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Sorption, Solubility, and Mass Changes of Hydroxyapatite-Containing Composites in Artificial Saliva, Food Simulating Solutions, Tea, and Coffee

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ABSTRACT: The purpose of this study was to evaluate the influence of preparation/storage conditions on the sorption, solubility, and mass changes of new proposed hydroxyapatite-containing resin-based composites. Seventy cylindrical samples of composite were prepared according to the ISO 4049 and stored in different storage solutions (distilled water, artificial saliva, 10% ethanol, 3% acetic acid, heptane, tea, and coffee) for 7, 14, and 28 days at 37°C. Principal component analysis and analysis of the variance were used to determine the impact of the preparation and storage conditions (e.g., curing time, storage time, and type of storage solution) on the changes of stability of examined material. Sorption, solubility, and mass changes of examined samples were specified. The tendency of these changes depending on the curing time, storage time, and type of storage solutions were presented. Due to the observed behavior, three groups of storage solutions were distinguished: "aqueous," acidic, and hydrophobic ("fat") solutions. Investigated properties changed in different way, characteristic for each of the above groups. No general tendency of the influence of storage and curing time was observed. The type of storage solution has the greatest impact on the sorption, solubility, and mass changes of examined material. The influence of the curing and storage time may be neglected. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39856.

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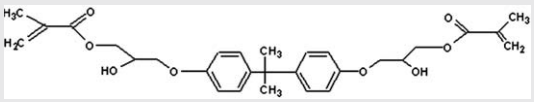
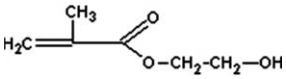
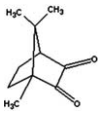
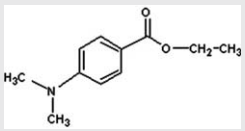
INTRODUCTION

For several decades, dental resin-based composites (RBC) have been one of the most commonly used materials for fillings and other dental applications (e.g., adhesives, sealants, crowns, bridges, and fixing cements).[1,2] They consist of two basic parts: the resin matrix and filler particles. Since 1965, following the Bowen's resin introduction, this resin (called Bis-GMA) is the most important component of the organic matrix. Full name of Bis-GMA is 2,2-bis[p-(2′-hydroxy-3′-methacryloxypropoxy)-phenyl]-propane. Due to the high viscosity of Bis-GMA, another monomer (comonomer), like hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate or other, is usually added. It allows to reduce the viscosity of the organic matrix by the dissolution the Bis-GMA in comonomer. Different aluminum-silicate glasses most often play the role of the filler in these composites.[1,3] RBC show several advantages in comparison with other groups of dental fillings, for example, low polymerization shrinkage,[2,3] low coefficient of thermal expansion, good mechanical properties,[3] ability to cross-linkings,[2] and high level of aesthetics.[4] The addition of the filler is designed to improve the mechanical, biological, chemical, or physical properties of RBC. Additionally, incorporation of fluoride component (or other antimicrobial substance) into matrix cause permanent fluoride release increasing cariostatic activity of the filling.[5]

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) is a natural constituent of bone, dentin, and enamel. Therefore, its application as a component of dental composites appears to be logically reasonable.[6] Some authors suggest that HA-reinforced RBC may be better than this commercial available because of the lower price of HA (compared with traditionally used fillers) and possibility of improvement the mechanical properties of filling by this material (less wear).[7,8] Another interesting feature of HA is that it is considered to be bioactive (not only biocompatible), what can be beneficial in the context of the release of calcium and phosphorus ions. These ions are responsible for the remineralization of enamel and the restoration of the mineral part (apatite) of the tooth.[9]

Table I. Components of the Examined Samples

Name	Formula	Function
Bis-GMA		Monomer
HEMA		Comonomer
HA	$Ca_{10}(PO_4)_3(OH)_2$	Filler
CQ		Initiator
EDMAB		Coinitiator

Two important factors in the application of dental fillings are their behavior and stability in the human mouth environment. We have selected several fluids simulating the natural conditions. This group can include such liquids as artificial saliva, food-simulating solutions, and so popular beverages as tea or coffee. Sorption, solubility, and mass changes during use are important predictors of the suitability and durability of dental materials. These parameters indicate the degree of absorption and dissolution of the material in the fluid used in given experiment. This allows to determine the total mass change, which occurs during the experiment as well as to estimate the direction of these changes (whether the sorption or degradation is predominant). This test will allow the investigation of the material behavior under conditions similar to natural ones occurring in human mouth. The applied procedure will also enable to define the type of solution which has the greatest impact on the material stability. Determination of these parameters in the above solutions at 37°C may bring the useful information about stability of examined system.[10–12] Many factors, like filler type, curing time, type of used solution, and storage time may affect the value of the determined parameters. Examination of all possibilities is time consuming, so it was decided to extract the factors responsible for the variability of the properties of studied materials. The aim of this article is to use the analysis of variance (factorial ANOVA) and principal component analysis (PCA) to estimate which one of these factors are most important (provide the largest changes) and which one can be ignored. ANOVA is able to determine the influential factors; PCA is used to detect similarities between samples, and variables describing the stability.

EXPERIMENTAL

Materials

Dental filling samples were prepared using 2,2-bis[*p*-(2′-hydroxy-3′-methacryloxypropoxy)phenyl]-propane (Bis-GMA; Sigma-Aldrich) as a basic monomer and HEMA (97%, Sigma-Aldrich) as a regulator of viscosity comonomer. Bis-GMA to HEMA ratio was 60 : 40 (m/m). HA (p.a. ≥90%, Sigma-Aldrich) was used as filler. Monomer to filler ratio was 50 : 50 (m/m). Camphorquinone (CQ; 97%, Sigma-Aldrich) and ethyl(4-dimethyl amino) benzoate (EDMAB; Sigma-Aldrich) were used as a initiator and coinitiator of polymerization, both in amount of 0.5% (m). Formulae of all compounds used in the preparation of the dental filling samples are given in Table I.

