

The effects of the curing regime and the composition on the absorption, solubility and the amount of residual monomers of dental flowable composites

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In the present work we investigate 13 samples of dental flowable composites with different ratios of fillers and different amount of polymerization initiator systems. This study summarises the formulation and physico-chemically analysis of such experimental composites, suggesting future work that will drive progress in this area into the future of dental composites research. The water absorption and solubility was determined according with ISO 4049/2000. The amount of residual monomers was determined by implementing two techniques: the high-performance liquid chromatography (HPLC) and the Nuclear Magnetic Resonance (NMR) relaxometry. The comparison of the results provided by the two techniques revealed good agreement. The advantages of NMR relaxometry as compared with HPLC technique are that it allows a faster determination of the residual monomer (6 minutes versus 10 hours), is completely noninvasive and does not require any sample preparation. The samples were investigated by infrared spectroscopy (IR), which provides information about atomic and molecular vibration unit. It is a standard analytical method which can provide information on various chemicals and guidance organization structures. Within the limitations of this experimental study, it was shown that the curing regime, the generic type of material and the ratio between organic and inorganic phase, affect the sorption, the solubility and the amount of residual monomers of dental flowable composites.

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1. Introduction

Dental composites are complex mixtures of ingredients and their knowledge is the key to a better understanding of their behavior and evolution. Each ingredient has, at a certain amount, a specific effect on bond strength, durability, shelf life and biocompatibility. An unbalanced mixture of ingredients, that can influence each other, may reduce these effects. Flowable composites are dental restorative materials based on resin with low viscosity and a low filler content leading to a lower rigidity and a smaller modulus than hybrid composites [1]. The advantages of flowable dental materials include the greater fluidity, which is useful when they are applied to teeth cavities that are less accessible. Also, the flexibility and the formation of the layered structure in a minimal thickness, excluding the possibility of inclusion or blocking air, being useful especially in pediatric dentistry.

The composition and the polymerization degree of flowable composites have a pronounced effect on the physico-chemical properties of dental biomaterials [2-6]. The higher the conversion degree the better the surface properties such as: hardness, modulus of elasticity, resistance to fracture and the diametral tensile strength. Thus, after polymerization of the dental materials the

monomer conversion is not complete in the polymerization reaction, and an amount of unreacted monomer is released into the environment in which the sample is immersed. High quantities of residual monomers influence the hydrolytical degradation of the samples causing a decrease of the mechanical properties [7-9]. Also, once in the mouth, these materials are in continuous contact with saliva and oral fluid. Therefore water absorption and solubility must be evaluated, because water absorption within the polymer causes expansion stress and influences its mechanical properties.

In the present work water absorption and solubility as well as the amount of residual monomers of 13 samples of dental flowable composites will be investigated. Absorption and solubility studies reflect degradation over a period of 3 months during which the samples were immersed in water and alcoholic solution (according to ISO 4049/2000). The residual monomers were determined using two techniques: the high-performance liquid chromatography (HPLC) and the Nuclear Magnetic Resonance (NMR) relaxometry [10]. The results of these investigations will be compared and discussed in relation with the final strength and durability of the dental materials.

C=C double bonds from unreacted methacrylate groups are determined quantitatively also by Fourier Transform Infrared measurements which highlight their presence after the end of polymerization reaction.

The novelty of this study is the use of NMR technique for determination of the residual monomer in dental materials, which is based on transverse relaxation measurements applying the Carr-Purcell Meiboom-Gill (CPMG) pulse sequence. The results of the NMR approach are compared with those of the high performance liquid chromatography (HPLC) technique.

