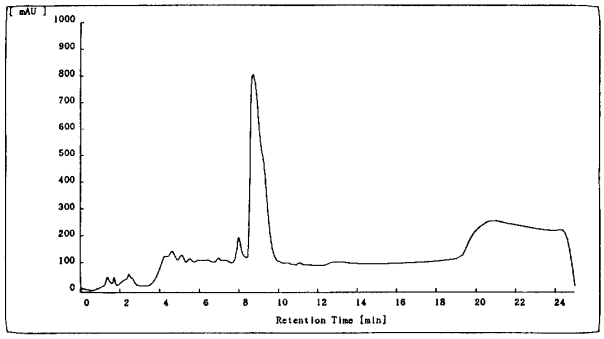

Fig. 7b The separation of aspartame in Coke Light on MSC column

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