Storage Solutions

The composition and function of all storage solutions used in this work for storage of the examined samples is given in Table II. Coffee solution was prepared by dissolving 1 g of coffee in 125 mL of hot water, while black tea solution was made by placing a tea bag in 125 mL of hot water for 3 min.

Sample Preparation

Samples (70; 35 for each curing time; 5 for each solution) were prepared in a PTFE mold (Ø 15 mm, thickness 1 mm-according to ISO 4049[14]), covered on both sides with PET foil. The curing process was carried out in 30 or 60 s (both sides) with two LED lamps (HILUX Optimax, 81W) emitting blue light of wavelength 470 nm (maximum absorbance of CQ).

Storage Conditions

After curing, each sample was weighed (in air – m_1 and in water – m_{w1}) and placed in 10 mL of storage solution (5 samples for each solution). After 7, 14, and 28 days of storage in 37°C samples were dried on surface by tissue (to remove water adsorbed on surface) and weighed (in air – m_2). After these samples were placed in dessicator and weighed till constant mass was obtained (m_3).

Sorption, Solubility, and Mass Changes

Sorption for each of tested solutions was calculated according to equations:

Table II. Composition and Function of Used Storage Solutions

Name	Function		Composition	
			Compound	Concentration (mg L^{-1})
SAGF[13]	Artificial saliva pH = 6.8		NaCl	125.6
			KCl	963.9
			KSCN	189.2
			KH_2PO_4	654.5
			Urea	200
			Na_2SO_4	763.2
			NH_4Cl	178
			$CaCl_2 \times 2H_2O$	227.8
			$NaHCO_3$	630.8
			H_2O	
FSS 1	food simulating solutions	Aqueous foods	Distilled water	
FSS 2		Acidic foods	3% Acetic acid	
FSS 3		Alcohol containing foods	10% Ethanol	
FSS 4		Fat foods	Heptane	
Tea	Black tea		Express black tea Saga by Unilever Poland S.A.	
Coffee	Instant coffee		Instant coffee Maxwell House by Kraft Foods Poland S.A.	

$$Sp_1 = \frac{m_2 - m_3}{V} \left(\mu g\, \mu L^{-1}\right) \quad (1)$$

or

$$Sp_2 = \frac{m_2 - m_3}{m_2} \cdot 100(\%) \quad (2)$$

while the solubility was calculated based on following formulas:

$$Sl_1 = \frac{m_1 - m_3}{V} \left(\mu g\ \mu L^{-1}\right) \quad (3)$$

or

$$Sl_2 = \frac{m_1 - m_3}{m_1} \cdot 100(\%) \quad (4)$$

where V is the volume of sample calculated from the density determined by pycnometric method:

$$V = \frac{m_1 - m_{w1}}{d_0} \quad (5)$$

where d_0 is the density of water (at temperature of measurement; g/mL).

Finally, mass changes were calculated according to eq. (6):

$$D_m = \frac{m_2 - m_1}{m_1} \cdot 100(\%) \quad (6)$$

Sorption (Sp) indicates the mass, which is reversibly absorbed during the storage in relation to the initial sample volume (Sp_1) or to the mass of swollen sample (Sp_2). Solubility (Sl) indicate the irreversible mass change, unchanged after drying in dessicator, in relation to the initial sample volume (Sl_1) or to the initial sample mass (Sl_2). D_m indicates the mass changes during the storage process, before drying, in relation to the initial sample mass.

These magnitudes are significantly correlated at the 0.05 level, (cf. Table III). The only exception is Sp_1, which does not correlate with Sl_1 and Sl_2 significantly. Our aim was also to determine which factor characterizes the mass changes at best.

CHEMOMETRIC METHODS

Variance Analysis (ANOVA)

A full factorial experimental design reveals the influence of various factors on the sorption, solubility, and mass changes, namely: curing time (30 or 60 s), the effect of solvent [water (*w*), SAGF (*s*), 10% ethanol (*e*), 3% acetic acid (*a*), heptane (*h*), tea (*t*), and coffee (*c*)] and storage time: 7, 14, and 28 days.

Although the model matrix (X) in a full factorial design is not singular, X'X can be inverted and the coefficients calculated. The significance of coefficients cannot be tested due to the lack of redundancy. Therefore, it is necessary to sacrifice of the third order interaction (curing time, storage time, and type of storage solution). We should keep in mind that the influence that one factor has on the response depends on the value of the other factors. ANOVA is a method for assessing effects of categorical factors and their interactions. At the same time, it gives a model for these effects, for this reason it is a member of the General Linear Model (GLM) family.[17]

Table III. Correlations Between Measured Parameters

Variable	Sp_1	Sp_2	Sl_1	Sl_2	D_m
Sp_1	1.0000	**0.5956**	0.1471	0.2355	**0.3374**
Sp_2	**0.5956**	1.0000	**0.3404**	**0.3322**	**0.6261**
Sl_1	0.1471	**0.3404**	1.0000	**0.9268**	**−0.4578**
Sl_2	0.2355	**0.3322**	**0.9268**	1.0000	**−0.5273**
D_m	**0.3374**	**0.6261**	**−0.4578**	**−0.5273**	1.0000

Marked correlations are significant at $P < 0.05000$, $N = 42$.

