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Analyses of benzophenones by capillary electrochromatography using methacrylate ester-based monolithic columns

Hsi-Ya Huang *, Chen-Wen Chiu, I.-Yun Huang, Szetsen Lee

Department of Chemistry and Center for Nano Technology at CYCU, Chung Yuan Christian University, Chung-Li, Taiwan 320, ROC

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Abstract

In this study, eight benzophenones, which are commonly used as UV filters in various cosmetics and plastics, were analyzed by capillary electrochromatography with a methacrylate ester-based monolithic column. The effects of the composition and pH of mobile phase, porogenic solvent ratio, and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) content on benzophenone separations were examined. For all benzophenones, separation performances were markedly improved in monolithic columns with larger 1-propanol ratio and higher AMPS content. Furthermore, a twofold increase in AMPS content almost reduced the separation time in half when a monolithic column had an adequately high surface area, i.e. monolithic column was produced in a higher ratio of 1-propanol. As well, the retention behaviors of these analytes in the monolithic column were strongly influenced by the level of acetonitrile in the mobile phase, and the pH of the mobile phase also had an apparent influence on separation resolution.

Keywords: Benzophenones; CEC; Methacrylate ester-based monolithic columns

1. Introduction

It has been well documented that ultraviolet (UV) radiation causes sunburn, premature aging of the skin, development of skin cancers and cataracts, immune suppression and activation of latent viruses [1]. The UVB (290–320 nm wavelengths) component of the solar spectrum presents the greatest degree of risk to the development of cutaneous neoplasm, while the UVA (in the range of 320–400 nm) has also been found to attribute to skin photoaging. In response to the increasing threat to public health, national and international health authorities have advised the public to take protective measures against the harmful effect of UV radiation. Of all the recommended protective measures, sunscreens are often the most feasible to use, particularly during outdoor activities [2].

Inorganic UV filters (such as titanium dioxide or zinc oxide) and organic UV filters (synthetic organic chemicals), which can reflect or absorb harmful UV radiation, are commonly added to various sunscreens and plastic products in order to reduce the harmful influence of solar light on skin or plastics, and to prevent damage to cosmetics and plastics by sunlight during storage. Benzophenones that have excellent absorbing abilities for solar light in the spectrum range of 320–400 nm (UVA) are now one of the most widely used organic UV filters [3–4].

Presently, the detection of these benzophenones is carried out mainly by high performance liquid chromatography (HPLC) with reliable results [5–6]. In addition, some papers have also reported that analytical methods of benzophenones have been established successfully based on micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) [7–11]. To our knowledge, the usage of capillary electrochromatography (CEC) methods for the simultaneous determination of these hydrophobic benzophenones has not been reported.

Capillary electrochromatography (CEC) is a hybrid separation technique which combines the features of HPLC and capillary electrophoresis (CE), and has gained much atten-

* Corresponding author. Tel.: +886 3265 3319; fax: +886 3265 3399.
E-mail address: hyhuang@cycu.edu.tw (H.-Y. Huang).

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tion in recent years [12–13]. Many studies had demonstrated the potential applications of CEC for the separation of a wide range of compounds including charged and neutral analytes [14–15]. To date, various polymeric monolithic columns such as acrylamide-, styrene-, and methacrylate ester-based monolithic columns have been developed and used as the separation column in CEC [16–22].

A methacrylate ester-based monolithic column has several advantages over the other columns due to its easily adjustable polymeric polarity, its fine control of porous property of monolith, and its high stability even under extreme pH conditions [12,21]. Previous reports have demonstrated that methacrylate ester-based monolithic columns have high reproducibility and stability whenever they were employed to separate analyte standards or to analyze real samples [23–27].

In this study, benzophenone separations by a methacrylate ester-based monolithic column were examined. In order to optimize the analyses, the effects of the composition and pH of mobile phase, porogenic solvent ratio, and AMPS content on benzophenone separations were examined. Finally, the qualitative and quantitative performances of the analyses of benzophenones by an optimized CEC method using a methacrylate ester-based monolithic column were discussed.

2. Experimental

2.1. Chemicals and reagents

Benzophenone-1 (2,4-dihydroxy-benzophenone), benzophenone-2 (2,2′,4,4′-tetrahydroxy-benzophenone), benzophenone-3 (2-hydroxy-4-methoxy-benzophenone) were purchased from ACROS (Geel, Belgium). Benzophenone-6 (2,2′-dihydroxy-4,4′-dimethoxy-benzophenone), benzophenone-7 (2,2′-dihydroxy-benzophenone) and benzophenone-10 (2-hydroxy-benzophenone) were purchased from Aldrich (Wisconsin, USA). Benzophenone-5 (4,4-dimethoxy-benzophenone) and benzophenone-9 (4,4′-dihydroxy-benzophenone) were bought from Merck (Darmstadt, Germany). These eight benzophenone standards were individually dissolved in methanol at a stock concentration of 1 mg/ml. Octyl gallate was used as the internal standard for the separation of the benzophenones, and it was added to each of the standard or sample solution at a concentration of 1000 μg/ml prior to injection. Uncoated fused-silica capillaries with 100 μm I.D. and 375 μm O.D. were purchased from Polymicro Technologies (Phoenix, USA). All other chemicals were of analytical-reagent grade, and were used as received except for ethylene dimethacrylate (EDMA) and butyl methacrylate (BMA), which were purified by distillation under vacuum prior to use.

