Chemical stability of orthodontic adhesives based on polymer network depending on external environment's pH

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Abstract: Chemical stability of composite adhesive systems is crucial for the safety of their use. The study assessed chemical stability of four light-cured orthodontic adhesives: Contec LC, Transbond XT, Transbond Plus, Resilience, depending on pH value of the external environment. Samples of polymerized orthodontic adhesives were treated with (high-performance liquid chromatography) HPLC-grade water solutions of phosphate-citrate buffer with pH values respectively: 4, 5, 6 and 7 at 36 °C. The eluates obtained after 1 hour, 24 hours and 7 days of sample incubation were analyzed for the presence of camphorquinone (CQ), bisphenol A (BPA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), bisphenol A diglycidyl methacrylate (Bis-GMA), ethylene glycol dimethacrylate (EGDMA), 2,2-dimethoxy-2-phenylacetophenon (DMPA) using ultra-high performance liquid chromatography (UHPLC). Out of the seven searchable substances, TEGDMA was present in eluates obtained from Contec LC, Resilience and Transbond XT materials and EGDMA in eluates obtained from Resilience adhesive. The eluates obtained from the Transbond Plus adhesive system were virtually free of the sought substances. The highest concentrations of TEGDMA in solutions were recorded after 1 hour of incubation regardless of the type of material. In the case of Contec LC material, an increase in TEGDMA concentrations was observed along with an increase in the solutions' pH, but only for the elution period of 1 hour and 7 days, the effect of the solvent's pH was statistically significant ($p \leq 0.001$). In the case of Resilience and Transbond XT, no significant differences in TEGDMA concentrations were observed with respect to pH of the external environment. In the conditions of the conducted study, a lack of chemical stability was confirmed for the majority of assessed orthodontic adhesive systems based on polymers, expressed in emission of component monomers to the external environment. The chemical compound identified in the study was TEGDMA, and for each pH of the solvent, statistically significant differences in its release were found between the materials. However, no explicit relationship was observed between chemical instability of the studied materials and pH of the external environment within the assumed range of assessment.

Keywords: orthodontic adhesive systems, HPLC, chemical stability, monomers, pH.

Stabilność chemiczna klejów ortodontycznych opartych na sieci polimerowej w zależności od pH środowiska

Streszczenie: Stabilność chemiczna kompozytowych systemów adhezyjnych jest kluczowa z punktu widzenia bezpieczeństwa ich stosowania. W badaniu oceniano stabilność chemiczną czterech światłoutwardzalnych klejów ortodontycznych: Contec LC, Transbond XT, Transbond Plus, Resilience, w zależności od wartości pH środowiska zewnętrznego. Próbki spolimeryzowanych klejów ortodontycznych poddano działaniu roztworów buforu fosforanowo-cytrynianowego na bazie wody o czystości HPLC, o wartości pH: 4, 5, 6 oraz 7 i temperaturze 36 °C. Eluaty uzyskane po 1 h, 24 h i 7 dniach inkubacji próbek analizowano metodą chromatografii cieczowej wysokociśnieniowej (HPLC) pod względem obecności kamforochinonu (CQ), bisfenolu A (BPA), dimetakrylanu glikolu trietylenowego (TEGDMA), dimetakrylanu uretanu (UDMA), bisfenolu A metakrylanu diglicydylu (Bis-GMA), dimetakrylanu glikolu etylenowego (EGDMA), 2,2-dimetoksy-2-fenyloacetofenonu (DMPA). Z siedmiu związków chemicznych identyfikowanych w roztworach potwierdzono obecność TEGDMA w eluatach uzyskanych z materiałów Contec LC, Resilience i Transbond XT oraz obecność EGDMA w eluatach z kleju Resi-

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lience. Eluaty otrzymane z systemu adhezyjnego Transbond Plus praktycznie biorąc nie zawierały poszukiwanych substancji. Największe stężenia TEGDMA w roztworach stwierdzono po 1 h inkubacji próbek ortodontycznych systemów łączących, niezależnie od rodzaju materiału. W odniesieniu do kleju Contec LC obserwowano wzrost stężenia TEGDMA wraz z wartością pH roztworów, ale wpływ pH rozpuszczalnika był istotny statystycznie ($p \leq 0{,}001$) tylko w wypadku czasu wymywania 1 h i 7 dni. W roztworach po inkubacji materiałów Resilience i Transbond XT nie stwierdzono istotnych różnic stężeń TEGDMA w zależności od pH środowiska zewnętrznego. W warunkach przeprowadzonego badania potwierdzono brak stabilności chemicznej większości ocenianych, polimerowych, ortodontycznych systemów adhezyjnych wyrażający się emisją tworzących je monomerów do środowiska zewnętrznego. W odniesieniu do każdej wartości pH rozpuszczalnika wykazano istotne statystycznie różnice w uwalnianiu TEGDMA pomiędzy badanymi materiałami. Jednocześnie w przyjętym zakresie oceny nie zaobserwowano jednoznacznej zależności stabilności chemicznej badanych materiałów od pH środowiska zewnętrznego.

