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# High-performance liquid chromatographic determination of unreacted monomers and other residues contained in dental composites

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**Abstract**

HPLC method was developed for determination of bisphenol A diglycidyl methacrylate (bis-GMA), bisphenol A diglycidyl acrylate (bis-GA), bisphenol A dimethacrylate (bis-DMA), glycidylmethacrylate (GMA) and triethylenglycol dimethacrylate (TEGDMA). Separation was carried out on a reversed phase Omnisphere 5 C18 column with a gradient mobile phase of $CH_3CN/H_2O$. UV detection was set at 205 nm and 275 nm parallel. The limits of quantification were found. The method has been applied for quantification of unreacted monomers trapped in polymer network of fillings.


*Keywords:* Bis-GMA; TEGDMA; HPLC; Dental composites

## 1. Introduction

Visible light-curable resin composites are now routinely used as filling materials in dentistry. These materials are based on dimethacrylate/acrylate resins along with silane coated inorganic fillers. The most frequently used monomers for the preparation of dental resins are 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxyphenyl]-propane (bis-GMA), 2,2-bis[4-(2-hydroxy-3-acryloyloxypropoxyphenyl]-propane (bis-GA), triethylenglycol dimethacrylate (TEGDMA). Usually the mixtures of these monomers are used. The selection of monomers strongly influences the reactivity of the monomer, mechanical properties, the water uptake and the swelling of the polymer [1]. In various studies it has been found that the degree of the monomer–polymer conversion of dental resin composites varies between 55% and 75% [2,3].

Unreacted monomers trapped in the polymer network may reduce the clinical longevity of the fillings. However, in aqueous environment restorations release unreacted monomers, additives and degradation products [1,4]. All these substances may penetrate through the dentin, irritate the soft tissues and promote allergic reactions [5]. It was found that released substances possess cytotoxic, genotoxic, mutagenic or estrogenic effects [6,7]. Therefore, the nature and the quantity of substances which may be segregated into the oral cavity during the clinical service of the fillings should be known.

The determination of the quality and quantity of the residual monomers eluted from polymerised dental composites is usually performed by using high-performance liquid chromatography (HPLC) [8–12]. Bis-GMA and TEGDMA were found to be the principal eluted monomers. Only few studies reported data about the release of degradation products [1,13,14]. The proposed analytical methods used in HPLC techniques vary between conditions established for determination of residual monomers. These methods have been used to study the release of residual monomers from laboratory polymerised specimens. The main point of concern was the amount of eluted monomers and the time needed for the complete elution. These parameters are affected by the polymerisation conditions, the solvent used for the elution and the chemical structure of the monomers. Some studies have suggested that the elution is completed in 7 days, while others found that it lasts for a long time (*e.g.*

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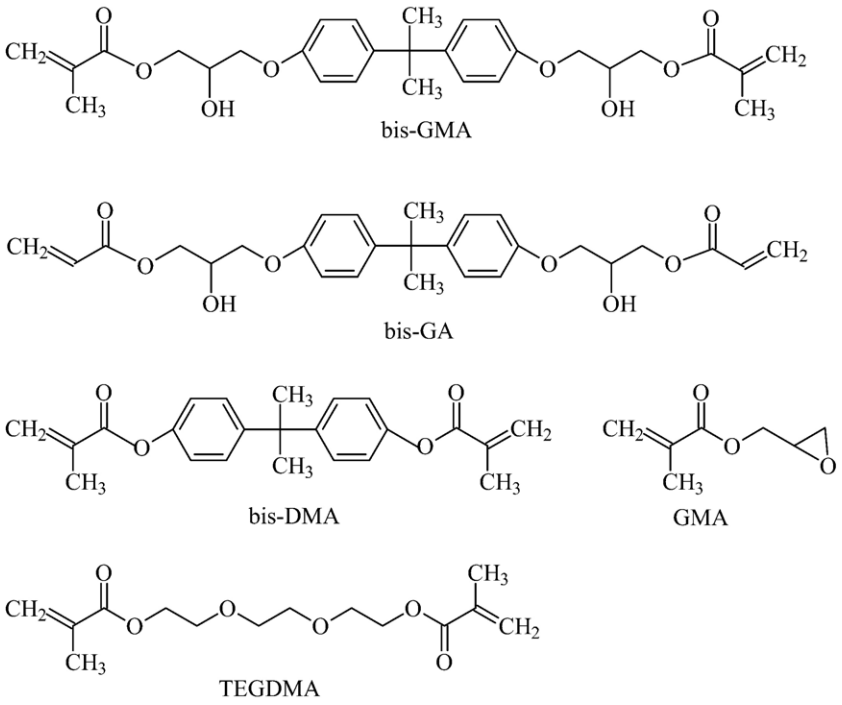


Fig. 1. Chemical structures of investigated compounds.

