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Separation of ethoxylated bisphenol A dimethacrylates in dental composite after derivatisation to ionisable amines by capillary zone electrophoresis

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Abstract

Bisphenol A ethoxylate dimethacrylates (Bis-EMA) are transformed into ionisable amines by derivatisation in order to make the analytes applicable to capillary electrophoresis. For this goal, piperidine was added onto the C=C double bond of the α,β-unsaturated ester group forming a tertiary amine with pK_a values between 9 and 10. Formation of the derivatives was confirmed by electrospray ionisation MS. Commercial Bis-EMA is a mixture of homologues with different number of ethoxy groups; it is characterised by the average number of the ethoxy groups in the chains. These homologues were resolved by capillary zone electrophoresis at pH 4. It is shown for the product with an average of four ethoxy groups per Bis-EMA molecule that about seven homologues can be baseline separated when differing by only one ethoxy group. For Bis-EMA with 30 ethoxy groups in average, about 23 homologues could be differentiated. The high resolution power of capillary zone electrophoresis enables characterisation of commercial dental composite material concerning the Bis-EMA constituents.

Keywords: Dental composites; Bisphenol A ethoxylate dimethacrylates; Ethoxylates

1. Introduction

Bisphenol A ethoxylate dimethacrylates [2,2-bis(4-methacryloxypolyethoxyphenyl)propane, Bis-EMA] are constituents of dental composite materials used for restoration of teeth, forming the continuous organic matrix after polymerisation. The main part of dental composite material consists of inorganic fillers, which are embedded into the polymerisable acrylates or methacrylates, representing up to 20% of the total material. Other constituents of the composite material are initiators for the radical-induced polymerisation of the acrylates, additives for stabilising the formulation before polymerisation and coupling agents on the interface of the filler particles [1–5].

Bis-EMA consists of the polymerisable methacrylate group, which is linked to bisphenol by oligo- or polyethoxy groups of different length (Fig. 1). The technical products are characterised mainly by the average number (m or n in Fig. 1) of ethoxy groups per phenol (EO/phenol), typical commercially available compounds have mean values for $m + n$ between 4 and 30.

It is obvious that the composition of the different composite materials and specimen is of interest for several reasons. Especially their monomeric constituents have relevance for technological and toxicological aspects [1,3,6–15], and therefore an adequate analytics is needed. Analysis is based so far mainly on chromatography occasionally in combination with mass spectrometry [6,8–10,15–20]. HPLC and HPLC–MS have been applied only for the homologue with 1 EO/phenol, and GC or GC–MS is mainly suited for small homologues due to the low volatility of the higher ones. Capillary electrophoresis (CE) was applied for the determination of the ionic methacrylic acid released from dental

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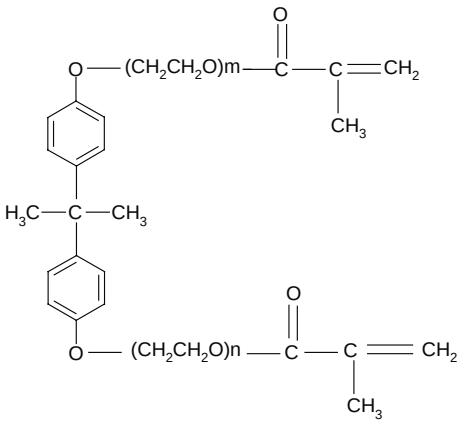


Fig. 1. Structural formula of Bis-EMA.

