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Determination of ethoxylated bisphenol A dimethacrylate monomers in dental composites by micellar electrokinetic chromatography

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Abstract

Separation of monomeric constituents of ethoxylated bisphenol A (BIS-EMA) with between 2 and 15 ethoxy groups per phenol in the molecule was investigated with micellar electrokinetic chromatography at different volume percentages methanol in the background electrolyte (10 m*M* sodium dodecylsulfate, 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0). The conditions allowed the differentiation of the lower from the higher BIS-EMA homologues and of isomers, and enabled the characterisation of commercial dental composite materials. The decay curve for the light induced radical polymerisation of BIS-EMA in composite specimens was determined. The content of leachable monomers after light curing was quantified, resulting in 6% of the initial value after the recommended curing time. The method is suited to determine monomer constituents in polymerised composite material in the ppm range.

Keywords: Dental composites; Background electrolyte composition; Bisphenol A dimethacrylate, ethoxylated

1. Introduction

Resin-based composites have been increasingly used by dentists for the restoration of posterior teeth due to the questionable biocompatibility of mercury containing alloys (dental amalgam). For this purpose new compounds have been developed with simplified handling procedures known as amalgam-alternatives with new formulations, which are constantly changed or improved by the manufacturers. Consumer demand for non-metallic restorations has also increased for aesthetic reasons.

Dental composites are pasty mixtures consisting basically of the following groups of components: (i) Polymerisable oligo- or polyfunctional acrylate or methacrylate monomers forming the continuous organic matrix after polymerisation. (ii) Glass, quartz, amorphous silica or other substances used as fillers, which are embedded in the organic polymerised matrix. (iii) Coupling agents on the interface of the filler particles. (iv) Initiators for radical-induced polymerisation of the matrix. (v) Additives to stabilise the formulations before polymerisation.

Whereas compounds from group iii–v are present at only low concentrations, group i and ii substances represent the main part of the composite material. Typically, the inorganic fillers range up to 70% and more, the content of the sum of the matrix forming

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acrylates and methacrylates can reach 20%, all w/w. These monomers are mainly either derivatives of bisphenol A or of different diols. It is obvious that even in case that a single substance is applied, it is not a chemically pure compound, but of technical quality; such products are typically mixtures of homologues and isomers.

The analytical determination of the monomer content in dental composite materials is of importance for a number of reasons. It has interest for product control, when the composition of the formulation has to be specified. It is clearly relevant also for consumer protection, since the exact composition of the materials is frequently not declared by the manufacturers. It is of interest also in the medical field, as the concentration of remaining monomers in the polymerised composite are considered to be responsible for toxic effects, which have been demonstrated by in vitro methods [1–3], and the toxic potential of a number of components of composite materials is at present unknown [3].

The present work deals with the determination of ethoxylated bisphenol A dimethacrylate (BIS-EMA) monomers, the one important class of matrix forming acrylates and methacrylates. Determination of leachable components from dental composites was carried out so far by chromatographic methods, occasionally in combination with mass spectrometry [4–13]. CE was applied for the determination of released methacrylic acid from dental composites [14,15]. GC and GC–MS, applicable for relatively volatile compounds, are not suited for the higher homologues of BIS-EMA, the compounds of interest in the present paper. With HPLC and HPLC–MS only the BIS-EMA homologue with one ethoxy-group per phenol was determined. We have taken into account that in practise a technical mixture consisting of a number of homologues and isomers might be applied. The present analytical method is based on micellar electrokinetic chromatography with sodium dodecylsulfate (SDS) as micelle formers, and should enable the quantitation of the BIS-EMA constituents, whereby there is no necessity to fully separate the individual homologues or isomers. The present method should provide determining monomeric BIS-EMA in commercial dental composites before an after light curing.

2. Materials and methods

2.1. Materials

Bisphenol A (99+%), bisphenol A ethoxylate (2EO/phenol, where EO=ethoxy group) dimethacrylate and bisphenol A ethoxylate (15EO/phenol) dimethacrylate (both technical quality) were from Aldrich (Steinheim, Germany). Boric acid, sodium dihydrogenphosphate monohydrate, sodium hydroxide and methanol (all analytical grade) were from E. Merck (Darmstadt, Germany). SDS was from Sigma–Aldrich (St. Louis, MO, USA). Commercial dental composite was Definite (Degussa-Dental GmbH., Hanau-Wolfgang, Germany). Water was double distilled from a quartz apparatus prior to use.