2.2. Apparatus

The CEC experiments were performed with a Beckman Coulter MDQ capillary electrophoresis system equipped with a photo diode-array detector (CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. A Waters instrument model 515 HPLC pump was used for washing and equilibrating the polymeric monolithic column. A scanning electron microscope S-4700 type (Hitachi, Japan) was used for morphology observation of the monolithic columns. A surface area analysis equipment model ASAP2020 (Micromeretics, USA) was employed for surface area measurement of the monoliths by a BET nitrogen adsorption and desorption method.

2.3. Preparation of polymeric monolithic column

Prior to the preparation of a polymeric monolithic column, the inner wall of a 100-μm I.D. capillary column was treated according to the following procedure. The capillary was conditioned by washing first with 0.1 M sodium hydroxide (2 min), followed by deionized water (10 min), and finally with methanol (2 min). After the capillary was dried by N_2 gas, it was filled by syringe injection with 3-(trimethoxysilyl)propyl methacrylate (MSMA) mixed with methanol in a volume ratio of 1:1. Both ends of the capillary were then sealed and submerged overnight in a 35 °C water-bath. Finally, the capillary was washed with methanol (5 min), then with water (5 min), and dried by N_2 gas.

After conditioning, the preparation of monolithic column was carried out as reported by Peters et al. [20]. Azobisisobutyronitrile (AIBN) (0.024 g, 1.0% (w/w) of monomer) and various ratios of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) (0.3–0.9% (w/w) of monomer, 0.007–0.022 g) were dissolved in 2.4 g of monomer mixture, which included 60% (w/w) BMA and 40% (w/w) EDMA. Ternary porogenic solvent, which consisted of water (0.36 g), 1,4-butanediol (1.09 g) and 1-propanol of various amounts (2.10–6.01 g, 59.2% (w/w)–80.6% (w/w) of ternary porogenic solvent), was slowly added to the monomer mixture. The solution was mixed under N_2 for 10 min until it became homogeneous, then it was used to fill the preconditioned capillary (33 cm) to a total length of 20 cm by syringe injection. The remainder of the homogeneous mixture was sealed in a glass vial. After both ends of the capillary were sealed with adhesive resin, the capillary and the glass vial were submerged in a 61 °C water-bath for 20 h. The monolithic column was then washed with methanol and mobile phase by a LC pump. A detection window was fabricated by using a microtorch to remove the polyimide coating at the 20-cm position on the column, where a polymer bed was absent. The monolithic polymer formed in the vial was Soxhlet extracted with methanol for 17 h, and then vacuum dried overnight.

2.4. Operation condition for CEC

The monolithic column was placed in the CE instrument and was equilibrated with the mobile phase under 15 kV applied voltage and 80 psi pressure at both ends of the column until a stable baseline was obtained. Samples and standards were electrokinetically injected into the capillary for 2 s at a

voltage of 5 kV. Separations were carried out using an electrical voltage of 25 kV, and the temperature of the capillary was maintained at 25 °C, while 200 nm was selected as the detection wavelength. Mobile phases were prepared by mixing acetonitrile and phosphate buffer (5 mM sodium dihydrogen phosphorate) in different volume ratios, and 1.0 M HCl was then added to mobile phase solutions until the desired pH was achieved (pH 3.0, 5.1, or 7.0).

2.5. Real sample and pretreatment

Commercial sunscreen samples were obtained from supermarkets in Taiwan. In order to be analyzed by CEC, 0.05 g of sunscreen lotion was mixed with 5 ml of acetonitrile. This mixture was sonicated for 15 min and was centrifuged for 10 min at 6000 rpm, and the resulting clear liquid was then ready for CEC analysis.

3. Results and discussion

3.1. Effect of the composition of mobile phase

A methacrylate ester-based monolithic column produced with 67.4% (w/w) 1-propanol and 0.3% (w/w) AMPS, which was able to produce an adequate EOF and had good separation ability in a previous report [27], was first employed to separate eight similar benzophenones in this study. First, a series of mobile phase composed with different percentage of acetonitrile was used in the following experiments.

Fig. 1 shows the electropherograms of eight benzophenone standards separated by different percentages of acetontirile in the mobile phase. The methacrylate ester-based monolithic column was unable to provide a good separation of the benzophenones when the percentage of acetonitrile was at 70% or 60%. A baseline separation, however, was achieved when the acetonitrile level was decreased to 50%. These results implied that a reversed-phase chromatographic effect was indeed followed in this system because the percentage of acetonitrile had an obvious influence on the partition behavior of analytes with the polymeric monolithic stationary phase. Even though separation resolutions for all analytes were greater than 2.0, however, the separation took a relatively long time (almost 24 min) when acetonitrile was maintained at 50% (Fig. 1(c)).