2. Materials and methods

2.1. Materials

The investigated samples were manufactured using the most common aromatic monomer on the market: Bis-GMA (Density: 1140; Viscosity at 60°C: 1300 CP; refractive index: 15100; synthesized in our laboratory) [11] and a diluted monomer: triethylene glycol dimethacrylate-TEGDMA (Aldrich, Germany), both forming the matrix. The inorganic phase of the samples consists of mixtures with different particle sizes. It was added to the mixture to increase packing density and filler

content for improving the strength. It also ensures that the material can be easily polished and decreases the polymerization shrinkage. For these samples we used 2 different ratios (55% and 65%) of fluoroapatite and tricalciumphosphate, which are important for dental tissue repair because of their similarity to minerals from the teeth, thus providing excellent biocompatibility and bioactivity. Quartz is added to give the strength and rigidity. The other ingredients are glass with strontium which gives good radioopaque effects and colloidal silica, which is added as a coupling agent between the phases.

The samples denoted S1-S6 have chemical initiation systems and were drawn in two-paste systems. Paste 1 contains the polymerization initiator which is benzoyl peroxide (BPO) (Merck, Germany). Paste 2 contains the curing accelerator N,N-dihydroxyethyl-p-toluidine (DHEPT) (Aldrich, Germany). Each of S1 to S6 samples have 2 ratios of initiation system, denoted *a* and *b* respectively. The sample denoted as SP is photochemically initiated, the polymerization being achieved using an Optilux lamp for 40 seconds. The composition of all samples is shown in Table 1.

Table 1. Composition of dental biomaterials

%	Organic Phase		Inorganic Phase					Chemical Initiation System	
	Bis-GMA	TEGDMA	Quartz	Glass with Sr	FAP	TCP	SiO ₂	DHEPT (% from Paste A)	BPO (% from Paste B)
S1a	24.5	10.5	26	23	6.5	6.5	3	1	2
S1b	24.5	10.5	26	23	6.5	6.5	3	0.7	1.2
S2a	31.5	13.5	22	19	5.5	5.5	3	1	2
S2b	31.5	13.5	22	19	5.5	5.5	3	0.7	1.2
S3a	26	9	26	23	6.5	6.5	3	1	2
S3b	26	9	26	23	6.5	6.5	3	0.7	1.2
S4a	34	11	22	19	5.5	5.5	3	1	2
S4b	34	11	22	19	5.5	5.5	3	0.7	1.2
S5a	19	16	26	23	6.5	6.5	3	1	2
S5b	19	16	26	23	6.5	6.5	3	0.7	1.2
S6a	25	20	22	19	5.5	5.5	3	1	2
S6b	25	20	22	19	5.5	5.5	3	0.7	1.2
SP*	34	11	22	19	5.5	5.5	3	CQ: 0.5	DMAEM: 1

* Photochemical Initiation System

Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl]propane (synthesized in Department of Polymer Composites laboratory); **TEGDMA:** Triethyleneglycol dimethacrylate (Aldrich, Germany); **FAP:** Fluoridated hydroxylapatite (synthesized in Department of Polymer Composites laboratory); **SiO₂:** Colloidal silica (Romania); **BPO:** Benzoyl peroxide (Merck, Germany); **CQ:** camphorquinone (Aldrich, Germany); **DMAEM:** dimethylaminomethylmetacrylate (Aldrich, Germany); **DHPET:** N,N-dihydroxyethyl-p-toluidine (Aldrich, Germany); **TCP:** Ca₃(PO₄)₂: Tricalciumphosphate (synthesized in Department of Polymer Composites laboratory).