Table IV. Mean Values (± Standard Deviation) of Sorption, Solubility, and Mass Changes of Examined Samples

Curing time (s)	Storage time (d)	Solution	Sp_1 ($\mu g\ \mu L^{-1}$) mean (±SD)	Sp_2 (%) mean (±SD)	Sl_1 ($\mu g\ \mu L^{-1}$) mean (±SD)	Sl_2 (%) mean (±SD)	D_m (%) mean (±SD)
30	7	w	92859.13 (±68534.69)	3.89 (±0.19)	−24910.99 (±25833.17)	−0.92 (±0.30)	5.00 (±0.18)
		s	88335.90 (±53520.44)	3.71 (±0.28)	−27006.31 (±15677.92)	−1.22 (±0.21)	5.13 (±0.22)
		e	47443.96 (±12487.30)	3.85 (±0.45)	−23065.12 (±8093.03)	−1.95 (±0.15)	6.03 (±0.56)
		a	73359.38 (±27007.43)	5.24 (±0.66)	26587.24 (±27659.55)	1.73 (±1.81)	3.69 (±1.26)
		h	1280.47 (±297.48)	0.09 (±0.02)	2743.87 (±1568.82)	0.19 (±0.10)	−0.10 (±0.10)
		t	57454.92 (±16458.59)	5.25 (±2.02)	−10576.36 (±6316.86)	−0.93 (±0.45)	6.56 (±1.84)
		c	38859.18 (±23593.72)	5.50 (±0.98)	−6547.27 (±4363.61)	−0.89 (±0.31)	6.77 (±0.96)
30	14	w	93291.50 (±70990.83)	3.87 (±0.16)	−20371.14 (±22265.06)	−0.73 (±0.32)	4.78 (±0.24)
		s	89516.60 (±52577.81)	3.79 (±0.18)	−24927.62 (±13451.46)	−1.15 (±0.21)	5.13 (±0.18)
		e	48807.58 (±13531.35)	3.94 (±0.36)	−24062.89 (±8711.38)	−2.03 (±0.18)	6.21 (±0.42)
		a	84171.88 (±32564.97)	6.02 (±1.16)	40921.87 (±37930.18)	2.75 (±2.42)	3.47 (±1.41)
		h	199.55 (±514.57)	0.01 (±0.03)	4157.37 (±1747.09)	0.29 (±0.12)	−0.27 (±0.10)
		t	53596.88 (±12488.13)	4.90 (±1.61)	−10809.17 (±6242.16)	−0.96 (±0.45)	6.18 (±1.37)
		c	36898.48 (±22704.55)	5.25 (±0.94)	−6268.53 (±4488.32)	−0.82 (±0.42)	6.41 (±0.86)
30	28	w	116522.91 (±90102.54)	4.79 (±0.20)	−249.44 (±6191.00)	0.12 (±0.30)	4.91 (±0.32)
		s	113895.45 (±63949.20)	4.87 (±0.19)	−7117.43 (±4224.02)	−0.36 (±0.22)	5.49 (±0.32)
		e	63458.17 (±17888.35)	5.09 (±0.26)	−14550.81 (±6512.10)	−1.21 (±0.23)	6.63 (±0.40)
		a	112551.78 (±43343.10)	8.08 (±1.28)	77530.05 (±52816.73)	5.37 (±2.95)	2.92 (±1.84)
		h	−548.77 (±511.21)	−0.04 (±0.04)	4373.56 (±1449.25)	0.30 (±0.09)	−0.34 (±0.11)
		t	61495.89 (±7058.39)	5.56 (±1.04)	−2062.06 (±3872.57)	−0.15 (±0.36)	6.06 (±0.83)
		c	42517.67 (±25612.54)	5.99 (±0.81)	−1594.85 (±1950.02)	−0.13 (±0.28)	6.52 (±0.91)
60	7	w	43516.55 (±20261.15)	5.28 (±0.60)	−5561.86 (±3282.05)	−0.67 (±0.25)	6.29 (±0.89)
		s	28307.76 (±7201.50)	5.38 (±1.50)	−5029.24 (±3583.79)	−0.89 (±0.38)	6.65 (±1.51)
		e	36362.97 (±11428.35)	4.84 (±0.19)	−13290.30 (±4081.35)	−1.90 (±0.10)	7.08 (±0.16)
		a	45547.59 (±7366.13)	5.93 (±0.78)	19266.74 (±6989.05)	2.66 (±1.06)	3.47 (±0.29)

TABLE IV. *Continued*

Curing time (s)	Storage time (d)	Solution	Sp_1 ($\mu g\ \mu L^{-1}$) mean (±SD)	Sp_2 (%) mean (±SD)	Sl_1 ($\mu g\ \mu L^{-1}$) mean (±SD)	Sl_2 (%) mean (±SD)	D_m (%) mean (±SD)
		h	958.11 (±465.95)	0.14 (±0.07)	1342.08 (±1382.91)	0.18 (±0.13)	−0.03 (±0.11)
		t	28866.14 (±6689.32)	4.28 (±0.12)	−6513.69 (±2628.74)	−0.97 (±0.21)	5.49 (±0.13)
		c	32809.51 (±10300.78)	4.04 (±0.17)	−8945.54 (±3852.92)	−1.11 (±0.15)	5.37 (±0.07)
60	14	w	36251.83 (±16730.74)	4.47 (±0.20)	−7125.50 (±4210.68)	−0.86 (±0.30)	5.57 (±0.10)
		s	25429.67 (±5739.36)	4.91 (±1.46)	−6345.34 (±3992.20)	−1.14 (±0.39)	6.38 (±1.42)
		e	34973.35 (±9908.03)	4.67 (±0.11)	−16264.26 (±4966.58)	−2.32 (±0.11)	7.34 (±0.20)
		a	52009.50 (±8641.20)	6.80 (±0.82)	30082.19 (±9225.33)	4.13 (±1.39)	2.85 (±0.63)
		h	374.16 (±262.38)	0.05 (±0.02)	1753.37 (±1670.37)	0.23 (±0.15)	−0.18 (±0.14)
		t	27279.11 (±5798.82)	4.06 (±0.22)	−8097.64 (±3344.68)	−1.21 (±0.26)	5.50 (±0.20)
		c	31949.72 (±9656.55)	3.95 (±0.21)	−10683.16 (±4782.09)	−1.31 (±0.23)	5.48 (±0.04)
60	28	w	37482.42 (±18314.47)	4.54 (±0.15)	−4679.54 (±3010.80)	−0.55 (±0.26)	5.33 (±0.35)
		s	24793.79 (±6610.55)	4.66 (±0.53)	−5298.87 (±3585.17)	−0.95 (±0.39)	5.88 (±0.19)
		e	36040.06 (±10184.14)	4.82 (±0.13)	−14915.15 (±4520.59)	−2.13 (±0.15)	7.31 (±0.26)
		a	60485.02 (±10826.86)	7.93 (±0.78)	44154.60 (±11440.26)	6.01 (±1.64)	2.08 (±0.93)
		h	64.76 (±322.41)	0.00 (±0.05)	2356.41 (±1772.15)	0.32 (±0.15)	−0.32 (±0.12)
		t	29469.18 (±6427.91)	4.38 (±0.20)	−6057.43 (±2802.58)	−0.89 (±0.28)	5.52 (±0.22)
		c	33535.33 (±10856.86)	4.10 (±0.10)	−9611.52 (±4243.10)	−1.19 (±0.18)	5.52 (±0.12)

