DENTAL POLYACIDS CONTAINING AMINO ACIDS AND METHACRYLATE MOIETIES

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Abstract
Glass-ionomer cements (GICs) are bioactive materials used in restorative dentistry due to their good adhesion, chemical bonding, low thermal expansion coefficients, less volumetric contraction, low shrinkage at the tooth–enamel interface, and often anticariogenic activity. Usually, the organic phase from these materials is composed of relatively weak acids, such as poly(acrylic acid), poly(acrylic acid-*co*-itaconic acid) or poly(acrylic acid-*co*-maleic acid), to name a few. To date, it has been demonstrated that (meth)acryloyl-modified amino acid monomers, such as *L*-glutamic acid, -alanine, glycine or proline can lead to GICs with improved properties concerning the mechanical parameters (compressive/flexural strength, diametral tensile strength). Another approach was focused on the hybrid materials which combine the properties of resin composite with those of the conventional glassionomers, the simplest forms incorporating a small quantity of 2-hydroxyethyl methacrylate or bisphenol A-glycidyl ether dimethacrylate (Bis-GMA) and/or urethane dimethacrylate, and triethylene glycol dimethacrylate (TEGDMA). Based on this information, we report herein some resin-modified glass ionomers carrying out poly(acrylic acid-*co*-itaconic acid-*co*-*N*-acryloyl-*L*-alanine/glycine) functionalized with (meth)acrylate moieties, BisGMA, TEGDMA and bioactive glass along with their properties including the degree of conversion, water sorption/solubility, and mechanical characteristics.

1. Introduction
Invented by Wilson et al. in early 1970 [1], the glass-ionomer cements (GICs) are widely used in restorative dentistry owing to their special properties such as good adhesion to moist tooth structure, biocompatibility, low toxicity, chemical bonding, low thermal expansion coefficients, low shrinkage at the tooth-enamel interface, and anticaries activity [2]. Typically, these materials are water-based cements that include an organic phase created from aqueous acid polymers or copolymers as poly(acrylic acid), poly(acrylic acid-*co*-itaconic acid), poly(acrylic acid-*co*-maleic acid) combined with a basic inorganic component consisting in ion leachable fluoride-containing glass powder, their generic name being derived from an acid-base reaction between the both partners in the presence of water [3]**.** Besides the mentioned advantages of the current commercial GICs, there are some major disadvantages related to the lack of strength and toughness, for which reason significant efforts have been made to enhance their physical and mechanical properties by modifying the polymer backbone, and not only. To respond this requirement a first approach has been focused on the use of N-vinylpyrrolidone (NVP) as co-monomer in free radical copolymerization of the acid monomers, the mechanical test results showing that the final GICs became stronger in function of the polymer

composition and the ratio between partners [3, 4]. It is generally agreed that the NVP molecules decrease the degree of steric hindrance of the acid groups and favour the ionic bond formation and subsequently, poly-salt bridge formation into the final set cement in comparison to the control group. Other direction has been taken in study the use of amino acid monomers, amino acid-functionalized copolymers and (meth)acrylate-functionalized amino acid monomers/copolymers [3, 5, 6] for making dental polymers, for which the optimization of the molecular weight of the acid copolymers together with the powder-to-liquid ratio (P/L) is crucial. Development of the visible visible light-curable (VLC) glass-ionomer systems, involved the mixture of VLC copolymer with 2-hydroxyethyl methacrylate (HEMA), water, initiator and activator to yield a polymer solution, which is then blended with Ca-fluoro-alumino-silicate (CaFAlSi) glass powder, the final products having both covalent crosslinking as well as ionic type crosslinking. A new route assumed the incorporation of conventional glass-ionomers and a small quantity of composite resins like HEMA, bisphenol A diglycidyl ether methacrylate (BisGMA) and/or urethane dimethacrylate, and triethylene glycol dimethacrylate, TEGDMA) [7, 8], and subsequent hardening. Notable is the option of preparation of the acrylic polyacids with methacrylate side chains grafted onto the polymer chain to generate visible light-curable polymers and resin-modified glass ionomers (RMGICs), respectively via an acid-base reaction and partly, photochemical polymerization [3, 9]. Although these materials are currently marketed, a better understanding of the setting chemistry and of their adherence to dental tissues is desired for clinical use. Contextually, there is an increasing need to enlarge the class of ionomeric compositions intended for a variety of dental applications ranging from water soluble to intermediary materials and insoluble compositions for achieving preformed structures, e.g. osseous tissue repair.