2.2. Instrumentation and procedures

The experiments were performed on a $HP^{3D}CE$ instrument (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector. Detection wavelengths were 200, 214, and 280 nm. The measurements were carried out at 20 kV in uncoated fused-silica capillaries (Supelco, PA, USA) of 36.5 cm (28 cm effective length)×50 μm I.D.×360 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 (150 mbar.s).

New capillaries were rinsed with 1 *M* NaOH for 20 min, 0.1 *M* NaOH for 10 min, distilled water for 10 min and background electrolyte (BGE) for 10 min. At the beginning of every working day the capillary was flushed with 0.1 *M* NaOH (10 min), water (3 min) and BGE (3 min). Between the runs the capillary was washed with methanol–water (30:70, v/v; 3 min), water (2 min), 0.1 *M* NaOH (3 min), water (2 min) and BGE (3 min). At the end of the day the capillary was washed with methanol–water (30:70, v/v; 10 min), 0.1 *M* NaOH (10 min) and water (3 min) and then air-dried for 3 min. These washing procedures turned out to be relevant for reproducible results.

Stock solutions of each analyte were prepared in methanol. Samples were made by dilution with solutions used for separation. The separation solu-

tions were prepared by dissolving 10 m*M* SDS in a mixture of 100 m*M* borate–50 m*M* phosphate buffer (pH 7.0), containing appropriate amount of methanol (from 5 to 20%, v/v). All samples and BGEs were filtered (0.45 μm, Minisart, Sartorius) prior to use.

Polymerisation of the composite material was carried out by irradiation with a blue-light source [Demetron Optilux curing light (Kerr, USA; light intensity: 550 mW/cm^2)] combined with an electric connection device for adjustment of exact time. The resulting cylindrical standard composite specimens (5 mm diameter, 2 mm thickness, mass about 55 mg each) were ground in an automatic agate mortar (Pulverisette, Fritsch, Germany) for 10 min to fine powder. The powder was transferred into a glass vial, a defined volume methanol was added, and the suspension was sonicated for 10 min at room temperature. The suspension was kept overnight, then centrifuged at 13 000 *g*, and the supernatant solution injected into the CE apparatus.

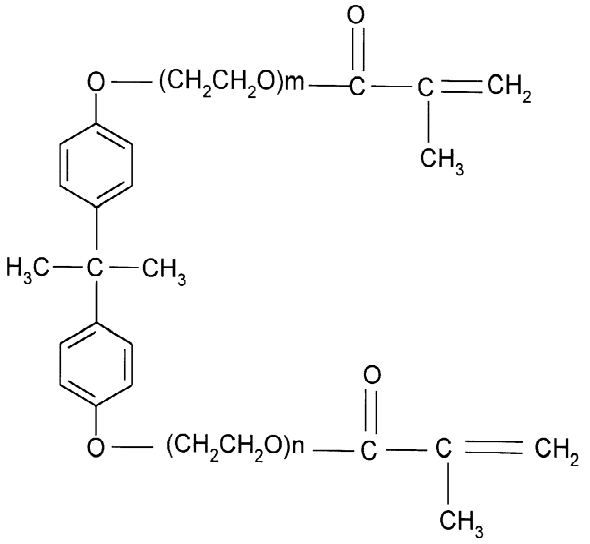

Fig. 1. Structural formula of BIS-EMA. *m* and *n* are the numbers of ethoxy repeats in the individual species.