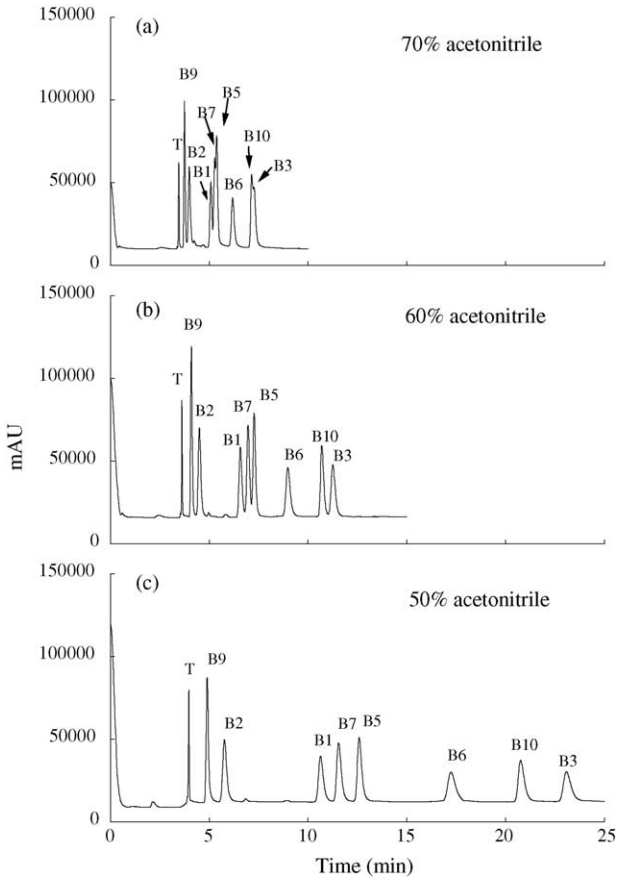

Fig. 1. Effect of acetonitrile level on the electrochromatographic properties of monolithic capillaries. Monolithic capillary was prepared by 67.4% (w/w) 1-propanol. Mobile phases of pH 3.0 were composed of phosphate buffer and acetonitrile in the volume ratio of (a) 30:70, (b) 40:60, and (c) 50:50. Separation conditions: 25 kV voltage was applied to a capillary tube of 33 cm (20 cm active length filled with monolithic stationary phase). Each analyte (500 μg/ml) was electrically injected by 5 kV for 2 s. Benzophenone-1 (B1), benzophenone-2 (B2), benzophenone-3 (B3), benzophenone-5 (B5), benzophenone-6 (B6), benzophenone-7 (B7), benzophenone-9 (B9), benzophenone-10 (B10), and thiourea (T).

3.2. Effect of porogenic solvent ratio

It is known that the pore size of a methacrylate ester-based monolithic column could be varied by changing the composition of porogenic solvent that is used to blend with the monomer mixture prior to polymerization [17]. In order to observe the porous property of the monoliths prepared in our laboratory, both BET method and scanning electron microscopy (SEM) were used to measure the monoliths. Similar to previous reports [12,17], the monoliths produced in our laboratory also had a smaller pore size and a higher surface area when 1-propanol level in the porogenic solvent was increased (Table 1). Since the pore size of polymeric monolith can effectively alter the separation performance in a CEC

Table 1
Surface area of various monolithic capillaries prepared in the study

AMPS level (%, w/w)	1-Propanol level in porogen solvent (%, w/w)	Surface area[a] (m^2/g)
0.3	59.2	3.8
	67.4	13.5
	71.3	22.7
0.6	67.4	15.2
	76.9	24.7
	80.6	33.6
0.9	67.4	19.7

[a] Values were measured by a BET method.

system, these methacrylate ester-based monolithic columns produced by different levels of 1-propanol were employed to separate eight benzophenones in the following experiments, in which AMPS was maintained at 0.3% (w/w), a widely used level in most reports related to methacrylate ester-based monolithic column [12,20–21]. The monolithic column prepared by 59.2% (w/w) or a lower 1-propanol level did not have any separation ability for the analyte standards, even though the level of acetonitrile in the mobile phase was reduced from 70% to 50% (Fig. 2(a) and the inset of Fig. 2(a)). On the other hand, baseline separations for the benzophenones were obtained when a higher level of 1-propanol was used (67.4% (w/w) or 71.3% (w/w)), in which the level of acetonitrile in mobile phase was maintained at 50% (Fig. 2(b) and (c)). Fig. 2 also shows the methacrylate ester-based monolithic column produced by 71.3% 1-propanol had a higher resolution and a longer separation time (34 min) when compared to the two columns produced with 59.2% (w/w) or 67.4% (w/w) 1-propanol. These results indicated that a smaller pore size and a higher surface area of monolithic stationary phase were