Słowa kluczowe: ortodontyczne systemy adhezyjne, HPLC, stabilność chemiczna, monomery, pH.

During orthodontic treatment, which usually lasts about 24 months, elements of orthodontic appliances are exposed to oral cavity environment and come into contact with its tissues. The presence of saliva, the influence of masticatory forces, the activity of microorganisms, the periodic presence of food and beverages, provide the oral ecosystem with the features of high humidity, pH variability, temperature fluctuations, electrochemical and enzymatic activity and the action of physical factors.

Environmental conditions undoubtedly affect the wear and degradation of materials used in dentistry, including orthodontics [1–3], which exposes them to the danger of losing their physical properties that are crucial for the treatment process [4]. Insufficient stability of chemical structure and strength of dental materials cannot be neglected due to the danger of a release of potentially harmful substances into the patient's organism [5].

Orthodontic adhesive systems based on composite materials are now widely used in treatment of patients with fixed appliances. The organic matrix of orthodontic adhesives is formed by "basic" monomers or oligomers that are derivatives of methacrylic acid. The most commonly used are: Bis-GMA (bisphenol A diglycidyl methacrylate), UDMA (urethane dimethacrylate), Bis-EMA (ethoxylated bisphenol A dimethacrylate). "Auxiliary" monomers with smaller molecules such as: HEMA (2-hydroxyethyl methacrylate), EGDMA (ethylene glycol dimethacrylate), TEGDMA (triethylene glycol dimethacrylate) or DEGDMA (diethylene glycol dimethacrylate) are added to increase plasticity of the material. Composite orthodontic resins also include inorganic fillers and a number of additional compounds with various functions, such as: polymerization initiators [camphorquinone (CQ), 2,2-dimethoxy-2-phenylacetophenon (DMPA)], catalysts,

T a b l e 1. Chemical names and abbreviations of substances included in the organic matrix of composite orthodontic adhesive systems

International abbreviation	Full chemical name
UDMA	1,6-Bis(methacryloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexane; urethane dimethacrylate
Bis-GMA	2,2-Bis[4-(2-hydroxy-3-methacryloxypropoxy)phenylene]propane; bisphenol A diglycidyl dimethacrylate
Bis-EMA	2,2-Bis[4-(2-hydroxy-3-methacryloxyethoxy)phenyl]propane; ethoxylated bisphenol A dimethacrylate
HEMA	2-Hydroxyethyl methacrylate
EGDMA	Ethylene glycol dimethacrylate
DEGDMA	Diethylene glycol dimethacrylate
TEGDMA	Triethylene glycol dimethacrylate
PEGDMA	Poly(ethylene glycol) dimethacrylate
DMPA	2,2-Dimethoxy-2-phenylacetophenon
CQ	Camphorquinone
BPA	Bisphenol A

T a b l e 2. Orthodontic adhesive systems evaluated in the study and the content of individual methacrylate resins, fillers and catalysts of polymerization reaction declared by the producer

Trade name	Basic ingredients	Filler content	Producer
Contec LC	17–19 wt % of Bis-GMA 22–23 wt % of TEGDMA	Silicates	Dentaurum GmbH & Co. KG, Germany LOT: 90370
Resilience Light-Activated Orthodontic Adhesive System	Bis-GMA TEGDMA Camphorquinone	No data	Ortho Technology, Inc. Tampa, Florida USA LOT: H002658
Transbond Plus Color Change Adhesive	5–15 wt % of PEGDMA 5–15 wt % of 1,2,3-propanetricarboxylic acid 2-hydroxy-reaction products with 2-isocyanatoethyl methacrylate 2 wt % of Bis-GMA	35–45 wt % of silane treated glass 35–45 wt % of silane treated quartz < 2 wt % of silane treated silica	3M Unitek Monrovia, Kalifornia USA LOT: N686102
Transbond XT Light Cure Adhesive Paste	10–20 wt % of Bis-GMA 5–10 wt % of bisphenol A bis(2-hydroxyethyl ether) dimethacrylate < 0.2 wt % of diphenyliodonium hexafluorophosphate	70–80 wt % of silane treated quartz < 2 wt % of silane treated silica	3M Unitek Monrovia, Kalifornia USA LOT: N619082

antioxidants, photostabilizers, plasticizers or dyes [5–9]. Full chemical names of the compounds mentioned in the publication are presented in Table 1.