PC Analysis

PCA is a projection method and dimension reduction of the data what can be achieved using a smaller number of PCs than that of original variables. The PCs are, in fact, linear combinations of the original variables. The linear coefficients of the inverse relation of linear combinations are called the component loadings, that is, the correlation coefficients between the original variables and the PCs. PCA is an unsupervised method of pattern recognition in the sense that no grouping of the data has to be known before the analysis. Still the data structure can be revealed easily and class membership is possible to assign in many cases.

PCs are uncorrelated and account for the total variance of the original variables. The first PC accounts for the maximum of the total variance, the second is uncorrelated with (orthogonal to) the first one and accounts for the maximum of the residual variance, and so on, until the total variance is accounted for. For practical reasons, it is sufficient to retain only those components, which account for a large percentage of the total variance.

PCA will show which variables and objects (samples, fillers, etc.) are similar to each other, that is, carry comparable information, and which ones are unique. The algorithm of PCA can be found in standard chemometric articles and textbooks.[15,16]

RESULTS

Mean values of sorption, solubility, and mass changes for all of examined samples after 7, 14, and 28 days storage in different solutions are given in Table IV. Results of ANOVA are shown in the Figures 1–5. According to the different units for part of variables ($\mu g\ \mu L^{-1}$ for Sp_1 and Sl_1 and % for Sp_2, Sl_2, and D_m) it was decided to compare them in pairs: Sp_1 with Sl_1 and Sp_2 with Sl_2. Figure 1 presents the Sp_1 values. In every case, the

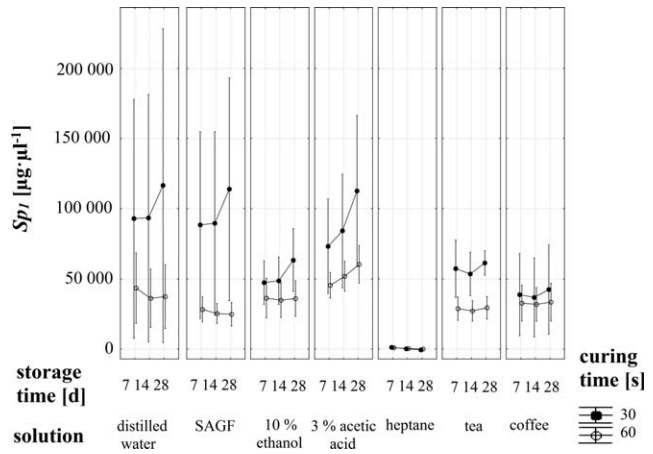


Figure 1. Weighted means of Sp_1 during experiment. F(12, 168) = 0.13659, $P = 0.99976$; effective hypothesis decomposition; vertical bars denote 0.95 confidence intervals.

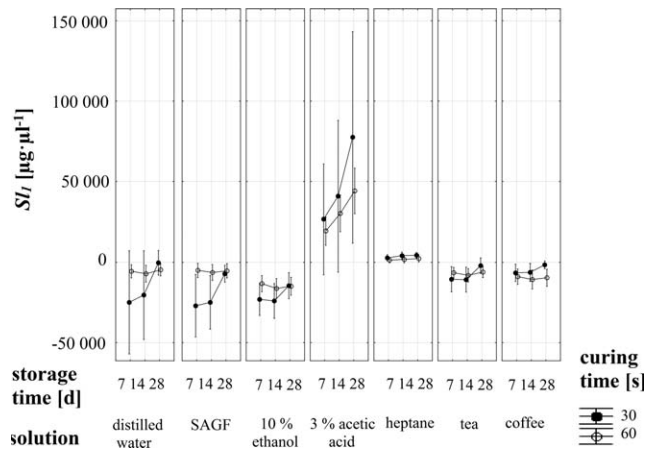


Figure 2. Weighted means of Sl_1 during experiment. F(12, 168) = 0.39718, $P = 0.96316$; effective hypothesis decomposition; vertical bars denote 0.95 confidence intervals.

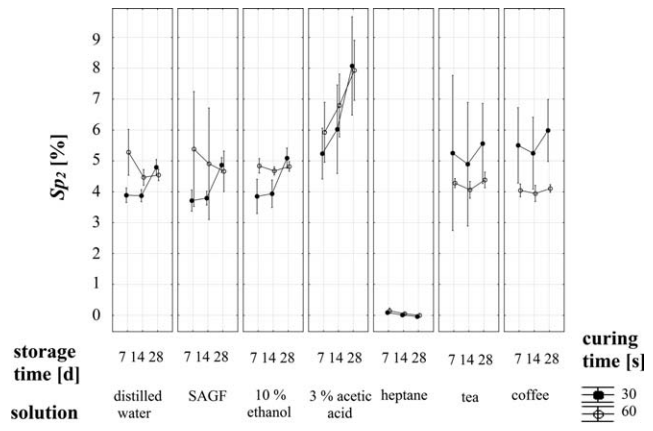


Figure 3. Weighted means of Sp_2 during experiment. F(12, 168) = 0.69617, $P = 0.75371$; effective hypothesis decomposition; vertical bars denote 0.95 confidence intervals.