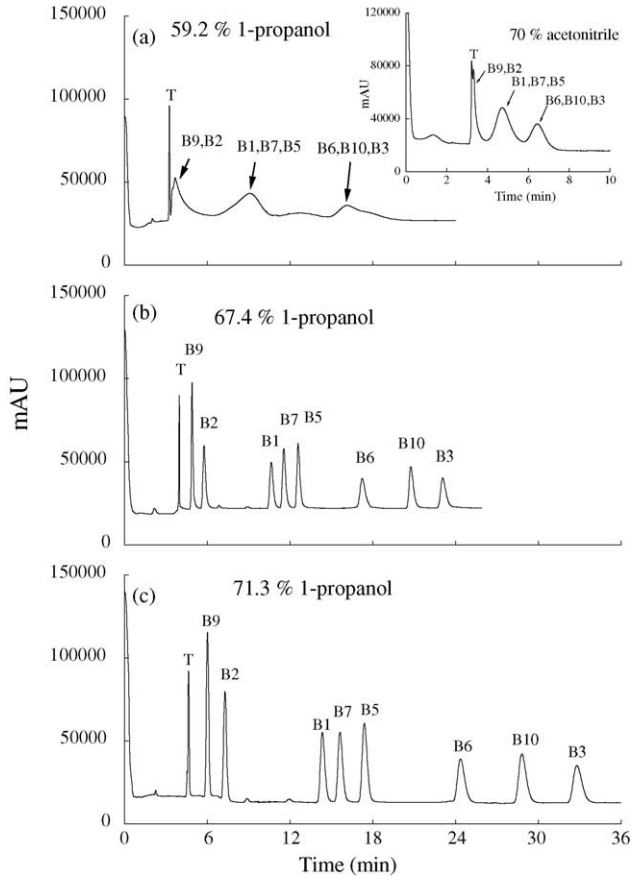

Fig. 2. Effect of percentage of 1-propanol on the electrochromatographic properties of monolithic capillaries produced by 0.3 wt.% AMPS. Monolithic capillaries were prepared by (a) 59.2%, (b) 67.4% and (c) 71.3% (w/w) 1-propanol, respectively. Separation conditions: pH 3.0 mobile phase was composed of phosphate buffer and acetonitrile in the volume ratio of 50:50 for Fig. 2(a)–(c), and 30:70 for the inset of Fig. 2(a). All other separation conditions were the same as in Fig. 1.

indeed able to improve separation behavior for the analytes. However, a longer separation time was needed to achieve a baseline separation for the eight benzophenones when a higher level of 1-propanol was used (for instance, 34 min for 71.3% (w/w) 1-propanol).

3.3. Effect of AMPS content

Even with the percentage of 1-propanol in the porogenic solvent and the level of acetonitrile in the mobile phase at the optimum levels, the time that was needed to separate these benzophenones was still too long. In order to speed up the benzophenone analyses and reduce peak spreading, the EOF in the monolithic column needed to be further increased. As the magnitude of EOF velocity in CEC is highly dependent upon the amount of charged sites on the surface of the stationary phase (in this case, the number of sulfonate group from AMPS determined the EOF strength), thus monolithic columns prepared by a higher level of AMPS (0.6% or 0.9% (w/w)) were used to separate the benzophenones. Similar to the separation behavior in monolithic column prepared by 0.3% (w/w) AMPS, benzophenone separations still took almost 22 min for 0.6% (w/w) AMPS, and 18 min for 0.9% (w/w) AMPS, when the level of 1-propanol was maintained at 67.4% (w/w) (Fig. 3). These results indicated that increasing the content of AMPS did not markedly influence the EOF velocity. Alternatively, by increasing both the levels of AMPS (from 0.3% (w/w) to 0.6% (w/w)) and 1-propanol (from 67.4% to 80.6% (w/w)) while keeping the acetonitrile content at 50%, the separation time of the eight benzophenones was shortened noticeably from 23 min to 13 min (Fig. 4). This result was relatively unusual because in previous reports and in the results reported above in this study it has been demonstrated that with an increase in the level of 1-propanol, the pore size of monolith was effectively reduced, and thus would cause a smaller EOF and a slower separation velocity (Fig. 2). In this case, the EOF and separation speed, however, were markedly enhanced by increasing the level of 1-propanol in porogenic solvent when the level of AMPS remained unchanged (i.e. 0.6% (w/w)) (Fig. 4). The SEM micrographs indicated that the monoliths were composed of linked nodules, and their diameters did not change significantly by the increase in the AMPS level (Fig. 5(a), 0.3% (w/w) AMPS and (b) 0.9% (w/w) AMPS while 1-propanol level was kept at 67.4% (w/w)). On the other hand, the diameters of the nodules were markedly reduced with an increase of 1-propanol (Fig. 5(c), 67.4% (w/w) 1-propanol and Fig. 5(d), 80.6% (w/w) 1-propanol while AMPS level was kept at 0.6% (w/w)). In order to examine the influence of AMPS level or 1-propanol content on the surface area of these monolithic columns, a BET method was used for surface analyses of the monoliths. The results indicated that the monoliths had similar surface area when only AMPS level was altered while 1-propanol was maintained at 67.4% (w/w) (for instance, 13.5 m^2/g for 0.3% (w/w) AMPS, 15.2 m^2/g for 0.6% (w/w) AMPS, and 19.7 m^2/g for 0.9% (w/w) AMPS) (Table 1).