2.2. Absorption and solubility methods

When the cured samples are immersed in water, some of the components such as unreacted monomers are dissolved and released from the samples into the solution, this resulting in weight loss measured as solubility, and which can cause irritation in the dental pulp. The study of the absorption and solubility was performed according to

ISO 4049/2000 and has revealed the dependence of the tested composites to their composition, the immersion time and the environment of immersion [12]. To determine the water absorption and the solubility, a teflon mold was used with the following dimensions: diameter $d = 15 \pm 1$ mm and the thickness $h = 1$ mm. Prior to weighing the specimens, these must be kept in a desiccators at 23°C until a constant weight is reached [13]. The samples were then immersed

in containers of distilled water, and alcoholic solution (concentration 50%) at a temperature of 37°C for 3 months [14]. The specimens were desiccated and weighed daily during the first 7 days and afterwards on day 14, 28, 60 and 90. The values of water absorption (A) and solubility(S) can be calculated for each disc as follows:

$$A = \frac{m_1 - m_3}{V} \quad S = \frac{m_2 - m_3}{V}$$

Here m_1 , m_2 are the masses of the specimen before and after immersion in water whereas m_3 represents the mass of the specimen kept in a desiccator until a constant weight is reached. V is the volume of the specimen. The data were analyzed by two-way ANOVA techniques [15] for multiple comparisons, which is useful when we desire to compare the effect of multiple levels of two factors and we have multiple observations at each level.

2.3. Determination of the residual monomers methods

To determine the amount of residual monomers the manufactured samples were investigated using two different techniques: the High Performance Liquid Chromatography (HPLC) and the Nuclear Magnetic Resonance (NMR) relaxometry. These two techniques will be shortly described in the following. A more comprehensive description can be found in refs. [16] and [10] respectively.

2.3.1. High Performance Liquid Chromatography (HPLC) technique

HPLC analysis was performed on a JASCO – 980 system equipped with: Intelligent HPLC Pump (PU-980), Ternary Gradient Unit (LG-980-02), 3-Line Degasser (DG-980-50), Intelligent Column Thermostat (CO-2060 Plus) Intelligent UV-Vis Detector (UV-980-975), soft ChromPASS. Separation was achieved on CARBOsep COREGel 87H3 (300 x 7.8 mm) column. The mobile phase was acetonitrile solution. The detection was 214 nm and the column temperature was 35 °C. The samples were prepared by curing them into a teflon mold, obtaining disc specimens with 8 mm diameter and 5 mm thickness. Subsequently they were immersed in 25 ml of chloroform for 8 hours at the boiling point of chloroform, and the extract was vaporized for 30 minutes. The out coming extract was diluted with acetonitrile and then injected into the HPLC. The residual monomer was identified by comparing their retention times with the corresponding standards from the HPLC calibration curves of TEGDMA monomers standards.

2.3.2. Nuclear Magnetic Resonance Relaxometry technique

The NMR relaxometry is a widely implemented technique for investigating the translational and rotational motion of molecules under confinement conditions or at the interface [10]. Using NMR relaxometry it is possible to identify both location and distribution of molecules inside heterogeneous media. Thus it can become an important tool in studying the curing process of dental materials [18]. In the present work the transverse relaxation measurements will be used to monitor the relationship between the amount of unreacted monomers and the polymerization time. The approach relies on the assumption that the unreacted monomers have longer transverse relaxation times as the solid matrix. This allows us to identify and quantify the amount of unreacted monomers during the polymerization reaction.

The transverse relaxation measurements are better performed with the well-known Carr–Purcell Meiboom Gill (CPMG) technique [10] which consists of an initial 90° radiofrequency pulse around x -axis which is followed by a train of 180° pulses around y -axis at time instants τ τ τ An echo train is then recorded from the signal appearing at time instants 2τ τ τ ... allowing us the identification of the characteristic transverse relaxation times. The main advantage of the CPMG technique as compared with other spin echo techniques is that it allows rapid multiple accumulations of the echo train signal - an important prerequisite for samples undergoing a rapid evolution.

All experiments were done here using a low field NMR instrument (MINISPEC MQ20, Bruker, Germany) operating at proton resonance frequency of 20 MHz. The samples were prepared at room temperature and immediately after were introduced into NMR tubes with 10 mm external diameter. The measurements were done at a temperature of 37°C specific for the environmental conditions in which these materials are used (oral cavity). The echo time interval in the CPMG experiment was set 100 μ s to avoid diffusion effects on echo train attenuation. A number of 1500 echoes were recorded for each experiment. The maximum duration of a complete echo train was 8 s which prevents for the sample changes during the experiment.

