AMINO ACID FUNCTIONALIZED IONOMERIC MATERIALS IN BIOMEDICAL APPLICATIONS

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Abstract: Several N-acryloyl and N-methacryloyl substituted amino acids have been prepared and copolymerized with acrylic acid and itaconic acid to prepare new polyelectrolytes . Due to an acid-base reaction, mixing of the water soluble copolymers with glass powders, having leachable Ca^{2+}, Al^{3+}, etc., type cations, produces inorganic-organic composites hardened by salt -bridge formation. These heretofore unexplored polyelectrolytes are shown to be very useful for preparing improved dental biomaterials.

INTRODUCTION

Inorganic-organic composites, formulated from water soluble polymers having pendant carboxylic acid residues and powdered metal oxides or silicate powders containing leachable Ca^{2+}, Al^{3+}, F^-, etc., type ions, are commonly called glass-ionomers (GIs). When the two components are blended in the presence of controlled amounts of water, an acid-base reaction occurs, generating metal cation - carboxylate anion salt-bridges, which subsequently bring about curing or hardening of the formulation. Their development has addressed some of the disadvantages of amalgams, dental composites and cements used in dental restorations. GIs have attractive characteristics for a variety of applications as dental biomaterials, due to such things as good adhesion to tooth and bone, fluoride release, biocompatibility, attractive coefficient of thermal expansion, etc. However, GIs suffer from having too low a modulus of elasticity, brittle failure and low abrasion resistance. If the latter deficiencies could be substantially improved GIs could have substantial growth in GI applications. Further, with significant curing time reduction, GIs could also become very useful in bone cement applications. As a route to improve conventional GIs, free radical polymerizable [visible light curable (VLC)] compositions / formulations have been developed, providing covalent crosslinking, along with salt-bridge formation, to give improved mechanical properties (1). But, mechanical properties of both conventional and VLC GIs still need significant improvement.

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The current copolymers used in conventional and VLC GIs are water soluble polyelectrolytes with carboxylic acid groups directly or very closely attached to the polymer backbone, such as the commercially used poly(acrylic acid-co-itaconic acid) or poly(acrylic acid-co-maleic acid) materials. We hypothesized that modification of the polyelectrolytes to broaden both the type (I^{o}, II^{o} and III^{o}), the distance of the acid group tethered from the backbone, and vary the pKa range of the carboxylic acid functionalities would provide increased availability of carboxylic acid groups for salt-bridge formation, affording a mechanism to improve the physical and mechanical properties of GIs. Further, we hypothesized that enhanced or optimum $A^{-}(CO_2^{-})_2$ Al^{3+}, $(CO_2^{-})_3$ Al^{3+}, etc., type salt-bridge formation, which could help to maximize the hardening by the acid-base reaction, could best be achieved at the point where a statistical distribution of the acid groups occurred within the cured organic matrix.

To support our hypothesis, we have used acrylic acid (AA), itaconic acid (IA) and N-acryloyl or N-methacryloyl substituted amino acids to prepared a wide variety of water soluble copolymers, having various ratios of the three monomers in the backbone, different molecular weights, type of acid, and distance of the acid group tethered from the backbone (2,3). In this paper, we focus principally on N-methacryloylglutamic acid (MGA), which is one of the more economically attractive monomers to prepare and use. Also, MGA is found to be particularly useful in this approach to design improved GIs, for conventional as well as VLC formulations.

EXPERIMENTAL

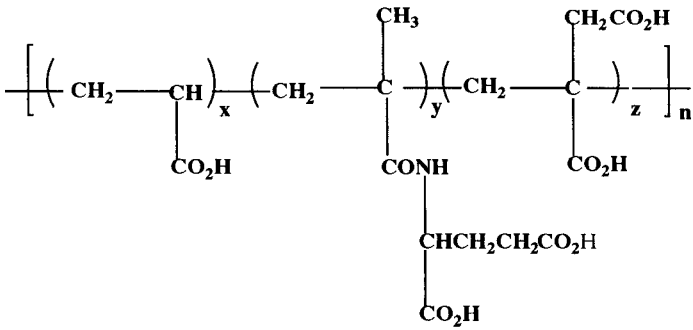
The methacryloyl chloride, glutamic acid, Diazald (N-methyl-N-nitroso-p-toluenesulfonamide), solvents, initiators, etc., chemicals were used as received from Aldrich Chemical Co.

N-Methacryloylglutamic acid (MGA) synthesis: A solution of 0.3 Mol. of NaOH and 0.1 Mol. of glutamic acid in 30 ml of distilled water was vigorously stirred and cooled to -10 ^{o}C, using an ice-salt bath. To this solution, 0.1 Mol. of methacryloyl chloride was added slowly with vigorous stirring over a 30 min. period. The reaction mixture was stirred at -10 ^{o}C for an additional 1.5 hr. Concentrated HCl was slowly added to the reaction mixture, until pH = 2.0 was reached. At this point, stirring was done for an additional 30 min. The resulting mixture was extracted several times with hot ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous magnesium sulfate. After filtration and removal of the ethyl acetate from the product, under reduced pressure, the crude product was obtained as a white solid in >75 % yield.

The product was recrystallized from ethyl acetate prior to use in modification of VLC glass-ionomer formulations. Crystalline MGA, mp. 130 °C, was confirmed by FT IR, and NMR (^{13}C and ^{1}H).