As a result of polymerization reaction, smaller molecules of monomers or oligomers combine into chains and networks, which is clinically manifested by hardening of the initially plastic or semi-liquid adhesive material that secures elements of the orthodontic appliance to the teeth. Numerous studies confirm that polymerization of orthodontic adhesive systems is not complete [10–12], and unpolymerized adhesive components and their decomposition products (*e.g.* BPA) can be released into the external environment both immediately after polymerization and as a result of degradation and aging of the material [5, 13].

Chemical compounds included in composite resins, polymer network degradation products or production impurities of materials are not indifferent to living organisms, and their harmful effects are manifold. In available literature there are many descriptions of studies confirming cyto- and genotoxicity of monomers and oligomers used in production of dental composite materials [2, 3, 14–16]. A negative effect of TEGDMA on the reproductive system and fertility of animals [17], and estrogenic activity of BPA and Bis-GMA [18–20] have also been confirmed. TEGDMA and EGDMA monomers also have the ability to stimulate growth of cultures of cariogenic bacteria [2], which may cause escalation of secondary caries around composite fillings. Composite materials used in dentistry, including orthodontic adhesive systems, can irritate surrounding tissues and cause allergic reactions in treated patients [21].

The aim of the study was to assess chemical stability of four light-cured orthodontic adhesives with respect to pH values of the solvent. Orthodontic adhesive systems evaluated in the study and chemical composition declared by their producers are presented in Table 2.

EXPERIMENTAL PART

Materials

Four light-cured orthodontic adhesives: Contec LC (Dentaurum, Germany), Transbond XT (3M Unitek, USA), Transbond Plus (3M Unitek, USA), Resilience (Ortho Technology, USA) were tested.

Samples preparation

Samples of the assessed materials were placed in teflon matrices with 5 mm diameter and 2 mm deep, previously purified with HPLC-grade water and methanol (Sigma Aldrich, USA). Orthodontic adhesive systems were then subjected to a 20-second polymerization with LED 55 curing light (TPC Advanced Technology, USA) at 1200 mW/cm^2 intensity. In the same way, 20 samples of each material were prepared, which were removed from the matrices and stored for 24 hours without access to light. Then the samples were weighed and placed in separate, aseptic tubes made of polypropylene with a total volume of 15 cm^3, closed with plugs. In order to avoid the influence of contamination during the course of the experiment, the tubes were pre-rinsed three times with HPLC-grade water. Samples of each of the assessed orthodontic adhesive systems were randomly divided into four groups of 5 samples in each. The tubes were filled with 10 cm^3 of phosphate-citrate buffer solution based on HPLC-grade water (Sigma Aldrich, USA) with pH values of 4, 5, 6 and 7, respectively, depending on the group, and then placed in an incubator shaker at 36 °C.

After 1 hour of incubation, the obtained eluates were collected and the tubes with the materials were filled again with 10 cm^3 of buffer solution with appropriate pH. The above procedure was repeated after 24 hours and 7 days of incubation. The control group in the study con-

T a b l e 3. Mean concentrations of TEGDMA detected in eluates of the tested orthodontic adhesives after 1 hour, 24 hours and 7 days of elution in a solvent at 36 °C and pH = 7

Material	1 h			24 h			7 days		
	Mean concentration μg/cm³	SD	Range μg/cm³	Mean concentration μg/cm³	SD	Range μg/cm³	Mean concentration μg/cm³	SD	Range μg/cm³
Contec LC	8.578 c	1.761	6.42–10.61	2.233 c	0.403	1.65–2.78	1.982 c	0.324	1.65–2.38
Resilience	2.640 b	0.377	2.23–3.11	0.513 b	0.198	0.39–0.87	0.342 b	0.145	0.25–0.60
Transbond XT	0.049 a	0.017	0.02–0.07	0.000 a	0.000		0.000 a	0.000	
p (based on the analysis of variance)	< 0.001*			< 0.001*			< 0.001*		

* – statistically significant differences between materials are present (as $p < 0.05$), SD – standard deviation, a–c – homogeneous groups.

sisted of buffered solutions with appropriate pH values, which did not contain samples of orthodontic adhesives. The eluates obtained at subsequent time intervals were frozen at -18 °C to minimize the probability of secondary polymerization reactions present in solutions of chemical compounds.