3. Results and discussion

3.1. Separation of isomers and homologues of BIS-EMA

In the descriptions of the composite materials as given by the producer the number of ethoxy groups of BIS-EMA is not specified. Therefore in the present work a wide range of possible homologues was taken into account, from about four to about 30 ethoxy groups in the formula given in Fig. 1. The reference compounds contain in average two ethoxy groups per phenol (2EO/phenol; $m+n=4$), and 15 ethoxy groups per phenol (15EO/phenol; $m+n=30$), respectively. It is obvious that even these reference samples are mixtures of homologues and isomers. 2EO/phenol in principle can form not more than two species, namely either the symmetrical one (2 and 2EO per phenol), or the asymmetric one (1 and 3EO per phenol). In addition, some homologues with different, whereas low numbers of EO groups per phenol can be present. In contrary, the 15EO/phenol sample has expectedly a much larger number of isomers (15:15, 14:16, 13:17 etc.) and homologues as well (e.g. 15:15, 16:15, 17: 15 etc.). Thus a certain distribution of the species of the individual reference substances can be expected.

Micellar electrokinetic chromatography (MEKC) for the mixture of the two standards (2EO/phenol and 15EO/phenol) is shown in Fig. 2. The lowest trace (0%) shows the profile obtained with a BGE as common in MEKC, consisting of 10 m*M* SDS in a mixture of 100 m*M* borate and 50 m*M* phosphate buffer (pH 7.0). In order to control the performance of the system, bisphenol A (BisA, a well-defined single compound) was always run together with the BIS-EMA mixture. The sharp peak of this standard (eluted at about 5 min) demonstrates the high separation efficiency (about 70 000 plates, i.e. 250 000 plates/m). However, the BIS-EMA mixture at this condition (ca. 8 min retention time) gives a single peak only. It can be assumed that the lack in resolution is caused by the large and undifferentiated extent of partitioning of the relatively lipophilic analytes into the micelles. This assumption is supported by the fact that dodecylbenzene, used as micelle marker, has the same retention time as the analytes (data not shown).

In order to increase the separation selectivity by reducing the interaction between the analytes and the micelle, methanol is added to the BGE as usual. Five

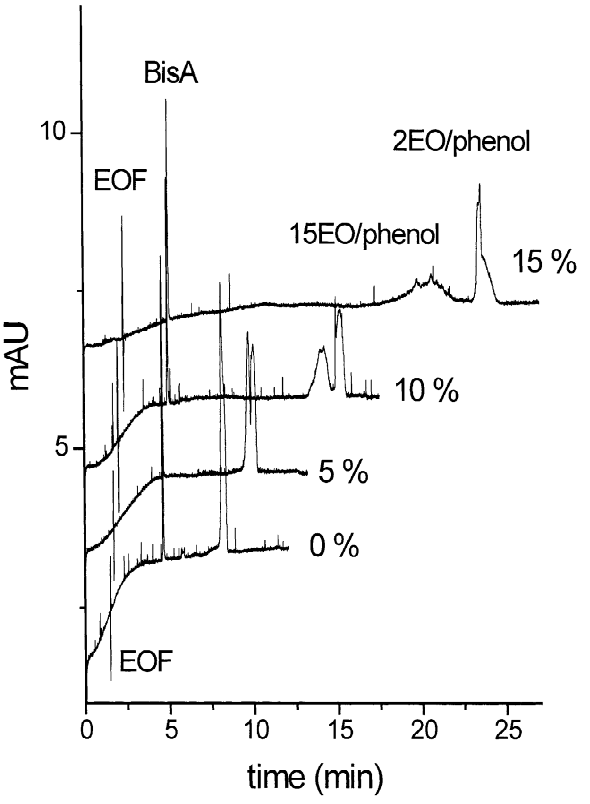

Fig. 2. MEKC of a mixture of BIS-EMA with two ethoxy groups per phenol (2EO/phenol) and 15 ethoxy groups per phenol (15EO/phenol) in average ($m+n$ from Fig. 1 is ~4 or ~30, respectively) at different percentage (v/v) methanol in the BGE. Conditions: uncoated fused-silica capillaries; 36.5 cm (28 cm effective length)×50 μm I.D.×360 μm O.D., cathode placed at the detector side. Temperature: 20.0 °C. Voltage: 20 kV. Pressurised injection for 150 mbar.s. Detection wavelength: 214 nm. Buffer: 10 m*M* SDS in a 100 m*M* borate–50 m*M* phosphate buffer (pH 7.0), containing different amount of methanol (from 5 to 15%, v/v). EOF, electroosmotic flow marker; BisA, bisphenol A.