Here, as an extension of our previous work concerning the dental monomers [10, 11] and acrylic copolymers [12, 13], we report the synthesis and exploration of water soluble acid copolymers such as poly(acrylic acid-co-itaconic acid-co-N-acryloylalanine or N-acryloylglycine) to be further modified with photopolymerizable moieties. Effect of structure of the amino acid and methacrylate units from acid copolymer on some specific properties of the experimental dental compositions is also discussed.

2. Materials and methods

2.1. Materials
Itaconic acid (IA), 4-amino-1-butanol, N,N′-dicyclohexylcarbodiimide (DCC), isocyanatoethyl methacrylate, alanine, glycine, acryloyl chloride, 1,1′-azobis(cyclohexanecarbonitrile) (ABCN), HEMA, BisGMA, TEGDMA, Irgacure 819 and hydroxyapatite were purchased from Sigma Aldrich Chemical Co. (Taufkirchen, Germany) and used without further purification. Acrylic acid (AA) was also purchased from Sigma Aldrich Chemical Co. (Taufkirchen, Germany) and was purified by vacuum distillation before use. The GC Fuji II LC Improved powder was purchased from GC Corporation, Tokyo, Japan.

2.2. Methods

2.2.1. Polymerization and functionalization procedure
Synthesis of alanine copolymer. AA (2.9 g), IA (1.3 g) and acryloylalanine (1.4 g), prior prepared in our laboratory, were copolymerized into a molar ratio of 4:1:1, in dioxane solution. The radical polymerization, initiated by ABCN, was performed at 80 °C for 72 h after degassing and purging the monomer solution with nitrogen. The formed acid copolymer (AP-Ala) was precipitated in diethyl ether, a fine powder being obtained after drying in vacuum for 12 h. Yield: 87 % (4.5 g). Similarly, it was obtained the corresponding copolymer with glycine moieties in its structure (AP-Gly).
For functionalization of the copolymers, 4 g of AP-Ala copolymer was dissolved in dioxane and cooled in an ice bath under stirring. Then, 1.4 g (6.5 mmol) DCC and 2 g (7 mmol) urea derivative (prepared according to a previously published procedure [12]) were added, keeping the temperature around 0 °C for about 2 hours. Further, the reaction mixture was heated to room temperature and stirred for 24 h under a dry nitrogen atmosphere. The crude product was separated by filtration,

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precipitation and dried in vacuum for 12 h. Yield: 59% (6.7 g).

2.2.2. Characterization

Fourier transform infrared (FTIR) spectra were recorded using a Bruker Vertex 70 FT-IR spectrometer, and ^{1}H (^{13}C) NMR spectra were recorded using a Bruker AC 400 instrument. The FTIR photopolymerization experiments were performed on the unfilled organic formulations (Table 1) subjected to blue light using a dental-curing unit (LA 500, Model Blue-light, Apoza Enterprise Co, Taiwan), and recording the FT-IR absorption spectra after different irradiation times. The conversion degree was determined from the differences appeared in the C=C stretching vibration at ~815 cm^{-1} after various curing periods. For all samples, the average DC values of the specimens under curing time were reported. The resin-modified glass ionomer specimens for water sorption/solubility, and mechanical determinations were prepared by using a two component system with a powder/liquid ratio (P/L) of 2.7/1. Each sample contains polyacid dissolved in distilled water (1:0.5 by weight), dental monomers, Irgacure 819 (as the initiator), and the inorganic filler (90 wt. % fluoroaluminosilicate glass Fuji II LC Improved and 10 wt. % hydroxyapatite).