Methods of testing

Chromatographic measurements

After the observation, the defrosted eluates were analyzed for the presence of CQ, BPA, TEGDMA, UDMA, Bis-GMA, EGDMA, DMPA using the ultra-high performance liquid chromatography method (UHPLC).

Chromatographic measurements were conducted with the use of NEXERA UHPLC system (Shimadzu Corporation, Japan) equipped with two LC-30AD pumps, SIL-30AC autosampler, SPD-M20A diode detector, CTO-20AC furnace and CBM-20A controller. During the analysis, Kinetex C18 columns and SecurityGuard ULTRA C18 2.1 mm ID (Phenomenex USA) precolumns were used. Phase A was HPLC-grade Chromasolv water (Sigma-Aldrich, USA) and phase B HPLC-grade Chromasolv acetonitrile (Sigma-Aldrich, USA). Analysis time of a single sample was 16 minutes and the phase flow rate was 0.3 cm³/min. The quantitative analysis was made at the wavelength of 205 nm.

For calibration, CQ, BPA, TEGDMA, UDMA, Bis-GMA, EGDMA, DMPA reference standards from Sigma-Aldrich (USA) were used.

Statistical analysis

Statistical analyzes were performed using Statistica 13 program (StatSoft, Poland). Comparisons of averages were conducted using the analysis of variance and multiple comparisons by the Fisher procedure (LSD). In order to determine the effect of pH on substance concentrations, a simple regression analysis was performed and Pearson's correlation coefficients were calculated. In all analyzes, the significance level was assumed at $p = 0.05$.

RESULTS AND DISCUSSION

Results

TEGDMA presence was confirmed in eluates obtained after incubation of samples of Contec LC, Resilience and Transbond XT materials, whereas EGDMA was detected in eluates from Resilience adhesive. The eluates obtained from Transbond Plus adhesive system were virtually free of the sought substances. Some of the chromatographic analyzes performed for Transbond Plus have peaks similar to the CQ standard, but their position is not clear.

In a solvent with pH of 7 the highest mean concentrations of TEGDMA were noted in solutions collected from adhesive samples after 1 hour of incubation, respectively: 8.578 μg/cm³ for Contec LC, 2.640 μg/cm³ for Resilience and 0.049 μg/cm³ for Transbond XT. In eluates obtained after 24 hours of incubation, the presence of TEGDMA with an average concentration of 2.333 μg/cm³ for Contec LC and 0.513 μg/cm³ for Resilience materials was observed, whereas the presence of TEGDMA in the eluates of Transbond XT was not confirmed. After 7 days of storage of adhesive system samples in solutions, the presence of TEGDMA with an average concentration of 1.982 μg/cm³ was confirmed in eluates from Contec LC orthodontic adhesive and of 0.342 μg/cm³ in eluates from Resilience material. No presence of the compound at measurable levels was found in eluates obtained from Transbond XT adhesive system. Data analysis shows statistically significant differences ($p < 0.05$) in the release of TEGDMA to the external environment depending on the tested orthodontic adhesive for all incubation periods at pH value of 7. Table 3 shows the mean values and concentration ranges of TEGDMA determined in eluates of the studied orthodontic adhesives for subsequent periods of observation in solutions with pH of 7.

In the case of incubation in solutions with pH values of 4, 5 and 6, the highest TEGDMA concentrations were observed in eluates obtained after 1 hour of sample storage, regardless of the type of assessed adhesive system. In subsequent periods of observation, a decrease in the concentrations of the said monomer was noted. Differences in TEGDMA concentrations recorded after

1 hour, 24 hours and 7 days of observation were statistically significant ($p < 0.001$) with respect to the type of adhesive system being evaluated regardless of the solution's pH. The highest concentrations of TEGDMA were noted in eluates obtained from Contec LC adhesive, significantly lower in the case of Resilience material. Significantly the lowest monomer concentrations were noted in eluates from Transbond XT. The comparison of mean concentrations of TEGDMA observed in eluates of the tested orthodontic adhesives for different periods of observation and pH ranges is presented in Table 4.