2.4. Infrared Spectroscopy (IR)

By using a Fourier Transform Infrared spectrometer (FTIR) can be made an quantitative determination of unreacted methacrylate groups using the absorption band at 1635-1640 cm^{-1} due to valence vibrations of the C = C double bonds from the methacrylate groups. The samples are polymerized in a teflon mold with a diameter of 5 mm and a thickness of 0.5 mm. The specimens were studied using JASCO FT-IR 610 at the Institute for Research in Chemistry "Raluca Ripan", Cluj-Napoca. The samples were examined immediately after polymerization reaction (reference value) and after 7, 14, 28, 60 and 90 days of immersion in water/alcohol solution.

3. Results and Discussion

The samples were physico-chemical analyzed in order to choose the right amounts of ingredients for obtaining a

biomaterial structure and texture appropriate to satisfy any conditions imposed for the use in dentistry.

Table 2. The amount of residual monomer determined by two independent techniques

Sample/ Technique	S1a	S1b	S2a	S2b	S3a	S3b	S4a	S4b	S5a	S5b	S6a	S6b	SP
NMR (%)	1.6	0.41	0.78	0.31	0.51	0.20	0.34	0.20	1.37	0.96	1.55	0.88	0.19
HPLC (%)	1.32	0.34	0.55	0.21	0.30	0.19	0.30	0.27	1.36	0.86	1.45	0.88	0.19

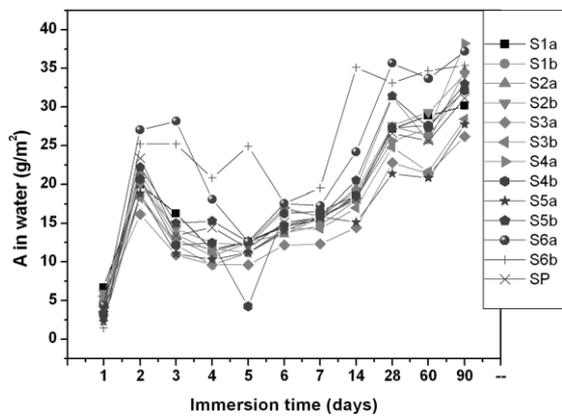


Fig. 1. Values for absorption of experimental dental flowable composites in distilled water

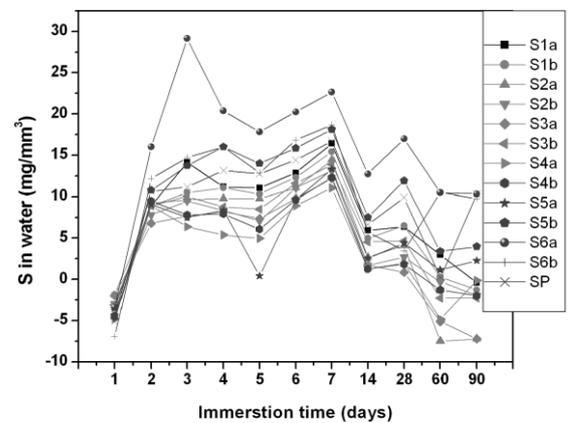


Fig. 3. Values for solubility of experimental dental flowable composites in distilled water

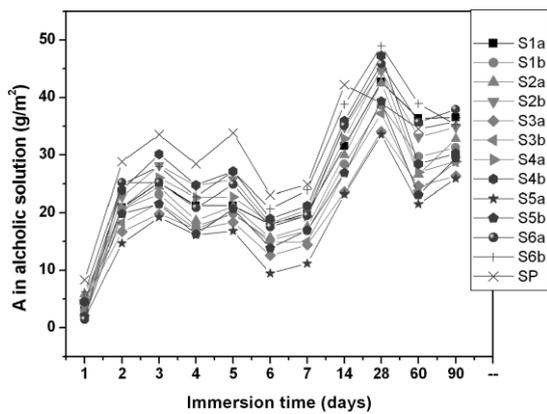


Fig. 2. Values for absorption of experimental dental flowable composites in alcoholic solution

