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Effect of preparatory conditions on the performance of photopolymerized sol–gel monoliths for capillary electrochromatography

Masaru Kato[a,*], Kumiko Sakai-Kato[a], Toshimasa Toyo'oka[a], Maria T. Dulay[b], Joselito P. Quirino[b,1], Bryson D. Bennett[b], Richard N. Zare[b]

[a]*Department of Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka, Shizuoka 422-8526, Japan*

[b]*Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA*

Abstract

We prepared different photopolymerized sol–gel (PSG) columns by varying the amount of monomer (methacryloxypropyltrimethoxysilane), porogen (toluene) and catalyst (hydrochloric acid) in the reaction solution containing a photoinitiator (Irgacure 1800). The effects of these variations on the chromatographic behavior of the PSG columns were studied. All of the columns studied exhibited reversed-phase character. The concentration of hydrochloric acid was important for the rigidity of the columns, although it did not affect the separation property. The ratio of monomer solution to porogen was a critical factor in controlling the through-pore size and the surface area of PSG, which were found to significantly affect the separation properties, such as permeability, theoretical plate number, retention time, and separation efficiency, of a mixture of test analytes—thiourea, benzene, and naphthalene. There was no change in the retention order for the test analytes. Short separation times were achieved on PSG columns made from a 10% monomer stock solution and 90% porogen with 1 *M* hydrochloric acid. Mixtures of polycyclic aromatic hydrocarbons and alkylbenzenes were separated with theoretical plate numbers greater than 100 000 plates/m.

Keywords: Monolithic columns; Stationary phases, electrochromatography; Capillary electrochromatography; Sol–gel; Alkylbenzenes; Polynuclear aromatic hydrocarbons

1. Introduction

Capillary electrochromatography (CEC) has become a very promising separation technique for the analysis of small sample volumes because of the high separation efficiencies that can be achieved [1,2]. There are few reports, however, on the application of CEC to the analysis of real samples, such as biological or environmental materials [3–5]. There remain some challenges in column preparation that need to be overcome. Because a very narrow diameter capillary (typically less than 100 μm) is used, elaborate work that includes the use of on-column frits is required to fabricate a stationary phase with good reproducibility and homogeneity. Monolithic stationary phases were developed [6–26] as alternatives to packed CEC columns. Gusev et al. [27] define a monolithic stationary phase as ''a continu-

*Corresponding author. Tel.: +81-54-264-5654; fax: +81-54-264-5593.

E-mail address: daikato@u-shizuoka-ken.ac.jp (M. Kato).

[1]Present address: Pharmacyclics, Inc., 995 E. Arques Avenue, Sunnyvale, CA 94086-4521, USA.

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ous unitary porous structure prepared by in situ polymerization or consolidation inside the column tubing and, if necessary, the surface is functionalized to convert it into a sorbent with the desired chromatographic binding properties''.

Monolithic stationary phases for CEC have been prepared from acrylamide [8–15], methacrylate [16–19], and alkoxysilane [20–26]. Monolithic stationary phases can be prepared in only a few steps with great facility, repeatability, and uniformity. The most noticeable advantage of monolithic column is the fritless design. Bubble formation or deterioration of the separation efficiency [28,29] has been observed in columns with frits.

Recently, we reported on a monolithic stationary phase prepared from methacryloxypropyltrimethoxysilane (MPTMS) [30]. MPTMS, which contains both methacrylate and alkoxysilane groups, was used to prepare a photopolymerized sol–gel (PSG) in a single-step reaction. One of the advantages of a monolithic column is the ability to control its morphology. The sponge-like silica monolith is comprised of micrometer-sized ''through-pores'' and a silica skeleton that possesses nanometer-sized ''mesopores''. The through-pores, mesopores, and the skeleton size of the monolithic column are controllable by the amount or type of monomer used, and by the ratio of monomer and porogen and catalyst [16,17,21,22]. Both Tanaka and co-workers [21,22] and Fréchet and co-workers [16,17] demonstrated the effect of monolith structure on the parameters of separation performance, including retention time, theoretical plate number, and pressure drop.

We describe the effects of varying the reaction conditions (i.e., concentration of hydrochloric acid and the ratio of monomer to porogen) on the morphology of the PSG monolith. We compare the separation of polycyclic aromatic hydrocarbons and alkylbenzenes on PSG columns prepared with different reaction conditions.