A comparison of mean TEGDMA concentrations recorded in solutions obtained from individual orthodontic adhesive systems depending on pH of aqueous solutions used in the study showed that for Contec LC material the correlation coefficient (r) had positive values for each observation time, *i.e.*, an increase in TEGDMA concentrations was observed with an increase in pH of solutions. For sample storage time of 1 hour and 7 days, the effect of solvent's pH was statistically significant ($p \leq 0.001$). For Resilience, average concentrations of TEGDMA in solutions reached the highest values for the solution with pH = 5, respectively, 2.814 μg/cm^3 after 1 hour, 0.554 μg/cm^3 after 24 hours and 0.378 μg/cm^3 after 7 days of observation, and the lowest values for the solution with pH = 4, after 1 hour and after 7 days, respectively, 2.182 μg/cm^3 and 0.171 μg/cm^3. On the other hand, after 24-hour observation period, the lowest TEGDMA concentration was recorded in the solution with pH = 6 at the level of 0.444 μg/cm^3. For Resilience material, the correlation coefficient (r) was positive for 1 hour and 7 days of observation, and negative for 24 hours, but the pH level of the solution did not statistically significantly impact the concentration of TEGDMA ($p > 0.05$).

T a b l e 4. Mean concentration (μg/cm^3) of TEGDMA detected in eluates of the tested orthodontic adhesives after different periods of observation and for pH = 4, 5, 6, 7

pH = 4			
Material	1 h	24 h	7 days
Contec LC	4.375 c	2.278 c	0.894 c
Resilience	2.182 b	0.545 b	0.171 b
Transbond XT	0.009 a	0.000 a	0.000 a
p (based on the analysis of variance)	< 0.001*	< 0.001*	< 0.001*
pH = 5			
Material	1 h	24 h	7 days
Contec LC	7.074 c	1.772 c	1.855 b
Resilience	2.814 b	0.554 b	0.378 a
Transbond XT	0.153 a	0.084 a	0.023 a
p (based on the analysis of variance)	< 0.001*	< 0.001*	< 0.001*
pH = 6			
Material	1 h	24 h	7 days
Contec LC	7.965 c	2.185 c	1.903 c
Resilience	2.620 b	0.444 b	0.315 b
Transbond XT	0.017 a	0.000 a	0.000 a
p (based on the analysis of variance)	< 0.001*	< 0.001*	< 0.001*
pH = 7			
Material	1 h	24 h	7 days
Contec LC	8.578 c	2.233 c	1.982 c
Resilience	2.640 b	0.513 b	0.342 b
Transbond XT	0.049 a	0.000 a	0.000 a
p (based on the analysis of variance)	< 0.001*	< 0.001*	< 0.001*

* – statistically significant differences between materials are present (as $p < 0.05$), a–c – homogeneous groups.

In the eluates obtained from Transbond XT material, the presence of TEGDMA was confirmed in all three periods of observation only in solutions with pH = 5, and the concentration values equaled 0.153 μg/cm^3 after 1 hour, 0.084 μg/cm^3 after 24 hours, and 0.023 μg/cm^3 after 7 days. In the other pH ranges, measurable TEGDMA concentrations were found only in the eluates obtained after 1 hour of observation. The lowest concentration of 0.009 μg/cm^3 was recorded for pH = 4, 0.017 μg/cm^3 for pH = 6, 0.049 μg/cm^3 for pH = 7 and the highest at 0.153 μg/cm^3 for pH = 5. The correlation coefficient (r) for all observation times was negative, but values of $p > 0.05$ indicated that the effect of solvent's pH level on concentrations of TEGDMA was not statistically significant for Transbond XT. Table 5 summarizes the distribution of mean TEGDMA concentrations in eluates obtained from Contec LC, Resilience and Transbond XT for three periods of observation depending on changing pH levels of the solutions.

The eluates obtained from Resilience orthodontic adhesive contained EGDMA after 1 hour of sample incubation for each pH range. The average concentration of EGDMA was the highest in the solution with pH = 7 and amounted to 0.018 μg/cm^3. This value was significantly higher than the concentrations observed at the level of 0.010 μg/cm^3 for pH = 4, 0.012 μg/cm^3 for pH = 5 and 0.011 μg/cm^3 for pH = 6. The concentrations of EGDMA in eluates with pH 4, 5 and 6 did not differ statistically. After 24 hours of incubation, no EGDMA was found in any of the solutions, and after 7 days of sample storage – only in the solution with pH = 7 at 0.005 μg/cm^3. Table 6 shows mean EGDMA concentrations (μg/cm^3) in solutions obtained from Resilience adhesive divided into subsequent pH values and observation time.

Discussion

In the conducted study, chemical stability of four orthodontic polymer-based adhesive systems commonly used in clinical practice was evaluated in *in vitro* conditions. Solutions with pH of 4, 5, 6 and 7 were used in the experiments, that is the range of values occurring in the oral cavity [22].