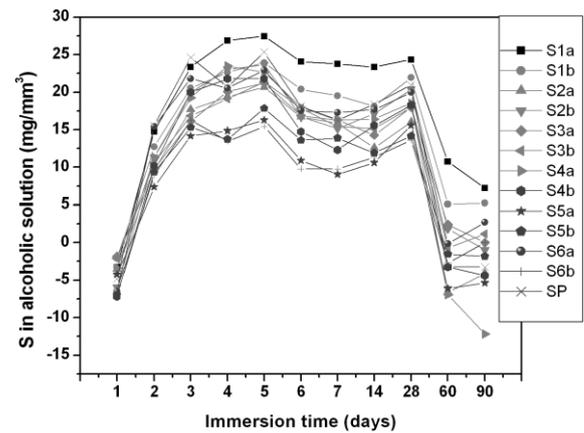


Fig. 4. Values for solubility of experimental dental flowable composites in alcoholic solution

3.1. Absorption and solubility

Water absorption and solubility of dental composite polymers after storage in water and alcoholic solution at 37°C for 3 months are shown in Figures 1, 2, 3 and 4 respectively.

High quantity of water absorption favors separation of fillers of the polymer matrix which afterwards is hydrolytically degraded and thus results the decrease in the mechanical strength of the biomaterial. Consequently, a prerequisite for clinical use of a dental composite is its insolubility. Composites denoted with *a* (larger amount of chemical initiation system) show a higher absorption compared to composites *b* (smaller amount of chemical initiation system). As it can be seen from Figures 1 and 2, the samples S2, S4 and S6, containing 45% organic phase, have a higher degree of absorption compared to composites S1, S3 and S5, containing 35% organic phase. Therefore, as the low molecular weight component has a higher portion, the samples show a higher solubility [18-20]. This is because the ethylene glycol unit from TEGDMA is an aliphatic hydrolytic group. The samples S3 and S4, which have the smallest amount of TEGDMA and a large quantity of Bis-GMA, reveal the lowest degree of absorption. This is due to the fact that Bis-GMA is a molecule with higher weight, so solvency is not very fast in water. Comparing the absorption values from both graphs in Figures 1 and 2, can be inferred that the samples showed higher water absorption in alcoholic solution compared with distilled water, which can be explained due to the different chemical affinity of the polymer matrix of the samples and the liquid in which they were immersed [21].

In the case of solubility analysis of the samples we can observe in Figures 3 and 4 that they have a similar behavior like that resulting from water absorption. The composite with the smaller amount of filler particles and basic monomers in the composition, like sample S6a, has been shown to have the highest degree of solubility in water. Instead, the sample S5a has the lowest degree of solubility in both water and alcoholic solution solubility. All absorption and solubility values fall within the maximum limit allowed by ISO 4049/2000 for all the analyzed composite samples.

The statistical studies were done by comparing samples S1a with S1b, S2a with S2b, S3a with S3b, S4a with S4b, S5a with S5b, and S6a with S6b, for solubility and absorption, using the ANOVA test with a significance level of consideration $p < 0,05$ [15]. Statistical analysis between groups with the same small amount of chemical initiation system, but with different ratios of monomers and fillers: S1a, S2a, S3a, S4a, S5a and S6a, indicate values of $p > 0.0004$ and the ratio value $F = 4.81$.

These indicate that there are statistical differences between samples, differences underlined by greater amounts of chemical initiation system addition. At the same time, for the group of samples with higher amount of initiation system (denoted by *b*), the *p* and *F* values ($p < 0.1336$, $F = 1.70$) indicate that no significant differences were found in absorption of water and alcoholic solution.