2. Experimental

2.1. Apparatus

All CEC experiments were performed on a Beckman P/ACE 5510 capillary electrophoresis system (Fullerton, CA, USA) with a UV-absorbance detector and a Hewlett-Packard ^{3D}CE system (Palo Alto, CA, USA) equipped with a diode array detector. A RPR-100 photochemical reactor (Ultraviolet Company, Branford, CT, USA) was used for the photopolymerization reactions. Scanning electron microscopy (SEM) analyses were performed on a Hitachi S-2500 scanning electron microscope (Tokyo, Japan).

2.2. Materials and chemicals

Fused-silica capillaries (75 μm inside diameter× 365 μm outside diameter) were purchased from Polymicro Technologies (Phoenix, AZ, USA). MPTMS was purchased from Tokyo Kasei (Tokyo, Japan). Toluene (C1), acetonitrile, thiourea, and benzene (C0) were from Kanto Kagaku (Tokyo, Japan). Naphthalene was received from Koso Chemical (Tokyo, Japan). Ethylbenzene (C2), butylbenzene (C3), and 1-phenylhexane (C6) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Propylbenzene (C4) was from Wako (Osaka, Japan). Irgacure 1800 was donated by Ciba (Tokyo, Japan). The water was purified on a Milli-Q system (Nippon Millipore, Tokyo, Japan).

2.3. Preparation of the PSG column

A PSG column was prepared using a previously described procedure [30,31]. Some modifications to this procedure were made to account for the use of a photoreactor with different lamp intensities and configurations to those used in the published procedure. Briefly, a monomer stock solution, a mixture of 750 μL MPTMS, 22.5 μL 1.0 *M* hydrochloric acid and 225 μL water, was stirred for 30 min at room temperature in the dark. Toluene (170 μL) was added to 30 μL of the monomer stock solution and stirred for 30 min at room temperature. Irgacure 1800 (photoinitiator) (8.9 mg) was added to the toluene mixture and stirred for 2.5 h at room temperature. This photoinitiator solution was flushed through a 30-cm long capillary with a 13-cm stripe of the polyimide coating removed in the middle of the capillary [30]. The filled capillaries were ir-

radiated in a photochemical reactor using 350 nm light for 20 min to form the PSG.

After irradiation, the capillaries were washed with methanol using a hand-held syringe to remove any unreacted reagents. A window was created immediately after the PSG monolith using hot sulfuric acid (>100 °C) to remove the polyimide coating. Once fabricated, the capillary was installed in a P/ACE cartridge without damage to the capillary. The PSG capillary was conditioned with the separation solution for approximately 5 min using a syringe and a hand-held vise. The column was further conditioned electrokinetically in the CE instrument by driving the mobile phase through the capillary at an applied voltage of 5 or 10 kV until a stable baseline was achieved.

2.4. SEM analysis

SEM was used to study the morphology of PSG. A PSG capillary was sectioned into 5-mm segments. These segments were sputtered with gold prior to SEM analysis.

2.5. Sample and solution preparation

A stock solution of each analyte was prepared at a concentration of 100 m*M*, except for alkylbenzenes, which were prepared at a concentration of 10 mg/mL. These samples were diluted 10 times in the separation solution. The separation solutions were degassed by ultrasonication and filtered (0.22 μm filter) before use.

3. Results and discussion

3.1. Effect of hydrochloric acid concentration

In a sol–gel reaction, the acid or base catalyst plays an important role in the hydrolysis and condensation reactions. Minakuchi et al. controlled mesopore sizes in a monolith prepared from tetramethoxysilane by varying the concentration of the ammonia solution [22]. By controlling the amount of catalyst used in the reaction, the size of the mesopores can be fine-tuned.