percent methanol leads to a splitting of the sample peak (Fig. 2); separation between the two BIS-EMA homologue mixtures is improved with increasing methanol concentration. The resulting first, broad peak is identified as 15EO/phenol, the second, sharper peak as 2EO/phenol by addition of the reference substances. The clear difference in peak shape is plausible concerning the composition of the samples, as pointed out above. Zone broadening is most probably not caused by a loss in separation efficiency (see the sharp peak of bisphenol A), but by the increased separation selectivity of the system. This assumption is confirmed finally by the resulting chromatogram in the BGE containing 20% methanol (Fig. 3), where the zone of the 2EO/phenol sample gives a considerably narrow zone (consisting of about 3 non-resolved compounds). The 15EO/phenol sample, on the other hand, is separated into more than 10 different peaks (see insert in Fig. 3 with an expanded view of the corresponding part of the chromatogram), seemingly the mixture of numerous isomers and homologues. The result shows that the MEKC system is suited for the separation and characterisation of the different BIS-EMA species.

3.2. Characterisation of the composite monomers

The chromatograms of one typical commercial composite material in the different BGEs are given in Fig. 4. According to the product description given by the supplier the composite material contains monomeric ethoxylated bisphenol A dimethacrylate in a non-specified concentration. Dodecandiol-dimethacrylate, also a constituent of the composite material, has a detector response more than an order of magnitude lower at the wavelength selected, and will be thus not recorded under the given conditions. An increasing resolution of the sample species upon addition of methanol can be seen. This main part consists of a number of non-resolved compounds,

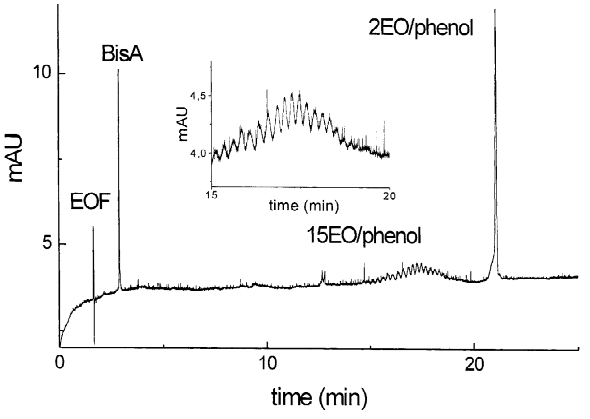

Fig. 3. MEKC of a mixture of BIS-EMA with 2EO/phenol and 15EO/phenol in average ($m+n$ from Fig. 1 is ~4 or ~30, respectively) at 20% methanol in the BGE. Experimental conditions as in Fig. 2, except voltage (25 kV). Insert: expanded view of the chromatogram between 15 and 20 min.

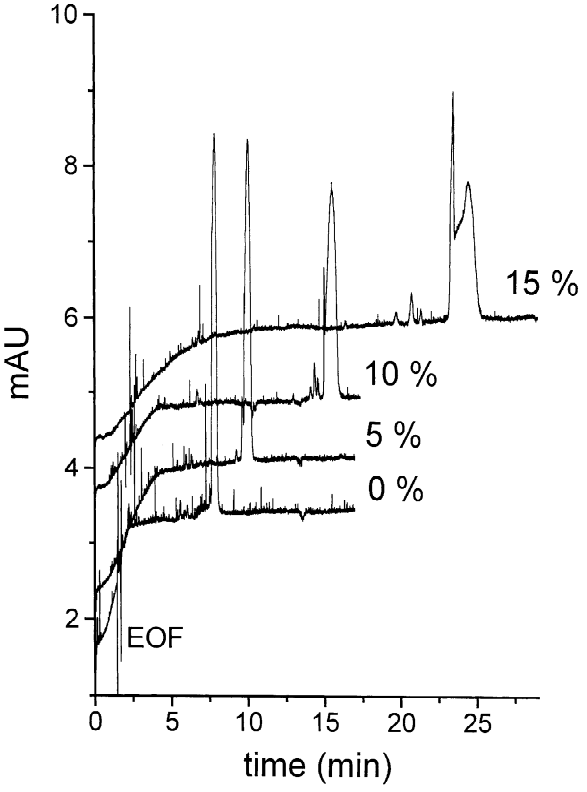

Fig. 4. MEKC of a composite material at different percentage methanol in the BGE. Experimental conditions as in Fig. 2.