With respect to the phenomenon of emissions of chemical compounds determined in eluates, the highest chem-

T a b l e 5. Distribution of mean TEGDMA concentrations in eluates obtained from Contec LC, Resilience and Transbond XT for three periods of observation depending on the changing pH of the solutions

Contec LC					Correlation coefficient (*r*)	Regression coefficient (*b*)	*p*
Leaching time	Mean concentrations, μg/cm³						
	pH = 4	pH = 5	pH = 6	pH = 7			
1 h	4.375	7.074	7.965	8.578	0.687	1.350	0.001*
24 h	2.278	1.772	2.185	2.233	0.046	0.013	0.857
7 days	0.894	1.855	1.903	1.982	0.716	0.331	< 0.001*

Resilience					Correlation coefficient (*r*)	Regression coefficient (*b*)	*p*
Leaching time	Mean concentrations, μg/cm³						
	pH = 4	pH = 5	pH = 6	pH = 7			
1 h	2.182	2.814	2.620	2.640	0.286	0.118	0.222
24 h	0.545	0.554	0.444	0.513	-0.128	-0.020	0.602
7 days	0.171	0.378	0.315	0.342	0.368	0.045	0.110

Transbond XT					Correlation coefficient (*r*)	Regression coefficient (*b*)	*p*
Leaching time	Mean concentrations, μg/cm³						
	pH = 4	pH = 5	pH = 6	pH = 7			
1 h	0.009	0.153	0.017	0.049	-0.016	-0.002	0.946
24 h	0.000	0.084	0.000	0.000	-0.113	-0.008	0.634
7 days	0.000	0.023	0.000	0.000	-0.122	-0.002	0.607

* – statistically significant differences are present (as $p < 0.05$).

ical stability in the conditions of the experiment was observed for Transbond Plus orthodontic adhesion system. This may be attributed to the fact that the manufacturer used different monomers than those identified in the study, as well as to the effective polymerization process of the material during sample preparation.

As far as the other adhesive systems evaluated in the study, the release of TEGDMA monomer to the external environment was confirmed. The presence of EGDMA was observed only in solutions obtained after storage of Resilience adhesive system samples.

It should be noted here that an analysis of chromatograms for solutions obtained from the assessed orthodontic adhesives revealed the presence of numerous chemical compounds, other than those identified in HPLC tests. This phenomenon confirms the lack of chemical stability of orthodontic adhesive systems in aqueous solutions.

T a b l e 6. Mean concentrations (μg/cm³) of EGDMA leached from Resilience adhesive in aqueous solutions at 36 °C and various pH values after 1 hour, 24 hours and 7 days of observation

	1 h	24 h	7 days
pH = 4	0.010 a	0.000	0.000 a
pH = 5	0.012 a	0.000	0.000 a
pH = 6	0.011 a	0.000	0.000 a
pH = 7	0.018 b	0.000	0.005 b
p (based on the analysis of variance)	0.001*		< 0.001*
Correlation coefficient (*r*)	0.650		0.760

* – statistically significant differences are present (as $p < 0.05$), a–b – homogeneous groups.

Numerous studies on the stability of composite materials used in conservative dentistry and orthodontics confirm that the main substance released into the external environment is TEGDMA monomer. Örtengren *et al*. in 2001, in a study assessing the chemical stability of 6 different composite materials used in dentistry, observed significantly the highest TEGDMA concentrations in water solutions, significantly lower concentrations of UDMA and the presence of Bis-GMA resin on the limit of detection. They did not confirm a presence of BPA in the assessed eluates [23].

Gioka *et al*. [24] used HPLC to analyze eluates obtained from two orthodontic adhesives stored in artificial saliva for 2 months. They confirmed the presence of TEGDMA at 13.2 ppm for a chemically polymerized material and 11.5 ppm for a light-curing resin. In this study, no Bis-GMA resin was found at the detection level assumed by the authors.

Moharamzadeh *et al*. [25] evaluated the release of monomers from experimental composite resins with high content of Bis-GMA, TEGDMA and UDMA, using the HPLC method. Only TEGDMA was identified in the eluates, while Bis-GMA oligomer and UDMA monomer were not detected. The mean TEGDMA concentration determined by the authors was 0.13 mg/cm³. Higher concentrations of

TEGDMA in solutions reported by Moharamzadeh *et al*. compared to the present study can be explained by the fact that the authors used samples of higher mass and that chemical composition of materials evaluated in both studies was different.