Five different concentrations of HCl, 0.001, 0.01, 0.1, 1 and 5 *M*, were used to prepare the PSG columns. The ratio of the monomer stock solution to toluene was adjusted to 15%. No monolith was formed when 0.001 or 0.01 *M* HCl was used. SEM micrographs (Fig. 1) show that a PSG monolith was formed in the capillary from reactions containing 0.1, 1, or 5 *M* HCl. It was considered that the concentration of HCl affected the hydrolysis reaction

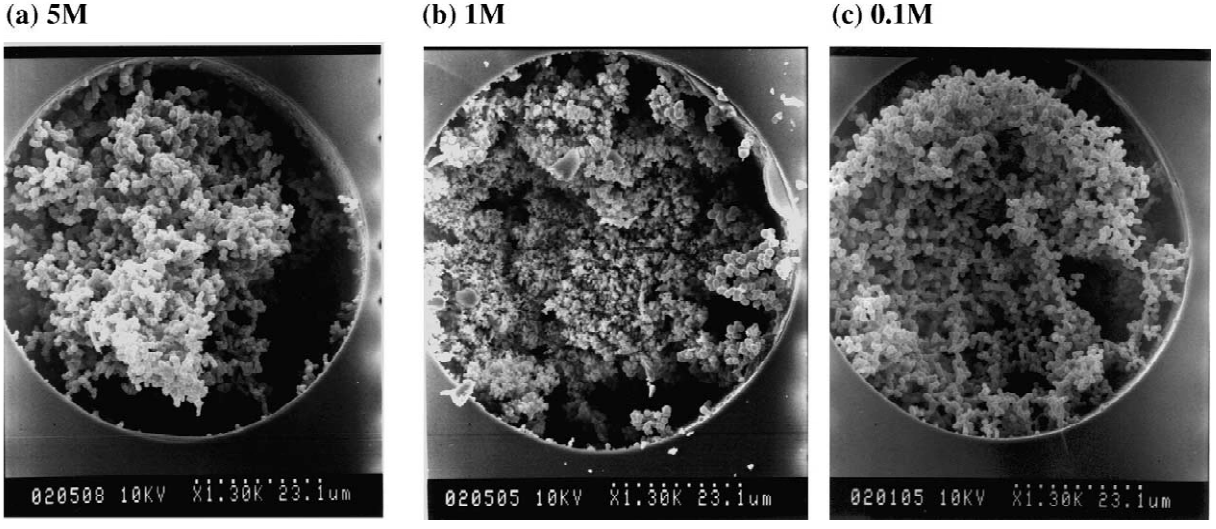

Fig. 1. Scanning electron micrographs of the PSG columns. (a) 5, (b) 1, and (c) 0.1 *M* HCl.

Table 1
Retention times of marker compounds on each monolithic column

HCl conc. (*M*)	Retention time (min)		
	Thiourea	Benzene	Naphthalene
0.1	3.04	3.18	3.37
1	3.35	3.54	3.82
5	3.52	3.71	3.87

and a low concentration of HCl does not produce enough silanol group to form a monolithic network. However, differences in the sizes of the mesopores could not be determined by SEM.

Table 1 shows the retention times of the three test compounds: thiourea, benzene, and naphthalene. The retention time of each analyte increased with increasing HCl concentration in the reaction solution. The durability of the PSG monolith was significantly affected by the concentration of HCl. The PSG column prepared with 0.1 *M* HCl readily formed cracks during separation of the analytes. These cracks were observed by SEM (about 1500× magnification). The cracks resulted in a dramatic reduction of the theoretical plate numbers by one-half or one-third. It is believed that, at low hydrochloric acid concentrations in the reaction solution, the rates of hydrolysis and condensation decrease. This results in a decrease in the rigidity of the PSG monolith. The best results were obtained with 1 or 5 *M* hydrochloric acid in the reaction solution.

3.2. Effect of the volume of monomer stock solution in the reaction solution

Photopolymers having morphologies with different permeabilities and surface areas were prepared by varying the ratio of the monomer stock solution to toluene (porogen). The porogen acts as a through-pore template and solubilizer of the silane reagent during the reaction [23,30]. The amounts of the monomer stock solution used were 5, 10, 15, 20, 30 and 40%. The amounts of photoinitiator and HCl were kept constant at 8.9 mg and 1 *M*, respectively. Fig. 2 shows SEM micrographs of three different PSG columns. Fig. 1a shows a capillary with the internal wall coated with PSG material when a monomer stock solution–toluene ratio of 5:95 (5% PSG column) was used to prepare the PSG monolith. No PSG material was formed in the center of the capillary. A PSG monolith is formed, however, when monomer stock solution–toluene ratios of 10:90 (10% PSG column), 15:85 (15% PSG column, Fig. 2b), and 20:80 (20% PSG column) were used. The SEM micrographs of each shows a structure comprised of an interconnecting network of about 1-μm spherical structures, throughout which micrometer-