60 days) [15]. The data about the amount of residual monomers in restorations with different longevity in human cavities is currently unknown. Generally, there is an interest to find out whether polymerised dental composites provide a chronic source of unreacted monomers to pulp and other oral tissues.

In this work a method for analysis of dental composite monomers and some of their degradation products eluted in aqueous medium is proposed. In order to fulfil the aim the method was first developed for the separation and determination of bis-GA, bis-GMA and TEGDMA concentration by optimising the experimental conditions and determining linearity for the investigated compounds. The method was used for the determination of the amount of unreacted monomers trapped *in vivo* made fillings.

## 2. Experimental

### 2.1. Reagents and chemicals

All solvents and chemicals were of analytical grade. Acetonitrile and ethanol were HPLC grade, bisphenol A diglycidyl methacrylate (bis-GMA), bisphenol A diglycidyl acrylate (bis-GA), bisphenol A dimethacrylate (bis-DMA), glycidylmethacrylate (GMA), triethylenglycol dimethacrylate (TEGDMA) were purchased from Sigma-Aldrich, Germany. The chemical structures of these compounds are illustrated in Fig. 1. Deionised water was used for preparation of mobile

Table 1
The time program of mobile phase gradient and detection wavelengths

| Time (min) | Eluents (%) | | Wavelength (nm) | |
|---|---|---|---|---|
| | $H_2O$ | $CH_3CN$ | Channel 1 | Channel 2 |
| Initial | 50 | 50 | 275 | 205 |
| 15 | 0 | 100 | | |
| 20 | 0 | 100 | | |
| 22 | 50 | 50 | | |

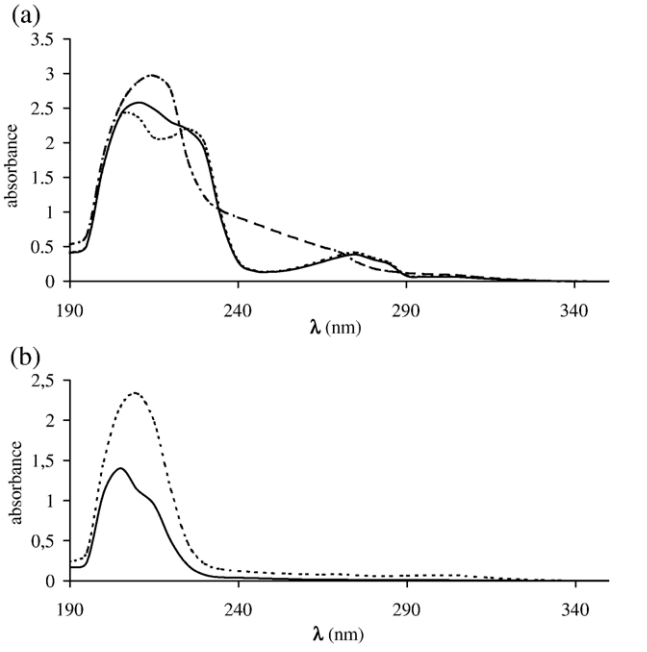


Fig. 2. UV spectra of (a) bis-GA (– – –), bis-GMA (—) and bis-DMA (- - —) (10 μg $mL^{-1}$) and (b) TEGDMA (—) and GMA (- - -) (10 μg $mL^{-1}$).