composites [7,21]. However, Bis-EMA cannot be analysed by capillary electrophoresis because the constituents are electrically neutral. We have therefore introduced recently micellar electrokinetic chromatography (MEKC) for the characterisation and determination of these polymerisable technical products, which gave indication of their multiple composition [22]. Indeed for commercial Bis-EMA with an average of 2 EO/phenol, 3 (non-resolved) peaks were obtained, in that with 15 EO/phenol about 10 peaks were separated. As the variability of the separation conditions in MEKC is not very wide, we intended to apply capillary zone electrophoresis (CZE) for a more detailed characterisation. Note that these two methods are based on different separation principles. Whereas MEKC uses the different extent of partitioning of the (commonly neutral) analytes between the aqueous phase and the micelles, separation in CZE is established according to the different electrophoretic mobility of the sample constituents in free solution. Thus, an obvious prerequisite for CZE is the presence of the analytes in an ionic form. It was therefore the goal of this work to transform the methacrylates into ionisable species. This was carried out by addition of a secondary amine to the C=C double bond of the α,β-unsaturated ester of the methacrylic acid in different Bis-EMA species. The resulting amines can be protonised in moderate to low pH background electrolytes (BGEs), and the charged ammonium ions separated without any further pseudo-stationary phases by CZE.

2. Materials and methods

2.1. Materials

Bisphenol A ethoxylate ($m + n \sim 4$; 2 EO/phenol) dimethacrylate and bisphenol A ethoxylate ($m + n \sim 30$; 15 EO/phenol) dimethacrylate (both technical quality) were from Aldrich (Steinheim, Germany). Formic acid was from Acros (NJ, USA), ammonium hydroxide solution from Fluka (Buchs, Switzerland), HPLC-grade acetonitrile (MeCN) from Baker (Deventer, The Netherlands), HPLC-grade ethanol from E. Merck (Darmstadt, Germany), piperidine from Sigma–Aldrich (St. Louis, MO, USA), sodium hydroxide for HPCE from Hewlett-Packard (Waldbronn, Germany). Commercial dental composite was from Degussa-Hüls, Hanau, Germany. Water was double distilled from a quartz apparatus prior to use.

2.2. Instrumentation

The electrophoresis experiments were performed on a ^{3D}CE instrument (Agilent, Waldbronn, Germany) equipped with a diode array detector. The measurements were carried out at +25 kV in uncoated fused-silica capillaries (Supelco, Bellefonte, PA, USA) of 61.5 cm total length (53.0 cm effective length, 75 μm i.d., 375 μm o.d.), with the cathode placed at the detector side. The capillary was thermostated at 20.0 °C. Samples were injected hydrodynamically from the anodic side of the capillary (30 mbar·s). Detection wavelength was 214 nm.

New capillaries were rinsed with 1 M NaOH for 10 min and bidistilled water for 5 min. At the beginning of every working day, the capillary was flushed with 1 M NaOH (10 min), water (5 min) and BGE (3 min). Between the runs, the capillary was washed with 1 M NaOH (3 min), water (1.5 min) and BGE (3 min). At the end of the day, the capillary was washed with pure MeCN (2 min), water (4 min), 1 M NaOH (5 min), water (5 min) and then air-dried for 3 min.

The BGE for final analysis consisted of 30 mM formic acid adjusted to pH 4.0 with ammonium hydroxide and 20% (v/v) MeCN (added after pH adjustment). BGEs were filtered (0.45 μm, Minisart, Sartorius) prior to use.

Electrospray ionisation (ESI) mass spectra were recorded with an instrument equipped with an ion trap analyser (Esquire3000plus, Bruker Daltonik, Bremen, Germany). The injected samples were diluted with 50% reaction solvent (see further) and 50% BGE to get the same conditions as with CZE. The samples were introduced through direct infusion with a syringe pump (74900, Cole-Palmer, London, UK) at 3 μl min^{-1}. The nebuliser gas (nitrogen) was set to 12 psi, the dry gas to 8 l min^{-1} at 300 °C (1 psi = 6894.76 Pa). Mass analysis was done in the standard resolution mode with m/z 13 000 s^{-1} scan rate.

2.3. Sample preparation

Derivatisation of the compounds was carried out following standard protocols (see [23]) with piperidine as recommended reagent. Different molar concentration ratios of piperidine to Bis-EMA were applied between 2:1 and 28:1 (note that each molecule Bis-EMA contains two methacrylate groups). For the 14:1 ratio, e.g. 1.08 g Bis-EMA, 2 EO/phenol (or 3.368 g Bis-EMA, 15 EO/phenol) was dissolved in 1.5 ml of solvent (named reaction solvent in the following to distinguish it from the BGE solvent);

the reaction solution for each compound consisted of 2.77 ml of piperidine and 0.6 ml of reaction solvent. The flasks containing piperidine dissolved in reaction solvent were cooled to about 10 °C, and the Bis-EMA solution was added very slowly under agitation. After mixing, the products were kept protected from light at room temperature (25 °C) for different times; finally, they were stored at 2 °C.