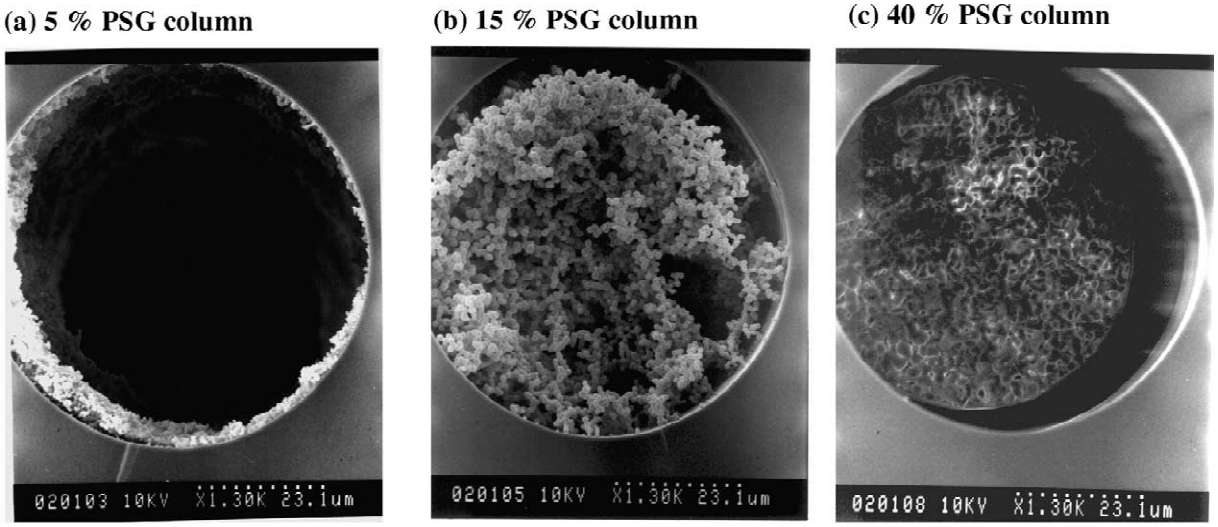

Fig. 2. Scanning electron micrographs of the PSG columns. (a) 5, (b) 15, and (c) 40% PSG column.

sized through-pores are interspersed. The morphologies of these PSG monoliths were similar to those previously reported [30]. The density of the PSG skeleton increased with decreasing through-pore size as the ratio of the monomer stock solution increased. A similar result was observed for monolithic columns prepared from tetramethoxysilane (monomer) and poly(ethylene oxide) (porogen) [23]. Fig. 2c shows a SEM micrograph of a monolith prepared from 40% monomer stock solution. There is a decrease in the number of through-pores due to the reduction of porogen (toluene), which serves as the through-pore template and a solubilizer during the reaction [23,30]. PSG columns with a 13 cm monolith positioned in the center of the capillary (total length, 30 cm; from inlet to detection point, 22 cm) were prepared to evaluate the separation properties of these columns. Monoliths that were formed from reaction solutions containing high percentages of monomer stock solutions resulted in much denser monoliths that were also less permeable than those made from lower percentages of monomer stock solution. Consequently, no liquid could be driven through the columns using a syringe because of the occurrence of significant back pressure in the columns. Liquid could be forced through a PSG column made from 18% monomer stock solution (18% PSG column) using a syringe. We reported recently, however, that a 27% PSG column was found to be permeable [30]. The differences in permeability between the 18 and 27% PSG columns may be due to differences in irradiation time and wavelength.