Table 1. Composition (wt.%) of the experimental formulations (each mixture contains 1 wt.% Irgacure 819).

| Formulation	AP-GlyM	AP-AlaM	Water	BisGMA	TEGDMA	HEMA
G1	50		25	12.5	6.25	6.25
G2	46.66		23.33	15	7.5	7.5
G3	40		20	20	10	10
G4		50	25	12.5	6.25	6.25
G5		46.66	23.33	15	7.5	7.5
G6		40	20	20	10	10

Compressive (CS) and diametral tensile strengths (DTS) were measured using a Shimadzu AGS-J testing machine, with a 5 kN load cell using appropriate specimens. A crosshead speed of 1 mm/min was applied in these tests. The compressive strength was calculated from the equation $CS = P/\pi r^2$, where P is the load at fracture and r the radius of the sample cylinder. DTS was estimated from the relationship $DTS = 2P/\pi dt$, where d is the diameter and t the thickness, respectively, of the cylinder. For water sorption/solubility measurements, disk specimens of reduced dimensions (6 ± 0.1 mm diameter, 2 ± 0.1 mm thickness)were prepared and further tested according the literature [12]. The microstructure of the cured samples in fracture was examined by using an environmental scanning electron microscope QUANTA200 (FEI Company, Hillsboro, USA). The composites were examined in low vacuum mode operating at 20 kV using an LFD detector.

3. Results and discussion

The structures of the acid copolymers synthesized by free radical polymerization are presented in Scheme 1. The both copolymers contain alanine or glycine moieties and methacrylate functions introduced by post-modification of the polymeric backbone based on acrylic acid, itaconic acid and acryloyl amino acid (molar ratio: 4:1:0.5) with N-4-hydroxybuthyl-N'-methacryloyloxyethyl (urea). Given the small content of carboxylic acid functionalized (~14 %), the resulting copolymers are soluble in water, an essential characteristic of such acid copolymers required of dental formulations for producing glass ionomer cements.

^{1}H-NMR analysis of these copolymers confirmed the chemical structure of AP-GlyM and AP-AlaM by the presence of peaks at 1.49 ppm attributed to the methylene protons from the

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pendant chain, while other signals at 1.52, 1.82 and 2.53 ppm, 1.9 ppm, 3.0 ppm, 3.4 and 3.6 ppm, 4.2 and 4.3 ppm belong to the methylene and methine protons from main chain, methyl protons from methacrylate, methylene protons connected to urea, methylene protons from ester group, methyne protons from glycine and alanine, respectively. Unsaturated protons give signals at 5.57 and 6.05 ppn, the urea protons appear at about 5.90 ppm and carboxyl protons can be observed at 12.25 ppm (Fig. 1).
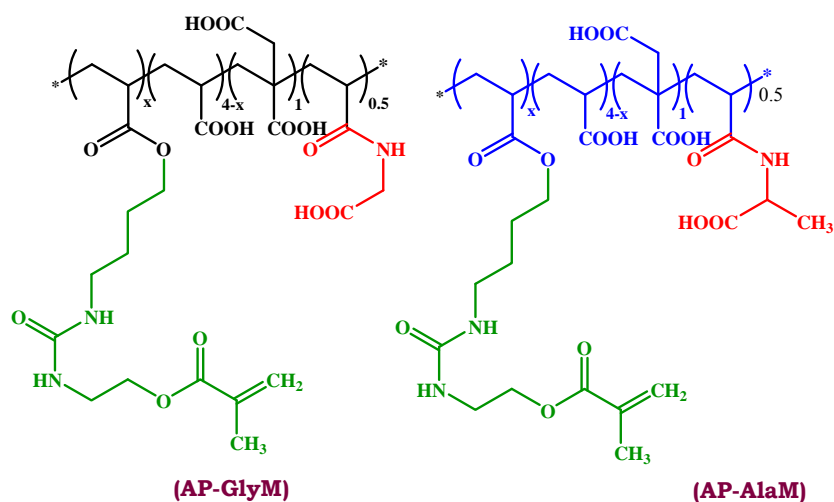
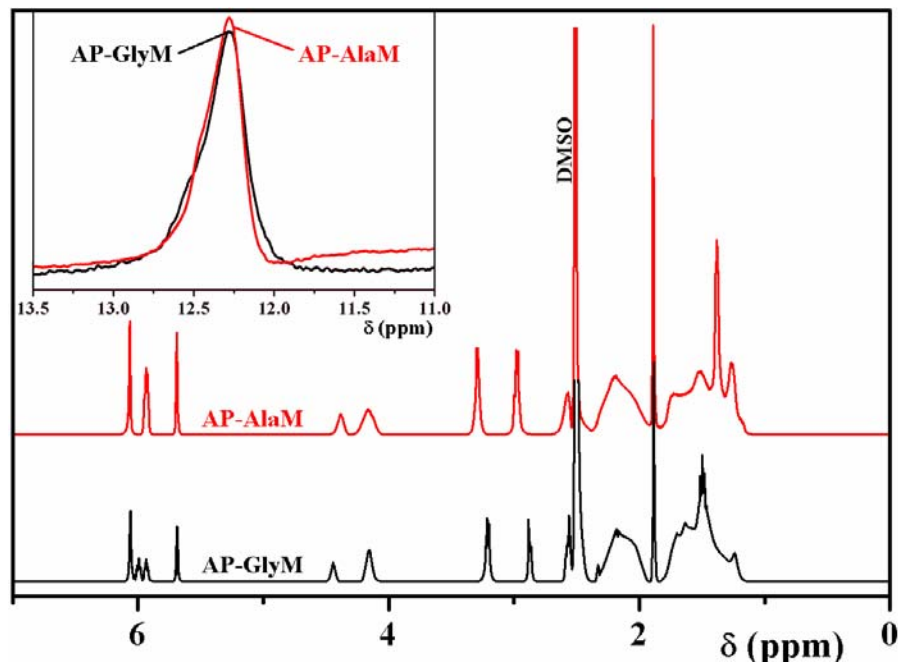**Scheme 1**. Structure of AP-GlyM and AP-AlaM copolymers with photopolymerizable sequences.