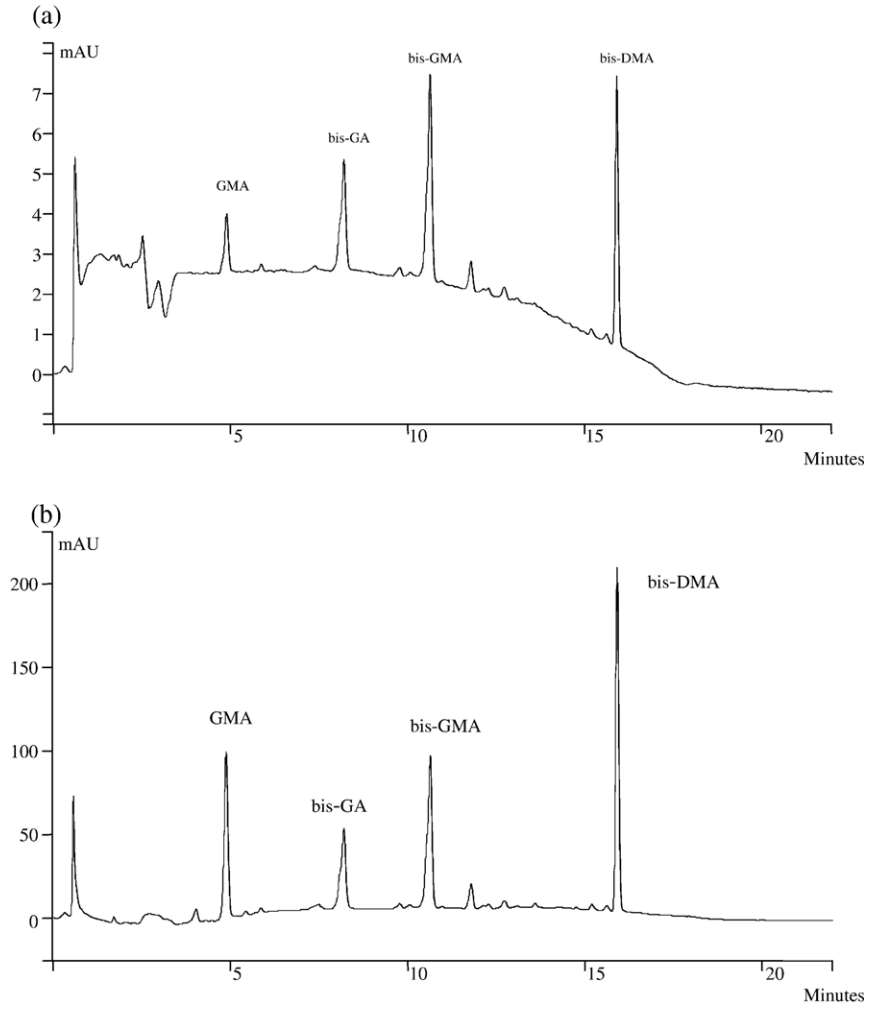


Fig. 3. Chromatograms of standard solution of all analytes (20 μg $mL^{-1}$) (a) at 275 nm and (b) at 205 nm. Chromatographic conditions: 20 μL injected volume, gradient mobile phase $CH_3CN/H_2O$, 1 mL $min^{-1}$ flow rate.

phase. The resin composites studied were the commercial products Solitaire 2 and Charisma, shade A2, both produced from Heraeus Kulzer, Germany. They were chosen because their resin matrix is based on the above monomers and represent condensable and microhybrid type composites respectively. Also nine restorations, which have been remained in human cavities for different period of time, were studied.

### 2.2. Equipment and chromatographic conditions

Quantitative analysis was performed on a Varian HPLC system consisted of a ProStar 230 solvent delivery module, a Rheodyne manual injector and ProStar 325 UV–VIS detector. The column used was reversed phase Omnisphere 5 C18 (250 × 4.6 mm, 5 μm particles), Varian. The mobile phase was a gradient prepared from acetonitrile and water. The time program given in Table 1 controlled the gradient elution and the detection wavelength. The flow rate was 1 mL $min^{-1}$ and the injector volume was 20 μL. UV detection was performed at 205 nm and 275 nm simultaneously. The components were identified by comparing the elution time with that of reference compounds and by their UV spectra.