Fig. 3a and b show electrochromatograms of thiourea, benzene, and naphthalene on the 5% and 15% PSG columns, respectively. All three analytes coeluted on the 5% PSG column (Fig. 3a). Poor separation occurs, because the PSG material forms only at the inner wall of the capillary. Fig. 3b illustrates the separation of the three analytes in less than 4 min on a PSG column made from 15% monomer stock solution. Separation of the analytes was possible because the monolith is formed throughout the capillary, as shown in the SEM (Fig. 2b). Similarly, the three analytes were separated on 10 and 18% PSG columns within 5 min. The theoretical plate numbers of thiourea, benzene and naphthalene on the 15% PSG column were approximately 150 000, 150 000 and 110 000 plates/m,

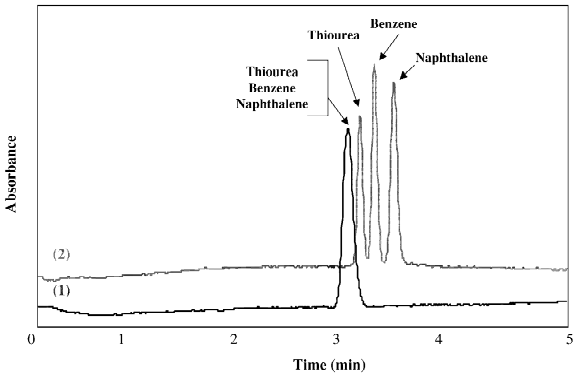

Fig. 3. Electrochromatograms of marker compounds. Mobile phase, 50 m*M* phosphate buffer (pH 8.0)–water–acetonitrile (1:3:6); capillary, (1) 5% PSG column, (2) 15% PSG column; packed length, 13 cm; applied voltage, 10 kV.

respectively. These theoretical plate numbers are comparable to or better than those previously reported for similar PSG columns [30].

3.3. Separation of alkylbenzenes by different PSG columns

Alkylbenzenes were used to study the separation mechanism on three PSG columns (10, 15, and 18% PSG). In our previous report, we demonstrated the reversed-phase nature of the PSG monoliths [30]. Separation solutions comprised of various ratios of ammonium acetate, water, and acetonitrile were used to separate a mixture of alkylbenzenes. Shorter elution times of alkylbenzenes were observed for the 10% PSG column as compared to the 15 and 18% PSG columns. The 10% PSG column is the most permeable of the three columns. An applied voltage of 5 kV was used for the 10% PSG column and 10 kV was applied for the other columns so that each alkylbenzene was eluted at a similar time on all PSG columns. Fig. 4 shows plots of carbon chain length vs. retention factor for the three different PSG columns and demonstrates the effect of different mobile phase conditions on the retention factors of the alkylbenzenes. The retention factor was determined using the equation:

$$k = (t_R - t_0)/t_0$$

where t_R is the analyte retention time and t_0 is the

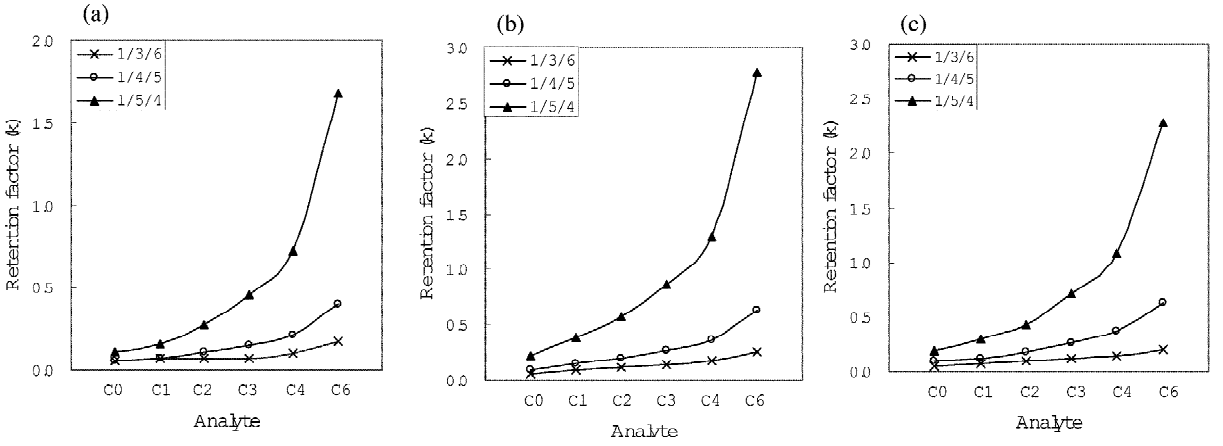

Fig. 4. Retention times of alkylbenzenes on PSG columns. (a) 10, (b) 15 and (c) 18% PSG column. The 50 m*M* ammonium acetate–water–acetonitrile ratios used were 1:3:6 (×), 1:4:5 (○) and 1:5:4 (▲).