Figure 1. ^{1}H NMR spectra for AP-GlyM and AP-AlaM copolymers (inset is given the signal for carboxylic protons from the copolymers).

A first evaluation of the above copolymers necessitated the study of their photobehavior taken in combination with commercial dental monomers upon exposure to visible irradiation in the presence of Irgacure 819 as photoinitiator (compositions given in Table 1). As can be seen in Figure 2 a, a

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monotone decrease of the absorption band of methacrylic groups at ~815 nm with irradiation time suggested that the photopolymerization process occurred, the degree of conversion (DC) attaining a value of ~87% (after 120 s of irradiation) in case of the unfilled G3 formulation. Using FTIR spectroscopy for the determination of the same parameter (Fig. 2b), higher conversions were found in the G1-G3 formulations containing glycine copolymer (DC: 64-87%) compared to those incorporating AP-AlaM (G4-G6, DC: 59-61%).
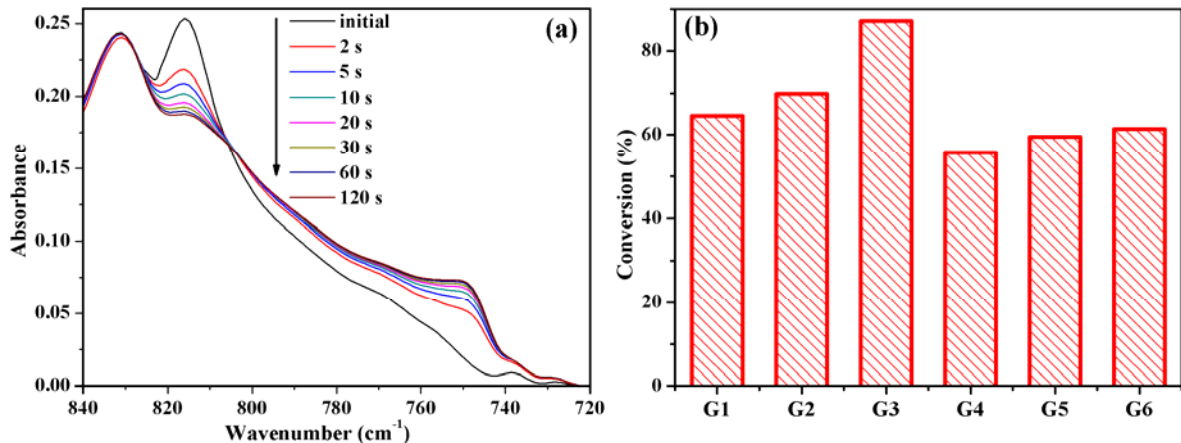

Figure 2. Evolution of the double bond absorption band from G3 formulation (a) and double bond conversion for the photopolymerized samples under visible light irradiation (b).