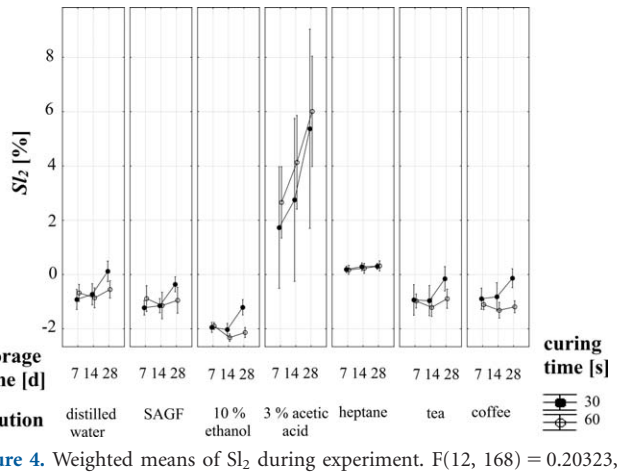


Figure 4. Weighted means of Sl_2 during experiment. F(12, 168) = 0.20323, $P = 0.99819$; effective hypothesis decomposition; vertical bars denote 0.95 confidence intervals.

sorption of the solution is higher for samples cured for 30 s than for 60 s. Sp_1 value usually increased with longer storage time for samples cured for 30 s; the exception are samples stored in tea and coffee, where this tendency is not observed. In addition, samples stored in heptane do not show this trend, values of sorption are more or less stable. Samples cured for 60 s present a different course, the Sp_1 values are rather stable with prolonging storage time (except samples stored in 3% acetic acid in which sorption increases after every period of time). Sorption (Sp_1) of the solution by examined samples can be aligned as follows: distilled water, SAGF > 3 acetic acid > 10% ethanol, tea > coffee > heptane (for 30 s curing time) and 3% acetic acid > distilled water, 10% ethanol > SAGF, tea, coffee > heptane (for 60 s curing time). All of the results are burden by large error (error bars), except samples stored in heptane. Values describing sorption are positive. Sl_1 values are shown in the Figure 2. Samples cured for 60 s are more soluble in distilled water, SAGF, and 10% ethanol than samples cured for 30 s. The opposite phenomenon is observed in 3% acetic acid. Solubility in heptane, tea, and coffee is approximately the same for both curing

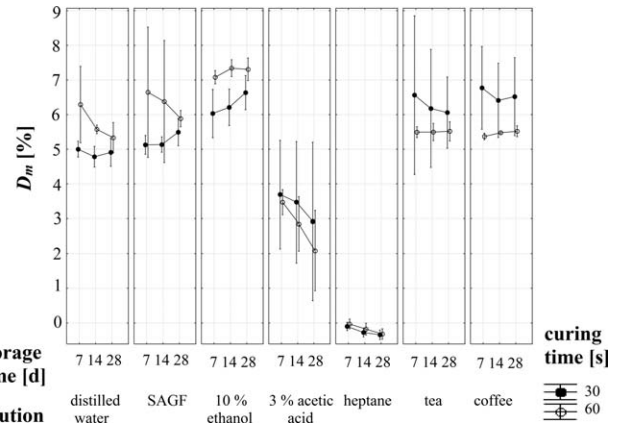


Figure 5. Weighted means of D_m during experiment. F(12, 168) = 0.48095, $P = 0.92385$; effective hypothesis decomposition; vertical bars denote 0.95 confidence intervals.

Table V. Loadings (Correlation Between the Initial Variables and the New Components)

Variable	Loading 1	Loading 2	Loading 3
Sp_1	−0.374	**−0.710**	−0.595
Sp_2	−0.458	**−0.839**	0.283
Sl_1	**−0.951**	0.155	0.179
Sl_2	**−0.976**	0.157	0.020
D_m	0.394	**−0.884**	0.244
Explained variance	2.362	2.039	0.526
Participation of total variance	0.472	0.408	0.105

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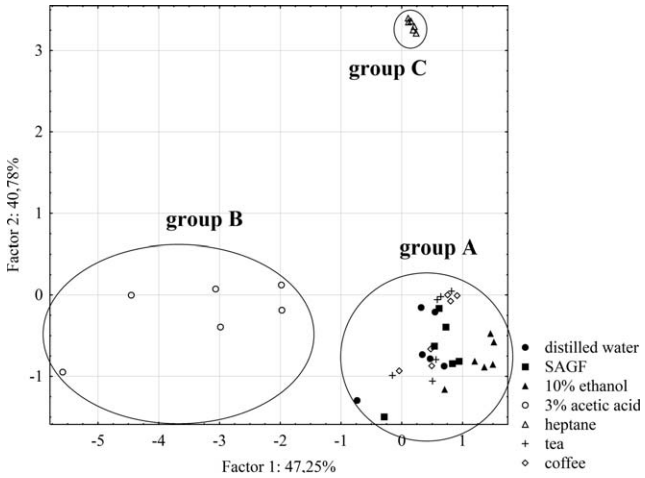

Figure 6. Effect of a storage solutions (scatterplot).

times. This value usually increase with storage time for samples with shorter curing time (30 s), whereas for longer curing time they are rather constant (except samples stored in 3% acetic acid). Solubility (Sl_1) of the samples in different solutions can be aligned as follows: 3% acetic acid > heptane > tea, coffee > distilled water, SAGF, 10% ethanol for both curing times. In this figure, large values of error bars are also found. For samples stored in distilled water, SAGF, 10% ethanol, tea, coffee Sl_1 values are negative while those for samples stored in 3% acetic acid, heptane are positive. Sp_2 values are presented in Figure 3. Sorption (Sp_2) of several