retention time of the unretained marker, the first eluting peak. The retention factors increased with increasing volume of water in the separation solution for all PSG columns. The 10% PSG column shows the lowest retention for all the alkylbenzenes. This suggests that the through-pore size affects the retention of the alkylbenzenes. It is expected that the retention time will decrease with increasing permeability. The increases in retention factors of alkylbenzenes were similar in both the 15 and 18% columns, as shown in Fig. 4b and c, respectively. The retention factor of alkylbenzenes seems to depend on the surface area of the PSG, not the amount of PSG, because the surface area of the 18% PSG column does not increase with the PSG content, compared with that of the 15% PSG column, despite the increase in the PSG content.

The more hydrophobic alkylbenzenes, i.e. those with longer alkyl chains, eluted from the column later than those that were less hydrophobic, as expected. Fig. 5 illustrates the effect of the number of alkyl carbon atoms (from 0 to 6) on the retention factors of alkylbenzenes. Plots of the alkyl carbon number vs. log *k* were linear for the 10, 15, and 18% PSG columns. The linearity of the plots is indicative of the reversed-phase nature of the separation mechanism. The slope of the line for the 10% column (slope 0.20) is slightly larger than that for the 15% (slope 0.18) and 18% (slope 0.18) columns. These results indicate that the separation efficiency of the 10% PSG column is better than that of the 15 and 18% columns. The greater permeability of the 10% PSG column may contribute to the increased separation efficiency, because permeability is considered to be related to the linear velocity, which affects the separation efficiency [23].

Table 2 lists the theoretical plate numbers of naphthalene, toluene, and ethyl benzene. The 10 and 15% PSG columns showed higher theoretical plate numbers as compared to the 18% PSG column, which has the lowest permeability. The low permeability of this column is thought to be the cause of the decrease in the theoretical plate numbers of the analytes. In this study, the optimum through-pore size was clearly formed by 10 or 15% PSG, as determined by the theoretical plate number, as well

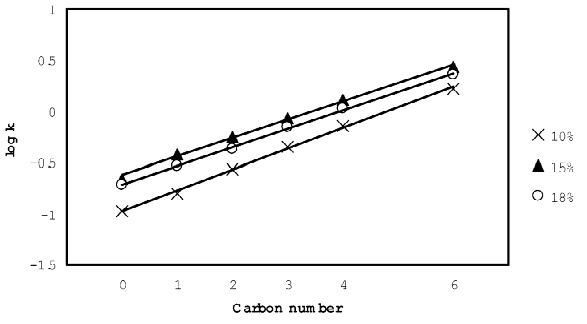

Fig. 5. Selectivity of alkylbenzenes on PSG columns. (×) 10%, (▲) 15%, (○) 18% PSG column. Mobile phase, 50 m*M* ammonium acetate–water–acetonitrile (1:5:4).

Table 2
Theoretical plate number of marker compounds on each PSG column

	Theoretical plate number		
	10%	15%	18%
Naphthalene	156 000	123 000	31 000
Toluene (C1)	165 000	168 000	40 000
Ethylbenzene (C2)	165 000	155 000	40 000

Mobile phase: 50 m*M* ammonium acetate–water–acetonitrile (1:3:6).

as permeability, retention time and separation factors.

4. Conclusions

In this study, PSG columns were prepared using different compositions of methacryloxypropyltrimethoxysilane, toluene, and hydrochloric acid. A reversed-phase mechanism was observed for all the PSG columns studied. The concentration of hydrochloric acid did not significantly affect the separation performance of the PSG columns. The durability of the PSG column, however, was improved by increasing the concentration of hydrochloric acid. The size of the through-pores and the surface area of the PSG monolith were controlled by varying the amount of monomer stock solution in the reaction solution. An optimum column permeability and surface area is achieved by controlling the size of the through-pores.

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