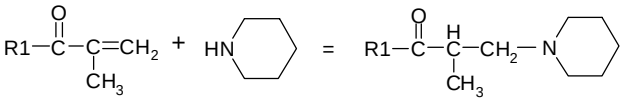
The samples were diluted just before analysis by reaction solvent; the final sample for CE analysis was dissolved in 1:1 volumes of reaction solvent and running BGE.

For sample preparation of commercial dental composite material, an amount of about 50 mg was dissolved in 200 μl of a solution consisting of piperidine and reaction solvent (volume ratio of 4:1) and sonicated for 10 min; the suspension was stored for 24 h at room temperature, protected from light and finally centrifuged at 11 000 × g. The supernatant solution was diluted following the same procedure as for standard samples and injected into the CE apparatus.

3. Results and discussion

3.1. Formation of derivatives

An amine, as a nucleophilic reactant, can add on the C=C double bond of an α,β-unsaturated ester under formation of an amine with one grade higher substitution; this reaction can be depicted for the present methacrylate and piperidine as reactants by the following reaction:



(1)

The tertiary amino group formed has a pK_a value around 9–10, and is thus protonated at not too high pH of the background electrolyte. As Bis-EMA has two methacrylate groups in the molecule, symmetrical bis-adducts can be formed.

Commercial Bis-EMA is of technical grade; it consists of a number of compounds, whereby the composition of the product is characterised by the manufacturer by an average sum ($m+n$) of ethoxy moieties per molecule. For Bis-EMA with, e.g. $m+n \sim 4$, we can expect as main compound that with $m = n = 2$, but also the isomer with 1 and 3 EO/phenol. Concerning its technical quality, homologues with $m+n =$ 3 (2 and 1) or 5 (3 and 2), can be expected as well, lower (1 and 1) or higher (4 and 2, and 3 and 3) homologues cannot be excluded.

The addition of piperidine will therefore not lead to a single reaction product, but will result in a larger number of constituents. For commercial Bis-EMA with higher numbers of $m+n$, even more homologues and isomers can be expected in the initial chemical. As they are not further specified, their ratio is unknown.

In order to select the appropriate reactions conditions for derivatisation, different parameters were modified: reaction time, concentration and mutual amount of reactants, solvent. The reaction yield was then derived assuming that it is proportional to the sum of the peak areas determined by CZE. The peaks were considered to stem from the reaction products when they were migrating ahead of the electroosmotic flow (EOF) marker in the electropherogram (as they must be cationic) and showed the same UV spectra like initial Bis-EMA. Residual, unreacted Bis-EMA could be distinguished as peak migrating with the EOF velocity.

Reaction was carried out at room temperature (25 °C) in the dark, because Bis-EMA seems to be sensitive to heat and light according to the description of the manufacturer. Aliquots were taken after different times of reaction, and analysed. It was found that at 25 °C, the reaction was not complete before 24 h. Two reaction solvents have been investigated, ethanol and MeCN; both turned out to be suitable for the reaction, but MeCN might be preferable as it leads to a slightly faster reaction.

In Fig. 2, the amount of products (expressed as the sum of the peak areas in the electropherograms of the different synthesis mixtures) formed upon reaction with Bis-EMA ($m+n = 4$) is given versus the molar excess of piperidine in relation to Bis-EMA applied (moles of piperidine to moles of Bis-EMA, taking a molar mass for the 2 EO/phenol homologue for the latter). As already mentioned, reaction was finally carried out at room temperature (25 °C) for at least 24 h. It can be seen that from a molar ratio of 14 on the yield remains about constant. This ratio was thus taken for further derivatisations. Under these conditions, the peak of the non-reacted Bis-EMA (migrating with the EOF) nearly disappeared in the electropherogram. Based on this finding,