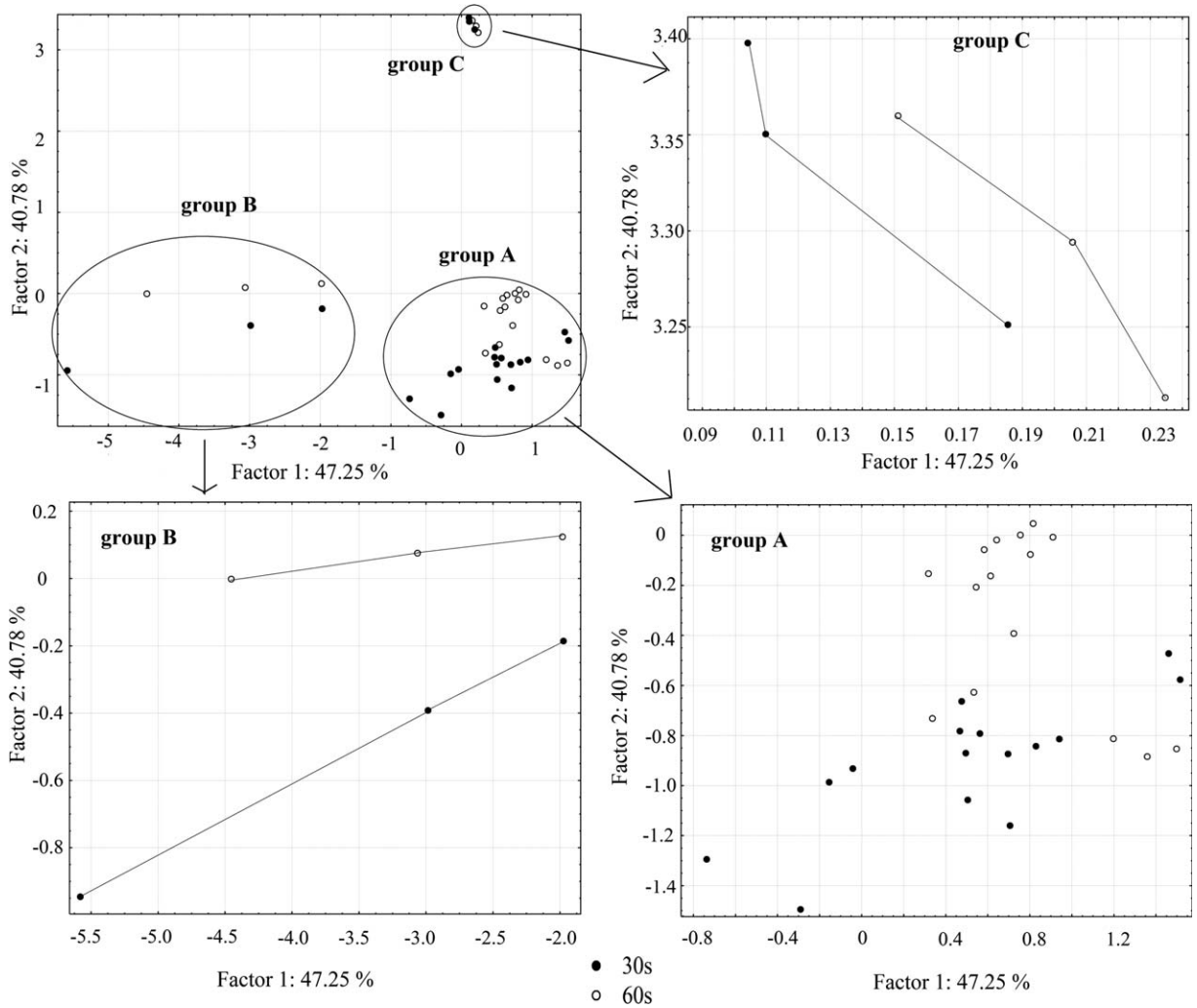

Figure 7. Effect of a curing time (scatterplot).