A third comparison was made for each sample, between *A* and *S* values from day 7 and day 14, yielding a value of $p < 0.0085$, much smaller than the significance level considered of 0.05, and the value $F = 2.75$, greater than 1. This suggests that the hypothesis of no major differences between day 7 and day 14 in absorption or solubility of each sample is rejected, the variation being explained by the differences between the groups.

3.2. Determination of the residual monomer

The amount of residual monomers extracted from the approach described above for the investigated samples increases as following: $SP < S3b < S3a < S4b < S4a < S2b < S2a < S1b < S1a < S5b < S5a < S6b < S6a$. The results are indicated in Table 2.

Figure 5 shows an example of CPMG echo trains recorded for different curing times in the case of Sample 6b. As can be observed the echo decay curves reveal a bi-exponential character in the beginning of curing time indicating the presence of two components. The component with longer relaxation time corresponds to the TEGDMA monomer characterized by a longer relaxation time associated with a higher mobility. It is also observed that by increasing the curing period the signal-to-noise ratio decreases due to the reactions of free radicals formed with dental methacrylate groups existing in the dental monomers from the sample. This reaction leads to the increase of macroradicals and to hardening of the samples [21]. To obtain the amount of residual monomer we extrapolated to zero the slowly decaying part of the echo decay curves (lines in Figure 5). The extrapolated value is proportional to the amount of monomer that has not reacted. Dividing the value found to the intensity of the first echo from the echo curve measured in the beginning of the polymerization reaction, we obtain the proportion of unreacted monomers from the samples.

It is clear from the evaluated data that the large amount of residual monomers is present in samples for which the polymerization initiation systems are 1% amine and 2% POB (denoted with *a*). Also, if the quantity of filler is higher and that of based monomer is smaller, the amount of residual monomer is lower.

The values of the amount of residual monomer extracted from chromatograms detected at a wavelength of 203 nm, specific for TEGDMA monomers reflect the fact that the amount of residual monomer in the sample S6a is 10 times higher compared with the sample SP or sample S3b.

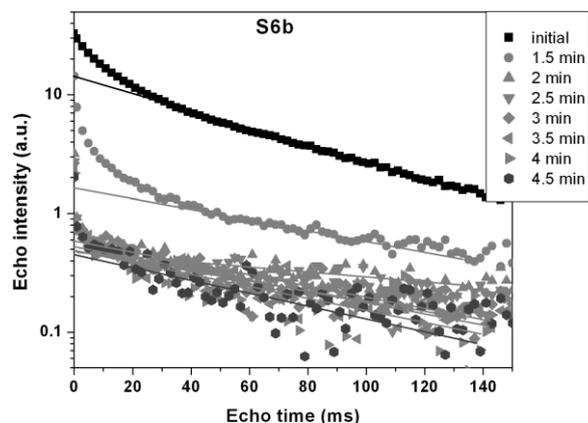


Fig. 5. The CPMG echo trains for the Sample 6b during the polymerization reaction.

The results are consistent with the evaluation by the NMR relaxometry. Comparing the absorption, the solubility and the amount of residual monomers, of the investigated samples we can infer that the samples with the higher values of absorption and solubility have a higher amount of residual monomers, so dental materials properties depend on their chemical structure, molecular weight, monomer ratio and the nature and the amount of fillers or of their initiation system.

3.3. Infrared Spectroscopy (IR)

The recorded spectra highlighted the main characteristic bands for the monomer mixture of composite and inorganic phase characteristic peaks. These bands are associated with stretching vibrations of urethane carbonyl C=O at 1717cm^{-1} , and at 1640cm^{-1} and 950cm^{-1} with characteristic bands of stretching and deformation vibration of C=C unsaturated bond in the acrylic component, while the absorption bands at $2918\text{--}2950\text{cm}^{-1}$ are associated with stretching vibrations of C-H and CH_2 's from the acrylic monomer structure.