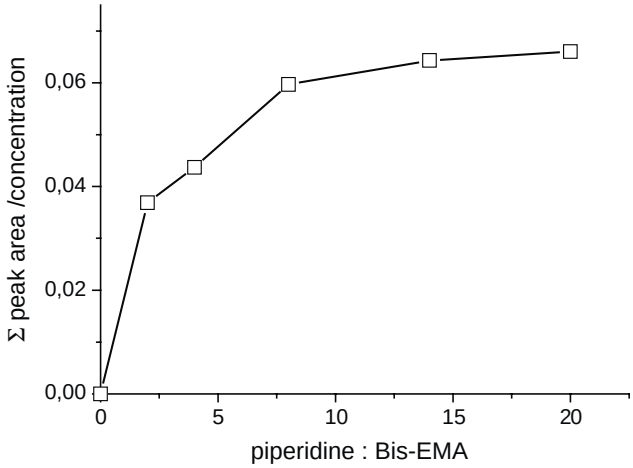


Fig. 2. Dependence of the sum of the peak areas of the reaction products on the molar ratio of piperidine to Bis-EMA (2 EO/phenol) in the reaction mixture. The areas, determined by CZE, were normalised to unit concentration (arbitrary units). Reaction temperature was 25 °C, reaction time was 24 h.

we concluded that the reaction yield was higher than about 95%.

3.2. Separation of derivatised homologues of BIS-EMA

For the development of an appropriate BGE for the separation of the analytes, the derivatisation products of Bis-EMA according to the reaction given in Eq. (1), a moderate or low pH must be chosen in order to charge the amines. This seems obvious due to the basic properties of the analytes. From the many possible BGEs, we selected formic acid/ammonium formate at pH 4, because this BGE is also suited to CE–MS. However, we have to take into account the relatively low water solubility of the derivatives; therefore, mixed aqueous–organic solvents may offer an advantage over pure aqueous BGEs. As MeCN is fully miscible with water, and has good solubility properties for organic compounds, it was added to the BGE at a concentration between 0 and 50% (v/v). Best separation was found with 20% MeCN; at higher content of the organic modifier resolution was worse, at 10% the same resolution was obtained as with 20%, but solubility problems arose.

The resulting electropherogram for Bis-EMA ($m+n \sim 4$; 2 EO/phenol) is shown in Fig. 3. A series of about normally distributed sharp peaks is seen between 6 and 8 min migration time (note that a second series of peaks—closer to the EOF marker—can also be observed, but they are present at much smaller quantity). These cationic compounds have mobilities between 21.46×10^{-9} and 17.07×10^{-9} $m^2\,V^{-1}\,s^{-1}$). The mobilities decrease nearly linearly ($r = -0.99947$; see insert in Fig. 3). All peaks show the same UV spectra as

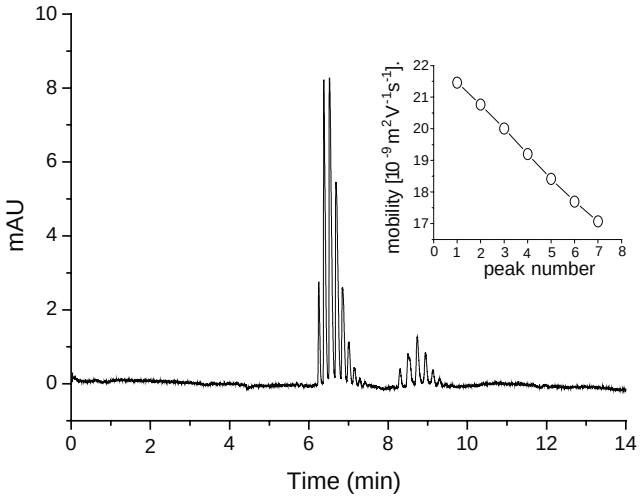


Fig. 3. Electropherogram of a derivatised sample of commercial Bis-EMA ($m+n \sim 4$). Insert: effective mobilities of the peaks migrating between 6 and 8 min, numbered from left to right. Conditions: uncoated fused-silica capillaries; 61.5 cm (53.0 cm effective length) × 75 μm i.d. × 375 μm o.d.; detector placed at the cathode side; BGE: 30 mM ammonium formate (pH 4.0) containing 20% (v/v) MeCN; temperature: 20.0 °C; voltage: 25 kV; pressurised injection: 30 mbar s; detection wavelength: 214 nm; concentration of Bis-EMA: 2 mg ml^{-1}.