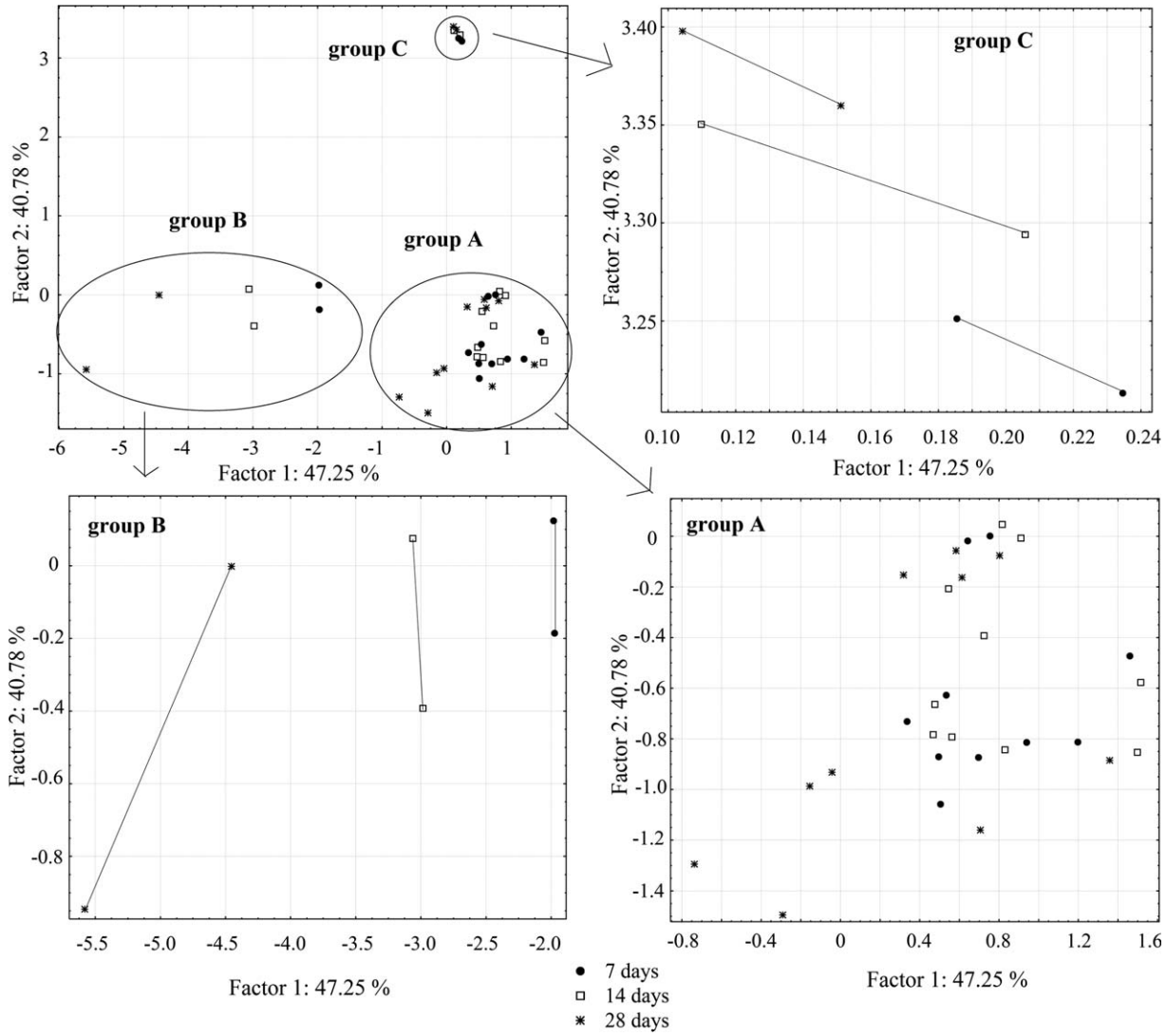

Figure 8. Effect of a conditioning time (scatterplot).

solutions is higher for samples cured for 60 s than for 30 s (distilled water, SAGF, 10% ethanol, 3% acetic acid), whereas for tea and coffee is smaller (in heptane approximately the same). Samples cured for 30 s absorb more solution after longer storage time except tea and coffee when no clear tendency is observed. Sp_2 values for sample stored in heptane are approximately stable. Sorption of water and SAGF decreases slightly with increasing storage time for samples cured for 60 s, For samples stored in 3% acetic acid Sp_2 values increased while no clear tendency was obtained for samples stored in 10% ethanol, tea, and coffee. It is also stable in heptane. The highest Sp_2 values were found for samples stored in 3% acetic acid, lower for distilled water, SAGF, 10% ethanol, tea, and coffee, while the lowest for samples in heptane. All Sp_2 values are positive. The second solubility parameter (Sl_2) is shown in the Figure 4. Most of the samples are more soluble when the curing time is shorter (30 s), the one exception are samples stored in 3% acetic acid. This solubility increases with storage time for samples cured for 30 s, while for samples cured for 60 s there is no clear tendency (exception: samples stored in 3% acetic acid). Solubility (Sl_2) of the samples in different solutions can be aligned as follows: 3% acetic acid > heptane > tea, coffee, distilled water, SAGF > 10% ethanol for both curing times. Sl_2 values are negative (for samples stored in distilled water, SAGF, 10% ethanol, tea, and coffee) and positive (3% acetic acid, heptane). Mass changes (D_m) of examined samples are shown in the Figure 5. These changes once are higher for samples cured for 60 s (in distilled water, SAGF, and 10% ethanol), while in other cases are lower, than for samples cured for 30 s. There is no clear tendency of the relationship between D_m values and storage time. The highest changes occur in 10% ethanol, distilled water, SAGF, tea, and coffee, smallest in 3% acetic acid and changes close to zero in heptane. Only these last values are negative.

Eigenvalues plot (scree plot) suggests three PC to be kept in the model. First two factors explained 88% of the total variance (Table V). The variance carried by Factor 3 explain about 10.5% of the total variance. Loadings 4 and 5 contain noise only and they are negligible. Loading 1 is strongly correlated with Sl_1 and Sl_2 so the variance explained by this component

depends mainly on the changes in the solubility of materials while Loading 2 describes mostly the variance in sorption and mass changes. The first PC is composed from Sl_1 and Sl_2, while second PC from Sp_1, Sp_2, and D_m. PCA scatterplot is presented in Figure 6. The solution effect is observed. The storage solutions can be grouped into three main groups, that is, group A: distilled water, SAGF, 10% ethanol, tea, coffee; group B: 3% acetic acid, and group C: heptane.

The nature of these solutions determines positions of corresponding points in the Figure 6. Effects of curing and conditioning times can be differentiated in PCA score plots. Figure 7 shows the effect of curing time with focus on every group of storage solutions. The points corresponding to curing time for either 30 or 60 s are connected by a line. For samples conditioned in acetic acid (group B) and heptane (group C) two lines corresponding to the different curing times can be seen in Figure 7.

The effect of storage time is shown in the Figure 8. There are clear distinctions of three pairs: corresponding to the 7, 14, and 28 days of storage of dental fillings immersed in group B and group C. No dependence on storage time was found for the samples kept in the group A.

DISCUSSION

ANOVA analysis of the obtained results demonstrates that sorption, solubility, and mass changes undergo changes during storage for 7, 14, and 28 days in different solutions at 37°C. Sp_1 and Sl_1 shows the greatest values of error bars (Figures 1 and 2). The variability of Sp_1 and Sl_1 might be explained by the procedure used for determination of Sp_1 and Sl_1. It is pycnometric method where the recommended mass of sample is in the range of 1–5 g (according to EN ISO 1183[18]), which is much greater than that used in our experiment (according to EN ISO 4049[14]). This can lead to generation of significant error and therefore high variability of the measured parameter. In such a case, it is appropriate to use parameters, which are not loaded by large error (Sp_2 and Sl_2). All results show that the examined material has the greater sorption (Sp_1 and Sp_2) of the solutions from group B (3% acetic acid) and group A ("aqueous solutions": distilled water, SAGF, 10% ethanol, tea, and coffee) than from group C (heptane). Examined material is the most soluble in 3% acetic acid, while in the "aqueous solutions" the solubility (Sl_1 and Sl_2) values are negative. It means that the samples placed in the solutions from group A have higher tendency to absorption from solution than to the dissolution. Both sorption and solubility in heptane have values close to zero. In addition, the variability of these parameters in heptane is very small. It means that the material has rather hydrophilic than hydrophobic characteristic. The mass changes (D_m) during storage are largest in "aqueous solutions," than in 3% acetic acid and heptane (near zero). The simultaneous interpretation of the values of all examined parameters allows to claim that acidic foods (imitated by 3% acetic acid) provokes larger changes in the examined material than artificial saliva (SAGF), aqueous foods (imitated by distilled water), alcohol containing foods (imitated by 10% ethanol), tea, coffee, and fat foods (imitated by heptane). The acidic solvents cause the greatest solubility and sorption of examined material. Changes occurred in heptane are so small that they may be considered as negligible. The effect of curing time on Sp_1, Sp_2, Sl_1, Sl_2, and D_m is small and there is no possibility to define the tendency, which it produces. In addition, the storage time seems to have a little effect on the changes during storage without no clear tendency. Only in one case, it has a large meaning, for samples stored in 3% acetic acid. In this case both sorption and solubility increase with storage time. Mass change decrease with storage time for samples stored in this solvent. It is probably caused by the significant weight loss in the earlier period of time.