In addition IR spectra highlights the characteristic bands for Si-O-Si bond at $\sim 1059\text{cm}^{-1}$ and Si-O- CH_2 . IR spectra revealed a dimming characteristic peaks of Si-O-Si bond (Figure 6, 7), this can be explained by the breakage of the Si-O-Si bond and not Si-C bond. Results show that in water, the bond Si-O-Si samples present in the composition Bis-GMA-based, TEGDMA and an inorganic phase consisting of glass, quartz and aerosil is more stable than other alcoholic solution.

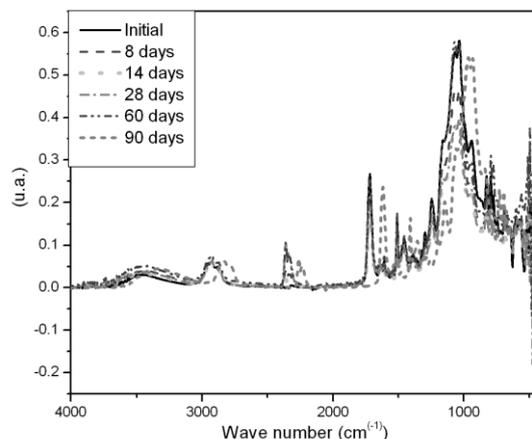


Fig. 6. IR spectra of S6a composite after immersion in alcoholic solution

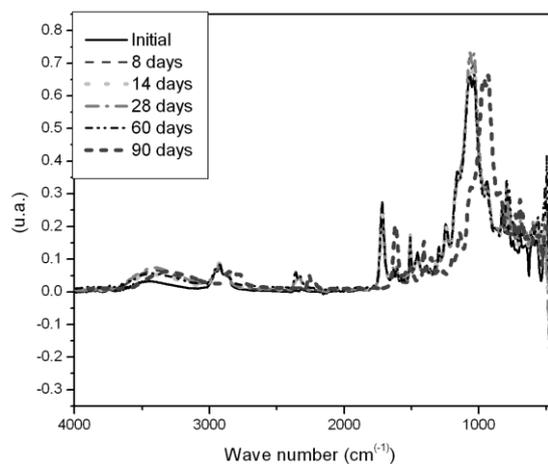


Fig. 7. IR spectra of S6a composite after immersion in water

4. Conclusions

The investigations presented here on the absorption and water solubility of some dental flowable composites have revealed the dependence of the tested samples to their composition, immersion time and the environment of immersion. Thus, as the amount of system initiation and the organic phase is higher, both the absorption and solubility are higher. With respect to the immersion time, if in the first days the absorption sharply increases, it becomes relatively constant in the last days of the 3 months of immersion and the sample reached the total elimination of residual monomer. The environment of immersion plays also a role as it was observed that the polymer matrix of the sample has a higher affinity for alcohol than distilled water, showing higher water absorption and solubility values.

To determine the amount of residual monomer we have implemented two independent techniques. One technique is the classical high-performance liquid chromatography (HPLC) often used for residual monomer determination in dental materials. The other technique is based on transverse NMR relaxation measurements using the well-known Carr-Purcell Meiboom-Gill (CPMG) pulse sequence which was used for the first time in this context. Both techniques provide comparable results. Note however that the NMR technique is significantly faster as compared with the HPLC technique (6 minutes instead of 10 hours), it is completely noninvasive and does not require any previous sample preparation. It can be also applied during the polymerization reaction or even in vivo using an NMR surface explorer [17].

The presence of unreacted methacrylate groups is also illustrated in the determination of composite materials hydrolytic degradation immersed in water and alcoholic solution. However IR spectra recorded for the same samples revealed no major changes in the effect of water and alcoholic solution over the components of experimental studied composite systems.

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