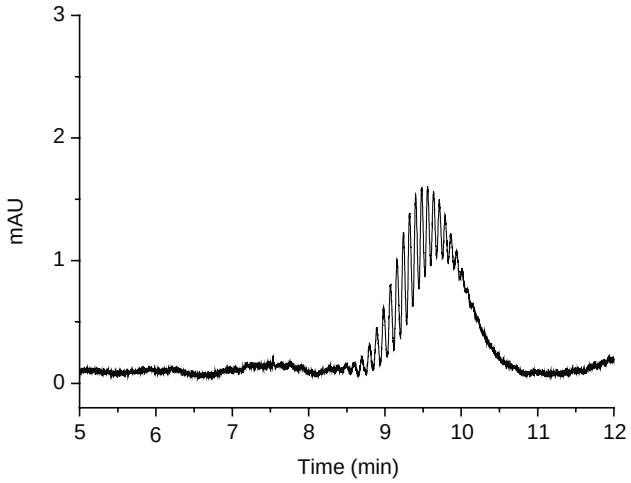


Fig. 4. Electropherogram of a derivatised sample of commercial Bis-EMA ($m + n \sim 30$). Conditions as in Fig. 3. Concentration of Bis-EMA: 2 mg ml^{-1}.

bisphenol A (spectra not shown). The number of the main peaks which can clearly be detected is about seven. The peak distribution has a maximum, probably from the homologue with 2 EO/phenol in average, but compounds with a lower average number of $m+n$— with higher mobility—and higher average number can also be distinguished, both with lower concentration. Given that all peaks represent indeed Bis-EMA derivatives, CE turns out to be an excellent separation method for these homologues.

It should be pointed out that separation does not change upon use of sodium in the BGE instead of ammonium (a BGE which is better suited for the combination of CZE with ESI-MS, which was, however, not used here).

The surprisingly high separation performance of CZE towards homologues is illustrated also for Bis-EMA (15 EO/phenol) with $m + n$ of about 30 in average, which has an even larger possibility to form homologues and isomers. The corresponding electropherogram (Fig. 4) indeed enables the differentiation of at least (whereas not fully resolved) 23 peaks. Taking into consideration the small differences between the homologues, e.g. with 30 or 31 EO groups in the molecules, the relatively high efficiency (expressed by more than 300 000 plates) allows the separation of two subsequent peaks (e.g. those at the left flank of the distribution) with a ratio of their actual mobilities of only 1.009.

3.3. Confirmation of amine formation by ESI-MS

In order to prove the formation of the derivatives, mass spectra were measured after electrospray ionisation. The sample was inserted into the mass spectrometer by infusion, not by on-line combination with the CE instrument. In the resulting superimposed mass spectra obtained from the reaction mixture (Fig. 5), main peaks with m/z 667.50, 711.51, 755.52, 799.53 are observed. Their difference is 44, which is equivalent to one CH_2CH_2O group. Indeed these masses