### 2.3. Calibration procedure

Stock solutions of reference standards (1 mg $mL^{-1}$) were prepared in ethanol. These solutions were stored in 4 °C. Working standards of the analytes were obtained by dilution with ethanol to final concentrations of 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 μg $mL^{-1}$. Calibration plots were produced using the standard solutions described above. Triplicate 20 μL injections were made for each standard solution and calibration curves were obtained by plotting the peak area *versus* concentration using linear regression analysis.

### 2.4. Sample preparation

Five specimen discs, 8 mm in diameter and 2 mm in thickness, of each composite were fabricated in an aluminum mould between two glass slides. The irradiation was carried out with a Bluedent LED Smart light curing unit (D and A Electronics, Bulgaria) with intensity of 800 mW $cm^{-2}$. The specimens were irradiated on upper side only, for 40 s (Solitaire2) and 20 s (Charisma) according to the manufacturer's recommendations. Immediately after the polymerisation,

Table 2
Regression equations of the calibration curves, retention times and detection limits of the investigated compounds at 205 nm and 275 nm

| Analyte | Regression equation | $R^2$ | LOD μg mL$^{-1}$ | LOQ μg mL$^{-1}$ | Retention times, min |
|---|---|---|---|---|---|
| Bis-GA | $Y_{205}=5.2086.10^5x-6.0193.10^4$ | 0.9986 | 0.120 | 0.300 | 8.16 |
| | $Y_{275}=3.0066.10^4x-5.3131.10^3$ | 0.9996 | 0.180 | 0.328 | |
| Bis-GMA | $Y_{205}=7.7768.10^5x-7.7908.10^3$ | 0.9995 | 0.095 | 0.286 | 10.55 |
| | $Y_{275}=4.3855.10^4x-2.8178.10^3$ | 0.9997 | 0.104 | 0.310 | |
| Bis-DMA | $Y_{205}=1.2292.10^6x-8.9962.10^4$ | 0.9993 | 0.097 | 0.304 | 15.77 |
| | $Y_{275}=3.9892.10^4x-3.1137.10^3$ | 0.9994 | 0.095 | 0.290 | |
| TEGDMA | $Y=6.4208.10^5x+4.0244.10^4$ | 0.9995 | 0.081 | 0.268 | 7.32 |
| GMA | $Y=5.1727.10^5x-4.5452.10^3$ | 0.9997 | 0.085 | 0.297 | 4.90 |

the specimens were immersed in 5 mL deionised water and left at 37 °C for 7 days. Samples were taken for HPLC analysis in 24 h, 72 h and 168 h. Nine restorations with different longevity in human cavities were ground with mortar. Fifty milligrams of powder was dissolved in 5 mL ethanol and left 24h at room temperature prior to the analysis.

## 3. Results

### 3.1. Chromatographic analysis

Detection was performed at the wavelength of 205 nm for all analytes because they exhibit significant absorption. The second wavelength of 275 nm for detection of bis-GA, bis-GMA and bis-DMA was used based on their UV spectra (Fig. 2). The chromatograms obtained are illustrated in Fig. 3.

The linearity of the response to analytes was established with six concentration levels. The equations obtained were shown in Table 2. The retention times of reference compounds were also reported in Table 2. The limits of detection (LOD) and quantification (LOQ) were determined using a calibration in the low concentration region (0.05–0.5 μg mL$^{-1}$) as $3\sigma$/slope and $10\sigma$/slope respectively for analysed compounds at a signal-to-noise ratio of 3. Sigma values were calculated as standard deviation of the intercept of regression equations ($n=6$).

Recovery experiments were performed to study the reliability and suitability of the method. One sample with known concentrations of the investigated compounds was analysed. Results are means of five replicates of the same sample (Table 3).

### 3.2. Sample analyses

Sample analyses were performed at the same chromatographic conditions as the standards. The results obtained show the highest concentration of eluted compounds after 168-hour extraction in all solutions (Table 4).

The chromatograms obtained from fillings are presented in Fig. 4. Concentrations of detected monomers are reported in Table 5.

## 4. Discussion

Our goal was to develop a simple method for analysis of four compounds used as main constituents in dental composites and some of their degradation products leached in aqueous medium. To obtain satisfactory separation mobile phases containing different proportions of acetonitrile and water were tested. The best results were achieved using the conditions shown in Table 1. The studied compounds were well separated in these conditions (Fig. 3).