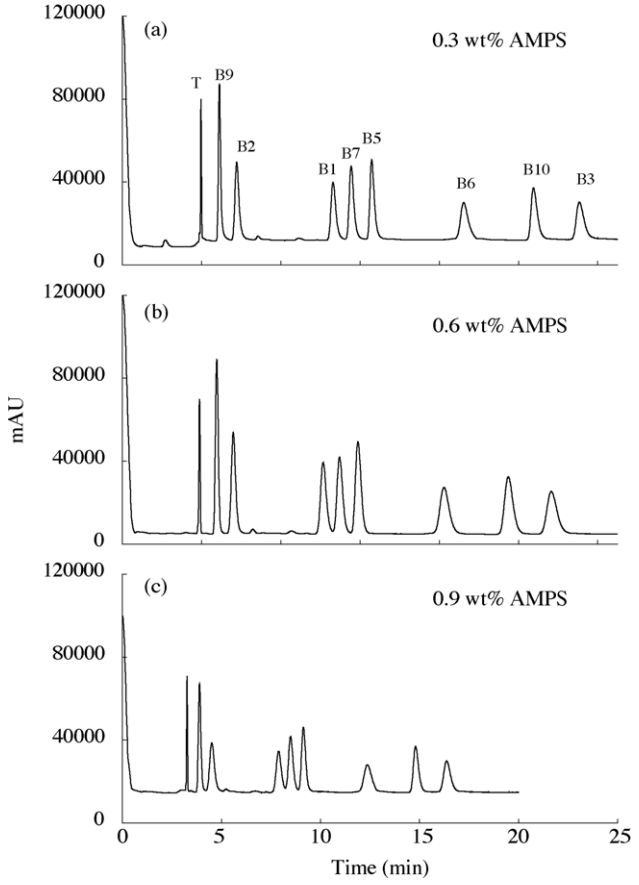

Fig. 3. Effect of AMPS content on the electrochromatographic properties of monolithic capillaries. Monolithic capillaries were prepared by (a) 0.3% (w/w) AMPS, (b) 0.6% (w/w) AMPS, and (c) 0.9% (w/w) when 1-propanol level was maintained at 67.4% (w/w). Separation conditions: pH 3.0 mobile phase was composed of phosphate buffer and acetonitrile in the volume ratio of 50:50. All other separation conditions were the same as in Fig. 1.

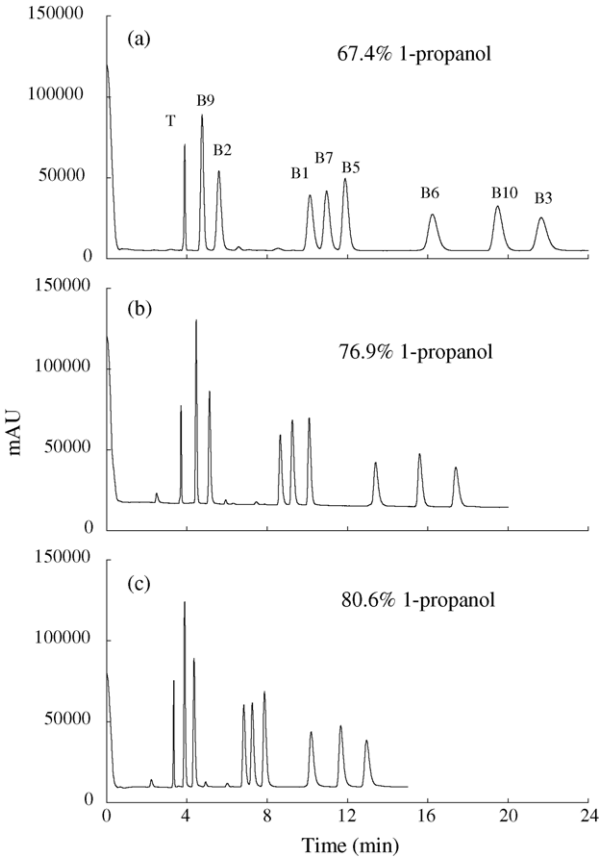

Fig. 4. Effect of percentage of 1-propanol on the electrochromatographic properties of monolithic capillaries produced by 0.6% (w/w) AMPS. Monolithic capillaries were prepared by (a) 67.4%, (b) 76.9% and (c) 80.6% (w/w) 1-propanol, respectively. Separation conditions: pH 3.0 mobile phase was composed of phosphate buffer and acetonitrile in the volume ratio of 50:50. All other separation conditions were the same as in Fig. 1.

Alternatively, the surface area of the monolith increased greatly from 15.2 m^2/g to 33.6 m^2/g, only by increasing 1-propanol from 67.4% (w/w) to 80.6% (w/w) with AMPS maintained at 0.6 wt.%. Hence, based on the results from SEM and surface areas, it was concluded that 1-propanol level had a greater influence than AMPS content on the size of linked nodules and the surface area.

Upon further examination of Figs. 3 and 4, it appeared that an increase in 1-propanol level had a more significant enhancement of EOF strength and benzophenone separation speed than an increase in AMPS content (the source of negative charge on the surface of monolithic column). A possible explanation for these unusual results could be the amount of AMPS that could be incorporated into a monolithic column was limited when a monolith had a lower surface area likely due to electrostatic repulsion between sulfonic acid entities (i.e. AMPS). For a monolithic column prepared by 67.4% (w/w) 1-propanol, in which a lower surface area was formed, only partial amount of AMPS could be allowed to chemically bond into the monolith, even though 0.6% or 0.9% (w/w) AMPS was added into the monomer mixture, as a result the magnitude of EOF and the migration speed of eight analytes were only slightly enhanced when compared to the addition of 0.3% (w/w) AMPS (Fig. 3). In contrast, if the surface area of monolith was high enough (for example, 33.6 m^2/g for 80.6% (w/w) 1-propanol), more AMPS monomers were able to actually attach to the monolith because no electrostatic repulsion is produced [19]. Consequently, the EOF and analytes' migration velocities could be sped up simply by simultaneously increasing the levels of 1-propanol and AMPS, for example, 80.6% (w/w) 1-propanol and 0.6% (w/w) AMPS, in which a baseline separation was achieved within 13 min.