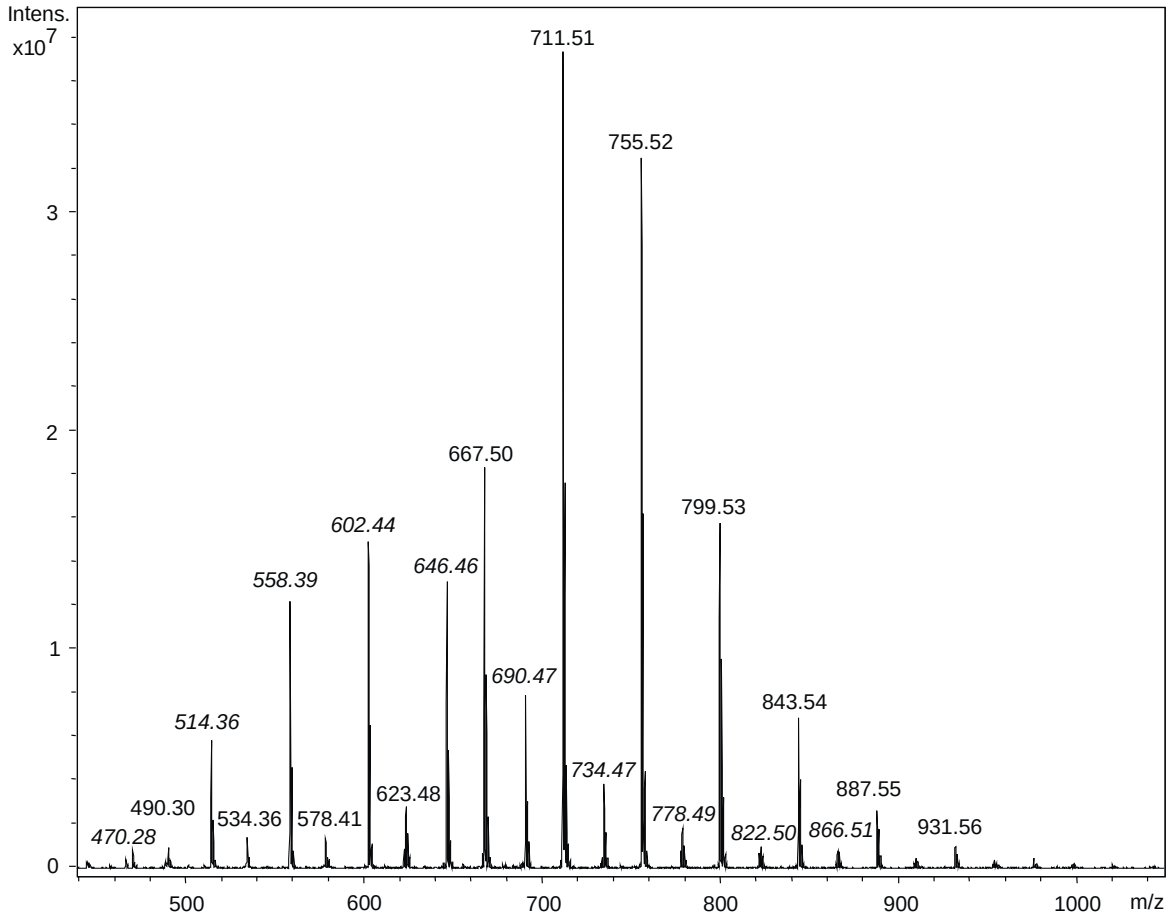


Fig. 5. Electrospray ionisation mass spectra of the derivatised commercial Bis-EMA ($m+n \sim 4$) sample. Sample mixture was introduced into the ESI-MS by infusion. For conditions see Section 2.

are identical with those of the di-derivatised dimethacrylates with $m + n$ equal to 3, 4, 5 and 6. Closer inspection of the mass spectra shows also peaks for $m + n$ equal to 2 and 7, 8 and 9 (623.48, 843.54, 887.55 and 931.56). This result confirms the formation of the di-derivatised Bis-EMA products by addition of piperidine onto the C=C double bond of the methacrylate group under formation of the tertiary amine according to Eq. (1).

We have mentioned earlier that a second series of (minor) peaks is seen in the electropherogram of Bis-EMA, 2 EO/phenol (Fig. 3). A second series of peaks appears also in the mass spectra (*m*/*z* 558.39, 602.44, 646.46, 690.47). These compounds seemingly belong to a series which also differ by 44 mass units. From their sum formula, it can be derived that they are mono-derivatised Bis-EMA compounds, which have only one methacrylate group attached at one ethoxy chain; at the second ethoxy chain the methacrylate group is substituted by H. As they can be also found in the reaction mixture diluted with non-aqueous acetonitrile (which excludes their formation by hydrolysis of the dimethacrylates), they are assumed to be present as contaminants in the initial technical Bis-EMA product, perhaps due to incomplete synthesis during production.