A linear detector response was observed in concentration range between 0.5 and 20.00 μg mL$^{-1}$ for two set wavelengths with correlation coefficients from 0.9986 to 0.9997 (Table 2). Recoveries were between 95.9% and 100.3% as reported in Table 3. The coefficients of variation ranged from 0.9% to 3.8%. This indicates that the proposed method is highly accurate and precise.

The detection limits at 205 nm are smaller than the ones at 275 nm. The *t*-test used for comparison did not show statistical differences between LOD at both wavelengths and between LOQ at both wavelengths. Detection limits reported in the present work are smaller then those reported by other authors [14,16]. It may be considered that both wavelengths may be successfully used for quantification of bis-GA, bis-GMA and bis-DMA.

The approximately twofold amount of monomers released by Charisma discs may be due to the different amount of the monomers in unpolymerised composite or result from insufficient irradiation time (20 s). It was found that the monomer–polymer conversion determines the quantity of leachable components, because the kinetics and mechanism of elution time processes depend on the composition and solubility parameters of the used solvent for extraction [14,15]. The diffusion through the polymer network is determined by the size and chemical characteristics of the leachable substances. TEGDMA is most flexible and mobile molecule compared to bis-GA and bis-GMA and more soluble in water, which is the reason we find it in extracts in high concentration.

Bis-GA was found only in the eluates from specimens made from Solitaire 2. It is a major component of this composite. The

Table 3
Confirmation of accuracy in the analytical technique by HPLC at 205 (275) nm

| Parameters | Bis-GA | Bis-GMA | Bis-DMA | TEGDMA | GMA |
|---|---|---|---|---|---|
| Real concentration (μg mL$^{-1}$) | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Concentration found (μg mL$^{-1}$) | 14.85 (14.81) | 15.05 (14.95) | 14.93 (14.95) | 15.18 | 14.39 |
| Recovery (%) | 99 (98.7) | 100.3 (99.7) | 99.5 (99.7) | 100.2 | 95.9 |
| CV (%) | 3.8 (3.7) | 1.1 (1.3) | 2.9 (3.4) | 2.6 | 0.9 |

The values in parentheses express results at 275 nm.

Table 4
Concentration in μg $mL^{-1}$ of eluted compounds from specimen discs

| Composite | Elution time (h) | Bis-GA | | Bis-GMA | | TEGDMA | GMA |
|---|---|---|---|---|---|---|---|
| | | 205 nm | 275 nm | 205 nm | 275 nm | | |
| Solitaire 2 | 24 | 2.62 (0.9) | 2.90 (0.96) | 0.79 (0.11) | 0.87 (0.37) | 21.25 (4.81) | 5.29 (0.73) |
| | 72 | 4.08 (0.30) | 3.89 (1.32) | 1.10 (0.11) | 1.12 (0.16) | 29.98 (4.26) | 11.43 (0.97) |
| | 168 | 4.06 (0.31) | 3.63 (0.55) | 2.66 (0.10) | 2.83 (0.08) | 28.65 (6.40) | 23.19 (2.86) |
| Charisma | 24 | | | | 0.76 (0.18) | 29.43 (6.5) | |
| | 72 | | | | 1.54 (0.04) | 33.88 (5.59) | |
| | 168 | | | | 4.46 (0.19) | 49.52 (6.32) | |

The values in parentheses express the standard deviation.

presence of glycidylmethacrylate in analysed solutions suggests that other reactions occur during the polymerisation and the extraction [1].

The fillings are subjected to chemical and temperature influences in the mouth. They absorb water and other oral fluids that increase the gaps between the polymer chains and small molecules are able to leave the polymer network. The question whether unreacted monomers are still present in polymer network of the restoration or all of them have leached was asked. The chromatograms obtained from the filling extracts show that the major monomer is present in the polymer network (Fig. 4).

The co-monomer TEGDMA and GMA were not found in any extract. This confirms the results reported by other authors [14,15,17], that smaller molecules leave the polymer network faster than the bigger ones.