Pelourde *et al*. [26] used gas chromatography and mass spectrometry (GC/MS) to determine the presence of chemical compounds released into solutions from Transbond XT and Transbond LR orthodontic adhesive systems.

Among various chemical compounds present in the solutions, they identified TEGDMA with simultaneous absence of both BPA and Bis-GMA in the eluates. The concentration of TEGDMA determined by the quoted authors at 13.12 μg/cm^3 in the eluates obtained from Transbond XT was significantly higher than the concentrations determined in the present study. The reasons for the mentioned difference should be found in a different method of sample preparation, different volumes of solutions in which materials were incubated, and the use of different analytical methods in both studies.

Many published studies on the stability of composite resins used in dentistry focus on the release of BPA and Bis-GMA.

Pulgar *et al*. [27] evaluated Bis-GMA-based dental composite resins used for fillings. Using the HPLC method they confirmed that BPA and Bis-GMA are present in the eluates obtained from polymerized and unpolymerized samples of most materials. Samples of 100 mg in 1 cm^3 of aqueous solvent were tested. For the applied method, the detection limit for BPA was determined at 0.2 μg/cm^3.

Eliades *et al*. [28] assessed the chemical stability of two different Reliance orthodontic adhesives. Samples of both adhesive systems after polymerization on the bases of metal brackets were treated with 15 cm^3 of 99 % alcohol. The obtained eluates were then analyzed by HPLC for BPA presence in solutions. At none of the time intervals adopted in the study did the chromatograms depict a peak characteristic for BPA at the limit of detection determined by the authors at 0.1 ppm = 0.1 μg/dm^3, which raises doubts as to the correctness of the units of measurements given by the quoted authors.

In a paper published in 2011, Eliades *et al*. [29] evaluated the stability of Transbond XT adhesive resin stored in aqueous solution in three time intervals: 10, 20 and 30 days. They confirmed the presence of BPA in the eluates with the use of gas chromatography/mass spectrometry (GC/MS). The highest concentration of BPA of 2.9 μg/dm^3 was observed after 30 days of incubation of material samples, *i.e*., outside the range of observation times adopted in the study.

Sunitha *et al*. [30] examined Transbond XT adhesive resin by exposing it to a 99 % alcohol solution. For samples of polymerized adhesive in conditions as close as possible to optimal, the presence of BPA in the eluates at 14.38 ppm after 24 hours and at 21.55 ppm after 7 days of observation was confirmed. In this study, the HPLC method was used, with the detection threshold at 0.1 ppm = 0.1 μg/dm^3, as in Eliades *et al*. from 2007.

Kotyk *et al*. [31] published the results of their research, in which they analyzed substance elution from – *inter alia* – Transbond XT adhesive system exposed to artificial saliva and variable temperatures in the 4–60 °C range, which was to intensify the resin degradation process. The eluates obtained from Transbond XT demonstrated the presence of BPA only after 3 days of observation with an average level of 2.75 μg/g. The GC/MS method was used in this study.

Purushothaman *et al*. [32] confirmed BPA release from samples of orthodontic adhesives polymerized on metal brackets, treated with a 99 % alcohol solution. The authors used research methodology similar to that described by Sunith *et al*. [30]. In the study of the quoted authors, the lowest concentration of BPA was noted in eluates of chemo-cured resin and it remained at the level of 0.18 ppm after 24 hours, and at 0.32 ppm after 21 days of observation. In the case of Transbond XT light-cured resin, the concentration of BPA in the eluates increased with the growing distance of the lamp tip during polymerization, reduction of exposure time and lower conversion rate of the tested samples.

Moreira *et al*. [33] evaluated BPA elution from 5 different orthodontic adhesive systems, including Transbond XT, *in vitro* and *in vivo*. As a solution in laboratory conditions, the quoted authors used an alcohol-water solution with volume proportion of 3 : 1. In order to evaluate the release of BPA from dental materials *in vivo*, the authors analyzed its concentration in saliva and urine samples. The tested fluids were assessed by gas chromatography. This study confirmed the release of BPA from all materials *in vitro* in all observation intervals. The authors noted an increase in the concentration of BPA in solutions with the duration of observation time, ranging from 28 ng/g after 30 minutes to 324.1 ng/g after one month of sample incubation. In the *in vivo* study, Moreira *et al*. [33] reported a significant increase in BPA level in saliva 30 minutes after fixing brackets of thin-wire permanent appliances with light-curied Transbond XT adhesive system. In urine samples, an increase in BPA concentration was significant after 24 hours and 7 days of observation.