3.4. Effect of the pH of mobile phase

Previous reports have confirmed that methacrylate-based polymeric monolithic columns are highly stable even when applied under extreme pH conditions (pH 2 or 12) [12]. Therefore, mobile phase pH would not be limited in this type of polymeric column, however, the influence of mobile phase pH on benzophenone separation had to be further evaluated.

For most CEC system, a sample is usually introduced into the capillary by electrokinetic injection, thus the amount of

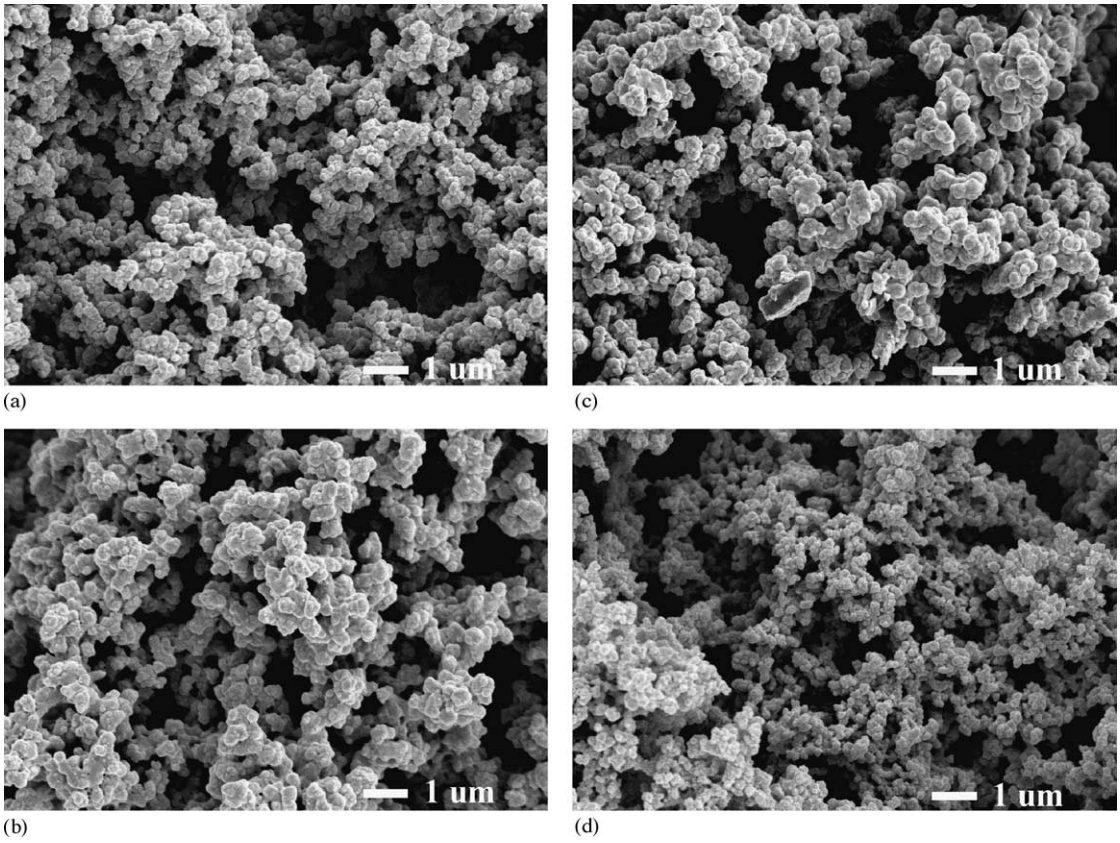

Fig. 5. SEM micrographs of methacrylate ester-based monolithic columns. Monolithic capillaries were prepared by (a) 0.3% (w/w) AMPS and (b) 0.9% (w/w) AMPS when 1-propanol level was maintained at 67.4% (w/w); (c) 67.4% (w/w) 1-propanol and (d) 80.6% (w/w) 1-propanol when AMPS level was maintained at 0.6% (w/w) AMPS, respectively. The magnification was 10 k for all graphs.

injected sample would probably be affected by the carried charges of analytes. All benzophenones were successfully electrokinetic injected into the monolithic column prepared by 0.6% (w/w) AMPS and 80.6% (w/w) 1-propanol when a pH 3.0 mobile phase was used because these benzophenones were all neutral (Fig. 6(a)). Although many benzophenones carry negative charges in higher pHs (for example, in pH 7.0, the ratio of the anionic analytes was almost in the range 24–61% for these benzophenones), however, the monolithic column prepared by 0.6% (w/w) AMPS provided an adequate EOF that allowed the benzophenones to be reproducibly electrokinetic injected in a pH 7.0 (Fig. 6(c)) or pH 9.0 mobile phase (data not shown). Previous reports have indicated that separation of acidic analytes on the current column can only be performed at acidic pH where they are neutral [27]. A wider pH range of mobile phase, however, was allowed to be used for acidic benzophenones if the monolithic column was prepared in a higher AMPS level.