PCA analysis allows to draw the same conclusions. Distinct impact of storing solutions is observed. The grouping of the samples according to the storage medium confirm the earlier statement, the three groups of solutions are distinguished (A: "aqueous", B: 3% acetic acid, and C: heptane). Effect of curing time and storage time is observable only in case of two groups: B and C. However, it should be noted that in case of C group the values of measured parameter are so small that this effect cannot be distinctly visible.

ANOVA and PCA analysis show that the greatest variability of the sorption, solubility, and mass changes depend mainly on the type of storage solutions. The effect of curing and storage time is minor and could be considered as secondary or even negligible.

Distilled water and artificial saliva are the fluids, which are most often applied in this type of experiments. The values of the distilled water sorption, for the examined material, are in the range of 3.87 ÷ 4.79 and 4.47 ÷ 5.28% for 30 and 60 s curing times, respectively. While for artificial saliva, they are 3.71 ÷ 4.87 and 4.66 ÷ 5.38% for 30 and 60 s curing times, respectively. Other authors have determined the values of this parameter for several experimental and commercial dental fillings. Skrtic et al.[9] investigated the sorption for several dental composites based on amorphous calcium phosphate and organic resin, after 30 days in 75% relative humidity air atmosphere. They have obtained the results at the level of 1–5%. Water sorption for commercial resin-modified glass-ionomer cements determined by Miettinen et al. after 7 days of immersion was in the range of 1 ÷ 9%,[19] while Kanchanavasita et al. have determined this value for another commercial RMGIC in water and artificial saliva at the level of 6.1 ÷ 15.3%.[20] Atai et al. have prepared several experimental Bis-EMA composites and determined its water sorption on the maximum level of 3%.[21] All of these data vary in the significant range, but the sorption values determined in this study are within this range. It means that, due to the water and artificial saliva sorption, investigated material is comparable to the commercial ones. Ferracane in its review article note that the sorption of composite materials reach the maximum values even 7%, which is in the accordance to our results.[22]

The solubility of the examined material in distilled water is in the range of −0.92 ÷ 0.12 and −0.86 ÷ −0.55%, for 30 and 60 s curing times, respectively. While the values of the solubility in artificial saliva are in the range of −1.22 ÷ −0.36 and −1.14 ÷ −0.89% for 30 and 60 s curing times, respectively. Kanchanavasita

et al. have determined the value of this parameter for several commercial RMGIC in much higher range from 0.4 to 9.6%,[20] while Ferracane suggested that the solubility of the dental composites reaches maximum value of 2% (and up to 7% when immersed in alcohol and other organic solvents).[22] Composite, which was examined in this study, shows much lower values of solubility than these mentioned above by other authors. It means that our composite is less soluble.

The values of the mass changes in distilled water, for the examined material, are in the range of 4.78 ÷ 5.00 and 5.33 ÷ 6.29% for 30 and 60 s curing times, respectively. While for artificial saliva, they are 5.13 ÷ 5.49 and 5.88 ÷ 6.65% for 30 and 60 s curing times, respectively. Musanje et al. have determined the value of this parameter for several commercial compomers, RBC and resin-modified glass-ionomer cements, which are used as a dental fillings. These values are in the range of −4 ÷ 4 and −1 ÷ 7% for distilled water and artificial saliva, respectively.[23] The values obtained for our composite are comparable with these mentioned above.

CONCLUSIONS

Analysis of the variance (ANOVA) and PCA suggest that the conditions of preparation and storage influence the stability (measured as a sorption, solubility, and mass changes) of dental fillings at most. The type of storage solution has the main impact on the stability of HA-containing dental composites. The greatest variability of sample properties occurs in 3% acetic acid. These fillings are least stable in acidic environment (e.g., acidic foods). The largest stability is observed in heptane simulating fat foods. Changes of samples properties are approximately similar in such storage mediums as distilled water (simulating hydrated food), artificial saliva, 10% ethanol (simulating alcohol-containing food), tea, or coffee, that is, samples are most vulnerable to acidic foods and beverages, afterwards to some aqueous (also coffee and tea) and alcoholic foods and finally to fat meal and drink. Fillings have hydrophilic character. It is also possible to group the samples according to the curing time. It has a secondary impact on the stability of dental fillings but a clear tendency could not be assessed. The effect of the conditioning time is the smallest. It can be observed mainly for acidic solutions (3% acidic acid), but in other cases this influence might be neglected. The values of the sorption, solubility, and mass changes are comparable with the results of other authors for several experimental and commercial dental fillings.

AUTHOR CONTRIBUTIONS

Zuzanna Okulus: experiment design, carrying out the experimental part, data interpretation, drafting the article, and approval of the submitted and final versions of article; Karoly Héberger: chemometrical calculations and interpretation of the results, revising the paper, approval of the submitted and final versions of article; Adam Voelkel: experiment design, data interpretation, revising the article, and approval of the submitted and final versions of article.

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