Considering potential applications in dentistry, the susceptibility of the photocured specimens to water contamination could be compromising for the cement introduced into a cavity. Thus, the water sorption and water solubility were determined. In Figure 3a is displayed the variation of these parameters for the final filled specimens, where it can observe that the water sorption had values between 103 μg/mm³ (G1) and ~ 42 μg/mm³ (G6), whereas the values of water solubility were of 20 μg/mm³ (G1) and 13 μg/mm³ (G6). It can appreciate that the materials with glycine in structure adsorbed more water than the light-cured alanine-containing specimens, most probably due to the hydrophilic nature of the former amino acid.
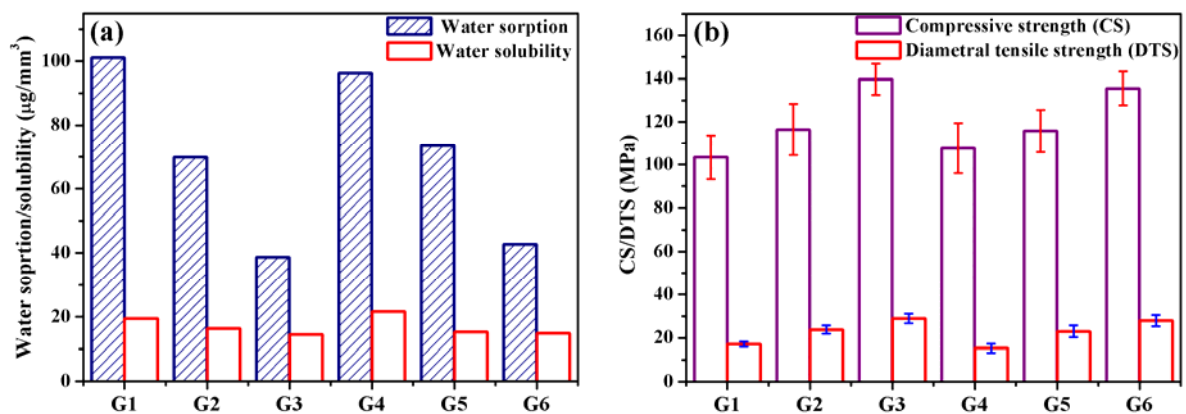

Figure 3. Water sorption/solubility (a) and mechanical parameters (b) measured for the experimental hybrid materials.

Compressive strength (CS) and diametral tensile strength (DTS) of the above resin-modified glass-ionomer materials have relatively close values (G1: CS – 103 MPa; DTS - 19 MPa; G4: CS – 109 MPa; DTS - 16 MPa) if the formulations include the same content of acid copolymer. Additionally, there is no significant effect of the amino acid structure on the measured mechanical properties. It was evidenced that a decreasing amount of polyacid is directly connected to an easy improvement of these

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characteristics (G3: CS − 140 MPA; DTS − 28 MPa; G6: CS -132 MPa; DTS − 30 MPa). Therefore, variations in compositions and the chemistry of such materials are responsible of their properties and potential clinical testing.

3. Conclusions

Two ternary polyalkenoates bearing alanine/glycine moieties on the acrylic backbone were synthesized and further modified with photopolymerizable groups to be used in dental materials of resin-modified glass ionomers (RMGICs) type. The copolymer structures were confirmed by spectral methods, and the aqueous polyacid solutions were formulated in dental ionomer compositions beside commercial dental monomers and 90% fluoroaluminosilicate/10% hydroxyapatite (powder/liquid ratio of 2.7:1). Upon exposure to visible irradiation in presence of Irgacure 819, the degree of conversion of the formulations varied between 59 and 87% (after 120 s of irradiation). With the addition of filler, the specific properties (water sorption, water solubility, compressive strength, diametral tensile strength) of the final materials are dependent on the chemical structure of amino acid and the content of acid copolymer present in each formulation. For the best balance of such parameters, supplementary investigations are necessary.

Acknowledgments

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