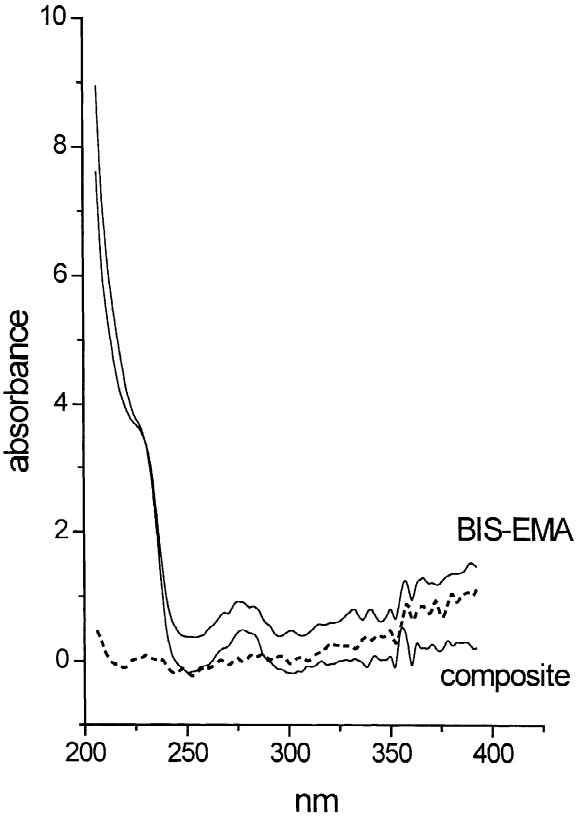

Fig. 5. Spectra of the peak of BIS-EMA (2EO/phenol) in Fig. 2, and the main peak of the composite sample in Fig. 4. Dotted curve: spectra of the BGE.

plausible regarding the technical quality of the composite material. Retention time (and standard addition) allows the conclusion that the main peak stems from a BIS-EMA material with a low number of EO/phenol. In addition, with 15% methanol a number of small peaks migrating before the main peak indicates that also BIS-EMA with a higher number of EO/phenol is present in the composite material, but at much lower concentration. Sample peak identity was confirmed by comparison of the UV–Vis spectra with the reference substances (Fig. 5), which have characteristic side-maxima at 230 and 260 nm.

We estimate the content of the short-chain monomers by relating their peak area to that of 2EO/phenol BIS-EMA after calibration with the latter. In this way a content of 2.7% (w/w) is found in the commercial sample.

3.3. Remaining monomer content of composite material upon light-induced polymerisation

The change of the monomer content of the composite material upon irradiation with blue light delivered from Demetron Optilux curing light is shown in Fig. 6. The total peak area of the zone identified as BIS-EMA was taken as measure of the monomer content. A typical decay curve with an initially sharp decrease of the peak area is observed. However, it is interesting that after 40 s irradiation time—the time recommended for polymerisation from the producers of the composite material for practical application with the patient—the peak area is still about 6% of the initial value. This is a considerably high residual content in the composite in practice given that the peak indeed represents monomers. Prolongation of the irradiation above the recommended time leads to

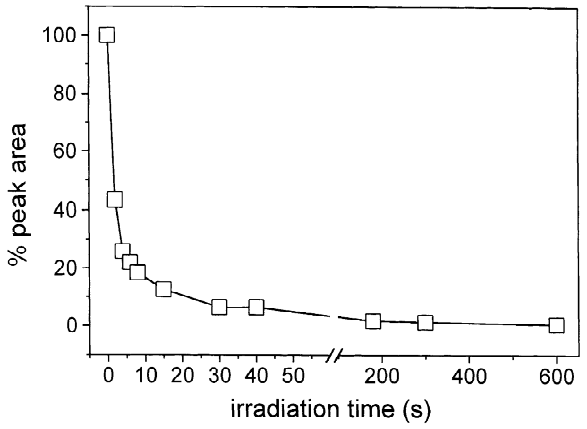

Fig. 6. Change of peak area of BIS-EMA in the chromatogram of the composite material with time of irradiation with the blue-light lamp to induce radical polymerisation.

a further reduction of the peak area, e.g. to about 2% after 180 s, and to slightly more than 1% after 5 min.