Further examination of the influence of mobile phase pH on the separation of these analytes indicated that an increase in mobile phase's pH caused some analytes to decrease in their separation resolution, especially for benzophenones-1, -5, and -7 (Fig. 6). The change in resolution with different pH was probably due to a variation in the ionized degree of the benzophenone with the pH of the mobile phase, e.g. a higher pH would cause a higher ratio of benzophenone anions to be produced. Hence, in addition to the chromatographic effect between the analytes and the methacrylate ester-based polymeric stationary phase, an electrophoretic effect of the charged analytes also played a role in the separation behavior of these benzophenones. Consequently, the separation behavior in a lower pH (for example, pH 3.0) was completely based on different retention factors of these analytes by the methacrylate-based polymeric stationary phase. The differences in analytes' electrophoretic mobilities also further altered benzophenone migration when a higher pH of mobile phase was employed, however, this led to a reduction in separation resolution of benzophenones.

3.5. Qualitative and quantitative performance

The above results demonstrated that a monolithic column prepared with higher 1-propanol and AMPS levels improved separation efficiency and shortened separation time for these benzophenones, and a mobile phase with a lower pH could enhance their separation resolutions. Therefore, a mobile phase with a pH of 3.0 and composed of acetonitrile and phosphate buffer in the volume ratio of 50:50 was chosen as the optimal condition for the analyses of eight benzophenones when methacrylate ester-based monolithic column produced

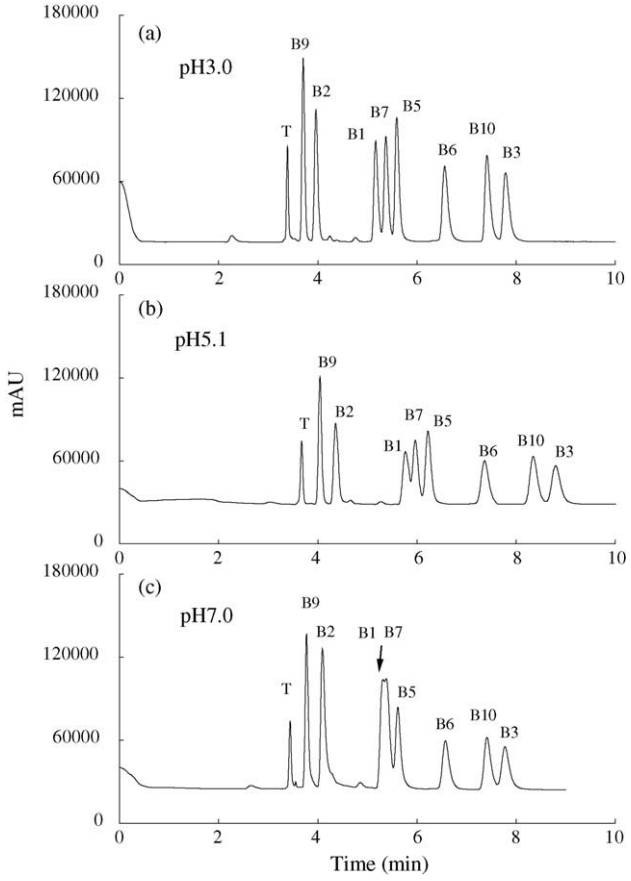

Fig. 6. Effect of buffer pH on the electrochromatographic properties of monolithic capillaries. Monolithic capillary was prepared by 80.6% (w/w) 1-propanol and 0.6% (w/w) AMPS. Mobile phases were composed of phosphate buffer and acetonitrile in the volume ratio of 40:60 at a pH of (a) 3.0, (b) 5.1, and (c) 7.0. All other separation conditions were the same as in Fig. 1.

by 80.6% (w/w) 1-propanol and 0.6% (w/w) AMPS was used as the separation column. Although all standards were completely separated under this condition, further examination of the qualitative and quantitative performances was needed. The repeatability of migration time and sample injection was examined based on three replicated injections of 2 s (5 kV) for 500 μg/ml standards, and the relative standard deviation (RSD) of migration time for the analytes were in the range of 0.29–0.45% (Table 2). The RSD of migration time for the analytes was in the range of 2.25–4.18% for three different columns prepared from the same polymerization mixture (Table 2). The above results indicated that the monolithic columns prepared in the study were highly reproducible and stable. The RSD of peak area for all analytes, which was represented as repeatability of sample injection, was in the range of 5.2–6.3%. Further to improve the repeatability of sample injection, an internal standard (octyl gallate) was used in the system, and the RSD of peak area was reduced to 0.58–1.05%, and this indicated that an internal standard could effectively enhance the quantitative ability of the CEC system. In addition, the correlation coefficients (r^2) of the calibration curves were greater than 0.998 for each of the analytes after internal standard calibration (Table 2). The detection limits for the benzophenones were in the range of 0.67–2.66 μg/ml based on S/N ratio of 3 (Table 2). The efficiencies for all analytes were in the range of 31 000–99 000 plate/m. These results indicated that this CEC method indeed provided relatively good qualitative and quantitative performances for the analysis of the eight benzophenones.