The above-mentioned studies on the stability of adhesive resins used in orthodontics and dentistry confirm the diversity of evaluated materials, adopted analytical methods, techniques of sample preparation, volumes and types of leaching solutions and applied units of measurement. These methodological differences do not allow a full comparison of results. The use of 99 % alcohol as an environment for incubation of dental materials, which is considered a medium significantly increasing composite materials' degradation, undoubtedly raises the probability of elution of components from polymerized samples of orthodontic adhesives compared to the elution capacity of water or saliva [27, 34, 35]. The influence of analytical method choice is also debatable. Some authors sug-

gest that the use of the gas chromatography technique for BPA detection in eluates may give false positive results. This is related to exposure of samples to a temperature of about 300 °C, which causes a breakdown of oligomers, *i.e.*, Bis-GMA, with a release of BPA, which would not take place in oral cavity environment [31, 35]. On the other hand, Hope *et al*. [34], after comparing commonly used analytical methods, *i.e.*, gas and liquid chromatography, suggest the possibility of obtaining false positive results when using the latter of these detection methods. The authors explain this phenomenon with a similarity of chromatographic curves of other chemical compounds to curves characteristic of BPA. Hope *et al*. [34] suggest the selection of mass spectrography as the detection method that increases sensitivity and specificity of identification of eluted substances.

Available literature offers few reports describing the dependence of release dynamics of composite resins' components used in dentistry in relation to pH of the environment.

Lee *et al*. [36] used 99 % propionic and acetic acid, distilled water and 75 % ethanol for specimen storage. They observed significantly higher leaching potential of these acids and ethanol compared to water.

Gusmão *et al*. [37] assessed the influence of external environment's pH level on water sorption by composite resins. They found that weight gain of some samples was significantly lower when they were treated with a solution with pH of 4.3, considered to be cariogenic, than in the case of samples stored in artificial saliva at pH = 7. This was explained by increased solubility of samples of composite materials used in dentistry, depending on low pH. At this point, however, it should be mentioned that increased solubility of dental materials in a lower pH environment does not have to be associated with losses in the organic matrix, but may depend on a loss of inorganic fillers.

In the present study, no explicit correlation was observed between pH of the environment and chemical stability of evaluated orthodontic adhesive systems, the measure of which was the concentration of TEGDMA monomer released into the solutions. This difference may result from a different test method, evaluation criteria and type of dental materials assessed in this study.

Pulgar *et al*. [27] evaluated the effect of aqueous solutions with pH values of 1, 7, 9 and 12 on dental sealants and materials used for fillings. They confirmed the effect of pH on the released amount of some monomers from polymerized samples of the majority of tested materials. According to the quoted authors, elution of BPA and other resin components increased with increasing pH and was the highest at pH = 9 and 12. The pH range of solutions for which Pulgar *et al*. [27] noted significant differences in chemical stability of dental composite materials was higher than adopted in the current study and going beyond the values observed in the oral cavity environment.

Örtengren *et al*. [38] evaluated two dental composite resins and found no significant relationship between their solubility and the level of pH. Only one of the Filtek Z-100 materials showed an increase in solubility with an increase in pH for the assumed values of 4, 6, 8. In another publication, Örtengren *et al*. [39] described further studies on chemical stability of Filtek Z-100 composite material in solutions with pH values of 4, 6, 8 in subsequent observation periods. The eluates were examined by fluorescence spectroscopy and GC/MS for the presence of six organic substances, including TEGDMA, EGDMA, BPA. They did not find a clear leaching pattern for individual substances depending on the duration and pH of the solution. The quoted authors reported a lower total leaching of material components at higher pH values after long storage [39].

Summary

In the current study, significant differences in the amount of TEGDMA monomer released from individual adhesive materials were observed. They may indicate different chemical stability of the assessed composites as well as different chemical composition of the orthodontic adhesives. On the other hand, the authors observed no commonly occurring relationship between the amount of monomer released into the external environment and the pH of solutions in which samples of materials were stored in the applied pH range. Only in the case of Contec LC material, which demonstrated the highest degree of TEGDMA monomer emission, a positive correlation could be observed between the concentration of TEGDMA and the increase in pH of the solution for the observation period of 1 hour and 7 days.

CONCLUSIONS

– Under the conditions of the study, orthodontic adhesive systems are not chemically stable.

– The obtained results suggest that pH level of the external environment influences the chemical stability of orthodontic adhesive systems. However, determination of accurate relationships requires further research.

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