The chromatogram obtained after 10 min irradiation is shown in Fig. 7, together with that after 40 s for comparison. The clear reduction of the peak size is visible by prolongation of the time. After 10 min, a small peak at the retention time of BIS-EMA is still present, corresponding to about 0.4% of the initial one. This is the concentration range found by other authors for leachable monomeric composite material [7,10]. In order to prove whether or not this remaining peak is the result of an incomplete penetration of the light into the core of the pellet, a thinner specimen was formed from the composite material (thickness smaller than 0.5 mm), and both sides of the resulting flat cylinder were irradiated for 5 min each. The resulting profile (upper trace in Fig. 7) is similar to that obtained for the standard pellet after the same irradiation time. It should be mentioned that the small shift of this peak compared to the two others in Fig. 7 is caused by a slight variation of the electroosmotic flow; the corresponding mobilities are identical. This leads to the conclusion that non-polymerised compound in the several per mille range is present even after excessive curing. It is obvious that it cannot be decided whether this is still monomeric BIS-EMA, or it is another constituent of the composite, that is eluted in the same range than the monomer, and does not react upon irradiation.

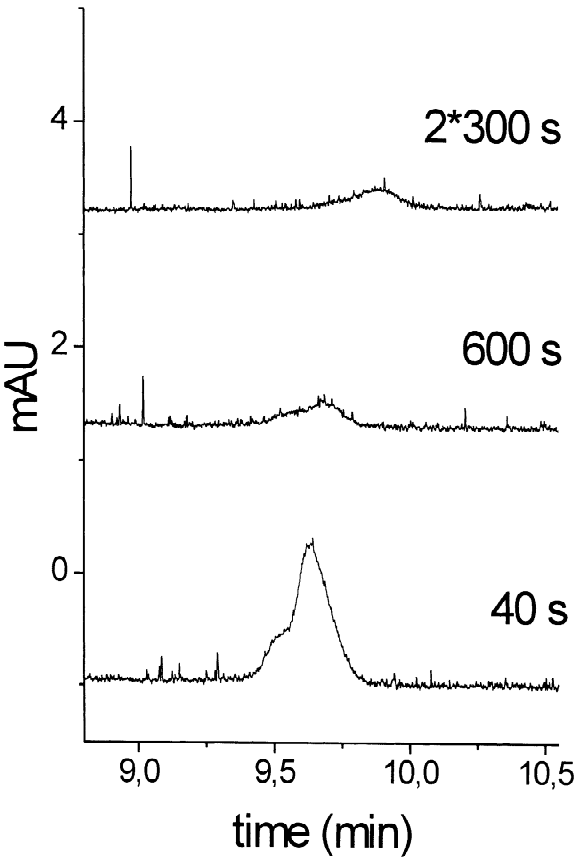

Fig. 7. MEKC of standard pellets from composite material after 40 and 600 s of irradiation (two lower traces). The pellets were ground and the monomers extracted with methanol from the resulting powder as described in the experimental part. The upper trace stems from a composite sample of the same mass, produced as a thin cylindrical layer (9 mm diameter, 0.5 mm thickness) which was irradiated at both sides for 300 s each. MEKC conditions as in Fig. 2.

The appropriate limit of detection (LOD) decides whether or not the method is suited to determine remaining monomers in the cured material even in trace quantities. The LOD is defined as signal being larger than three times the standard deviation of the noise of the baseline (without spikes, which have clearly differing frequency). Based on peak height in chromatograms from reference standard solutions as those shown in Fig. 2 the LOD is about 160 ng/ml or 290 n*M*, respectively, under these experimental conditions. This means that the method should enable determining monomeric constituents in the composite material in the ppm range and below.

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