The results presented in Table 5 demonstrated that regardless of the longevity of the restoration unreacted monomers are trapped in the polymer network. The results are presented as μg per mg polymerised resin. The presence of monomers in the

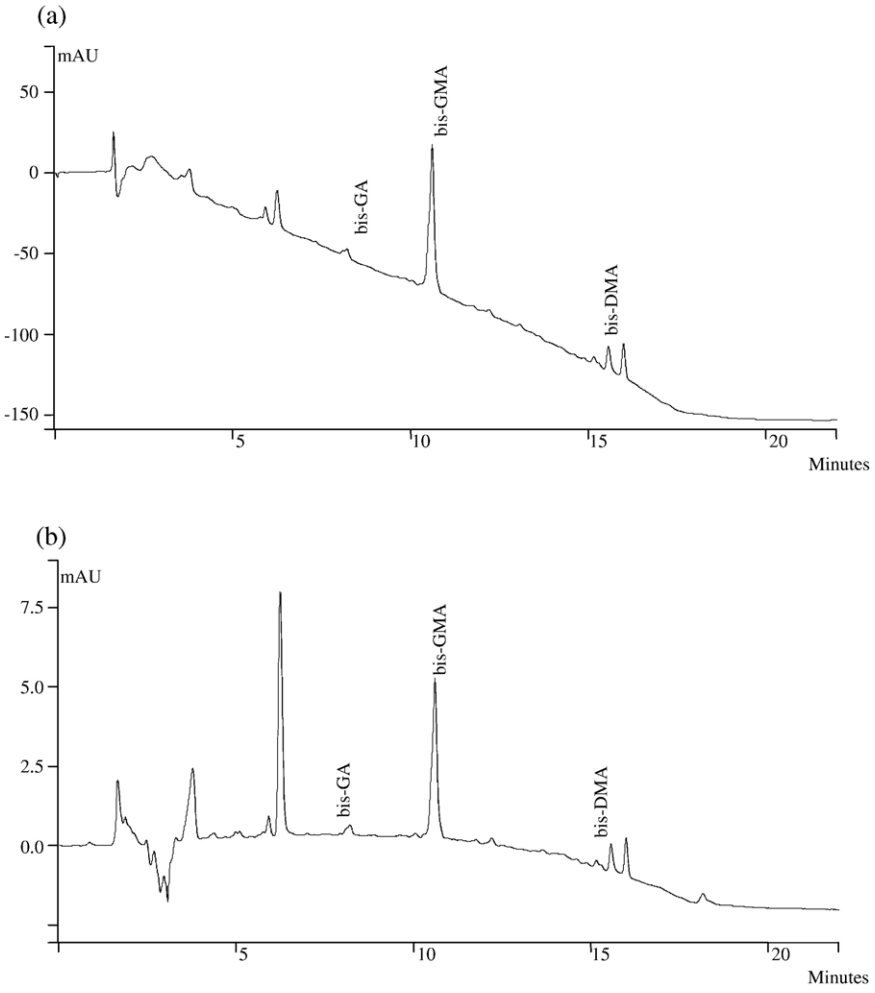


Fig. 4. The chromatograms obtained from restoration made from Evicrol solar composite (a) at 205 nm and (b) at 275 nm. Solutions were diluted three times.

Table 5
Concentration (μg $mg^{-1}$) of eluted monomers from restoration polymer network

| Composite | Longevity of the restoration | Bis-GA | Bis-GMA | Bis-DMA |
|---|---|---|---|---|
| Evicrol solar | 3 years | 0.37 | 2.60 | 0.45 |
| Valux plus | 6 years | | 0.107 | |
| | 9 years | 1.05 | 0.305 | 0.06 |
| | 1 year | | 0.13 | 0.65 |
| Polofill | 7 years | | 0.14 | 0.12 |
| Pecalite | 7 months | | 1.13 | |
| | 8 months | | 0.30 | |
| | 7 months | | 0.44 | |
| Unknown | | | 2.15 | 0.66 |

restoration network is a prerequisite for their leaching that may cause permanent pulp and soft tissues irritation.

## 5. Simplified description of the method and its application

The proposed HPLC method for analysis of the residual monomers in polymerised dental composites is simple and rapid. The coefficients of variation between 0.9% and 3.8% confirm the precision of the method. It is suitable for identification and quantification of the monomers bis-GMA, bis-GA, bis-DMA and TEGDMA as well as GMA from various samples.

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