3.4. Analysis of composite materials

One typical commercial dental composite material has been analysed after the derivatisation procedure as given earlier. It has the typical composition with fillers, reaction starters, etc. as described in Section 1. According to the product sheet, this material contains Bis-EMA, but in an unspecified form. The resulting electropherogram of the material is shown in Fig. 6. Several sharp peaks can be distinguished (note the extended time scale). Comparison with the mobilities derived for the Bis-EMA samples (compare with Fig. 3) allows the conclusion that the main peak in Fig. 6 stems from aminised Bis-EMA with $m + n = 4$, accompanied by those with $m + n = 5$, 6 and 7. The separation of these homologues is remarkable, as they have the same charge number and differ in mass only by about 1%.

A quantitative estimation for the composite material by comparing the sum of the peak areas with those obtained from the product Bis-EMA with $m + n \sim 4$ gives a content of Bis-EMA of about 6.9% (w/w). This is in the commonly given composition range. More detailed data about reproducibility of quantitation, etc. are not of interest for the present topic, they are thus not given here.

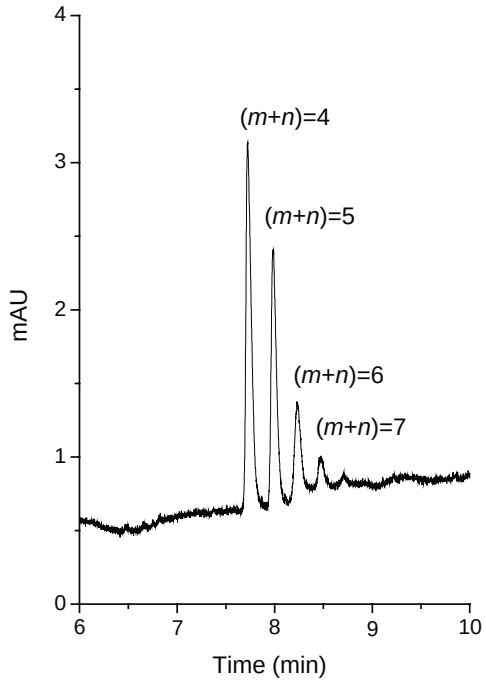


Fig. 6. Electropherogram of commercial composite material. Experimental conditions as in Fig. 3. Concentration of composite material: 2.5 mg ml^{-1}.

4. Conclusions

Transformation of the electrically neutral methacrylates by addition of an amine to ionisable amino derivatives make them suitable to electrophoresis. The high resolving power of CZE enables separation of the derivatised homologues of Bis-EMA differing only by one ethoxy group in the chain. Separation could be carried out under moderately acidic conditions, with MeCN added to the BGE. The separation is relatively indifferent concerning changes in the kind of electrolyte constituents of the BGE. Compared to MEKC, an about two times larger number of analytes can be differentiated for the same sample.

Some critical comments should be added in the following. One is related to the handling of the samples, which must take into account the limited solubility of Bis-EMA in aqueous solutions. To enhance solubility, mixed solvents were used mainly with MeCN as organic constituent. However, even in this case the final concentration should not exceed 3 mg ml^{-1} for Bis-EMA (2 EO/phenol) and 17 mg ml^{-1} for Bis-EMA 15 EO/phenol; higher concentrations led to solubility problems in the final injection solution (consisting of BGE and MeCN, 50%, v/v, each). For the same reason, the final concentration of the commercial composite material should not be higher than 5 mg ml^{-1}.

Problems arise sometimes with the reproducibility of the peak shapes, which might also be related to the solubility of the analytes. In these cases, distorted and asymmetric peaks were observed with worse resolution. We assume that this effects are caused by precipitation or adsorption of compounds at the capillary wall, especially at the injector side. This problem could be eliminated by flushing the capillary with pure MeCN from the detector side. Normally, the electropherograms were highly reproducible over weeks, with the usual precision of the migration times and peak areas, which is—expressed by the relative standard deviation—in the low percentage range.

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