3.6. Real sample analyses

The electropherograms of commercially available lotions separated by CEC under optimal separating condition are shown in Fig. 7(a). Benzophenone-3 was determined without any interference in these products. The content of benzophenone-3 in the two products were in the range of 36.7–65.6 mg/g, and the RSD of the contents were in the range of 3.06–3.76% with triplicate measurements, thus indicating this method did provide a good quantitative reproducibility. In order to examine the separation ability for other benzophenones, two oil-based lotions spiked with other benzophenones were also separated under optimal condition. Consequently, benzophenones-1, -2, -5, -7, and -10 were

Table 2
Average migration times, reproducibility of column-to-column, theoretical plate numbers, detection limits and calibration curves of benzophenones standards[a]

Benzophenones	Migration time (min, %)[b]	Reproducibility of column-to-column[c] (%)	Theoretical plate numbers (N/m)	Detection limits (μg/ml)	Calibration curves[d]	r^2
Benzophenone-9	3.88 (0.45)	4.18	99000	0.67	$Y = 0.0013X + 0.2143$	0.998
Benzophenone-2	4.35 (0.39)	4.03	31000	0.97	$Y = 0.0033X - 0.0487$	0.999
Benzophenone-1	6.82 (0.34)	3.14	66000	1.56	$Y = 0.0031X + 0.0335$	0.999
Benzophenone-7	7.24 (0.32)	2.99	56000	1.37	$Y = 0.0026X + 0.0242$	0.999
Benzophenone-5	7.85 (0.32)	2.67	60000	1.38	$Y = 0.0034X + 0.0297$	1
Benzophenone-6	10.16 (0.31)	2.36	57000	2.24	$Y = 0.0029X - 0.0132$	0.999
Benzophenone-10	11.63 (0.29)	2.62	65000	2.05	$Y = 0.0030X + 0.0290$	1
Benzophenone-3	12.91 (0.30)	2.25	67000	2.66	$Y = 0.0028X + 0.0206$	1

[a] Separation conditions: a pH 3.0 solution composed of a phosphate buffer (5 mM) with acetonitrile in a volume ratio of 50:50 was used as mobile phase, temperature was fixed at 25 °C, and 25 kV voltage was applied to a monolithic capillary tube with 21 cm of effective length.
[b] Values were means of three intra-day replicates. The value in parenthesis indicates the RSD of migration time in percentage.
[c] The RSD of migration time for three different columns prepared from the same polymerization mixture represented reproducibility of column-to-column.
[d] The calibration curves were constructed from five replicate measurements at each concentration in the range of 50–1000 μg/ml. Octyl gallate (1000 μg/ml) was used as internal standard (IS) in the method.

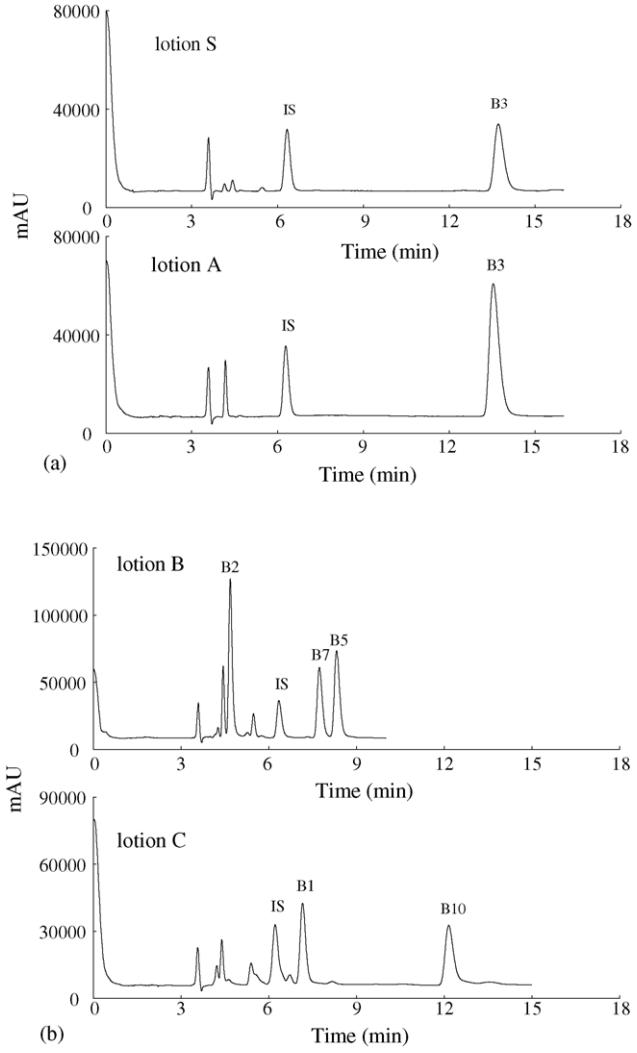

Fig. 7. The electropherograms of commercial products determined by monolithic capillary CEC method. Monolithic capillary was prepared by 80.6% (w/w) 1-propanol and 0.6% (w/w) AMPS. Mobile phases (pH 3.0) were composed of phosphate buffer and acetonitrile in the volume ratio of 50:50. Octyl gallate was used as internal standard (IS).

determined in the lotions without any interference (Fig. 7(b)), and the recoveries of these spiked benzophenones were between 97.7% and 99.0%. These results demonstrated that the sample pretreatment used in the study was suitable, and the CEC method indeed possessed enough separation ability to analyze the benzophenones in these commercial products.

4. Conclusion

In this paper, a CEC method using methacrylate ester-based monolithic column was developed for analyzing eight benzophenones commonly used as UV filters in commercial products Surface area of monolith was found to have strong influence on the amount of AMPS that can be bonded to the monolith. In addition, increases in AMPS level can effectively shorten separation time only when the ratio of 1-propanol in the porogenic solvent is simultaneously raised. Finally, a methacrylate ester-based polymeric column produced by higher 1-propanol and AMPS levels was able to produce a better separation for the eight benzophenones under optimal condition.

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