Preparation of poly(AA-co-IA-co-MGA): Copolymers with a monomer ratio of 7:3:3, acrylic acid:itaconic acid:N-methacrolylglutmic acid (Figure 1) were prepared by standard free-radical polymerization techniques (4). The three monomers, in the feed ratio shown, were combined with deionized water and varying amounts potassium persulfate, $(NH_4)_2S_2O_8$, in a suitable reactor. Various amounts of isopropanol were also added to the reactor to act as a chin transfer agent, helping reduce or control molecular weights. With stirring under an N_2 sparge, the contents of the reactor were heated for 3-4 hrs. at 95-100 °C. The terpolymers were isolated by standard freeze-drying techniques, using Edwards High Vacuum Int., Sussex, UK equipment. The polymers were further purified thorough dissolution / precipitation with methanol / diethyl ether. Elemental analysis, FT IR and NMR (^{13}H and ^{1}H) confirmed the structures. Viscosities of the copolymer solutions, at 50 % solids, were measured on a CarriMed CSL^2 Controlled Stress Rheometer (TA Instruments) at 25 °C. The Molecular weight of one of the MGA copolymers, copolymer D in Table 1, was estimated by GPC, using Waters GPC Equipment, tetrahydrofuran (THF) solvent and a polystyrene (PS) standard. To achieve solubility in THF for GPC characterization, copolymer D, Table 1, was treated with diazomethane, generated from Diazald, to substantially esterify the copolymer. The poly(AA-co-IA-MGA), copolymer D, had a GPC estimated MW of 40,000 and polydispersity of 2.4.

Figure 1. Poly(AA-co-IA-co-MGA), 7:3:3 Copolymer



Preparation of free-radical curing copolymer: The poly(AA-co-IA-co-MGA), with MW of 40,000, was treated with 2-isocyanatoethyl methacrylate (IEM) in THF ,

per a reported procedure (1). The IEM treated copolymer used in the study for VLC formulations had about 15% of the carboxylic acid groups converted to methacryloyl functionalized moieties, as shown by the simplified structure in Figure 2, Elemental analysis (% N), FT IR and NMR (^{13}C and ^{1}H) confirmed the structure.

Table 1. Viscosities of MGA and AGA Terpolymers

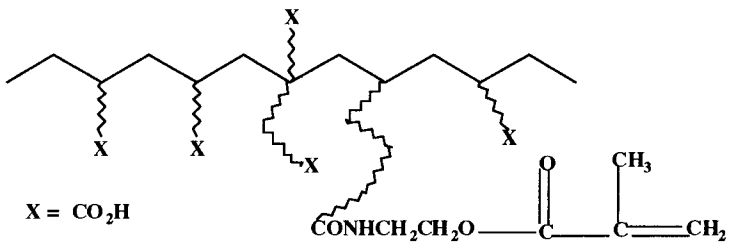
System	Copolymer Type (X, Y and Z Ratios)	*Viscosity, cp
A	AA-IA-AGA (7:3:3)	2200
B	AA-IA-MGA(7:3:3)	650
C	AA-IA-MGA(7:3:3)	3050
**D	AA-IA-MGA(7:3:3)	2500

*Viscosity for a 50/50(wt./wt.)H_2O solution at 25 °C

**GPC estimated MW = 40,000 and dispersivity = 2.4

Preparation of chemically-cured glass-ionomers: The 7:3:3 poly(AA-co-IA-co-MGA), along with 2 % by wt. of (+)tartaric acid, was dissolved in deionized water to form a concentrated solution having 50 % by wt. solids. The curing reaction was activated by blending the aqueous solution of the copolymer with the glass powder used in the commercial Fuji II (GC Int., Tokyo, Japan) conventional glass-ionomer. The powder/liquid ratio used in the formulation was 2.7/1, the same as used in Fuji II. Further, mixing and manipulation of the experimental system was the same as recommended by manufacturer of Fuji II. Fuji II was selected as a control due to its good acceptance by the dental community as a conventional glass-ionomer.

Figure 2. IEM Modified Poly(AA-co-IA-co-MGA)



Preparation of VLC glass-ionomers: For VLC systems, the aqueous solutions were formulated to contain ca. 48 % terpolymer, 20 % 2-hydroxyethyl methacrylate

(HEMA), 0.5 % camphoro-quinone (CQ) and 1.0 % diphenyliodonium hexafluorophosphate (wt./ wt.).The type of glass powders and powder / liquid (P/L) ratios used in the various formulations are shown in Tables 2 and 3. After mixing, per Fuji II LC and Vitremer Tricure procedures, and placing in suitable molds, samples for testing were cured for 1-3 Min., using an Elipar VLC Lamp (ESPE, Seefeld, Germany).

Mechanical properties: Determinations of flexural strength (FS) and compressive strength (CS) were accomplished per NISI ADA specification No. 66 for GIs, with n=8. Fracture toughness (FT) determinations (n=8) used the compact disc method described by Kovarek et al.[5] All samples were conditioned at 37 ^{o}C. and 100 % relative humidity or in water at 37 ^{o}C for set time intervals.

CS, and FS characterization: For CS cylindrical test samples 6 mm by 12 mm were fabricated for testing. Formulas used in the calculations were CS = P / pr^2. The Instron was set at a crosshead speed of 1.0 mm / min. For FS, the samples were fabricated to be 25 mm long x 3 mm wide x 3 mm thick, with formula for calculation being FS = 3PL / $2bd^2$.Testing on the Instron was at a crosshead speed of 0.1 mm / min.

Fracture toughness characterization: For fracture toughness testing samples were fabricated in suitable metal molds having a glass plate covering, giving testing specimens with dimensions 6.3 mm x 6.6 mm x 1.7 mm thick. The specimens configuration and test fixture were modified from ASTM Standard E399-83 for compact test specimens (5). A precrack and notch was formed during fabrication, using a razor blade. A measuring microscope was used to check dimensions of the polished specimens.

Setting and working time: These variables were evaluated per standard procedures, using a 400 gram indenter having a flat head of 1.0 mm for ST and a 28 gram indenter with a flat head of 2.0 mm for WT evaluation.

RESULTS AND DISCUSSION

We find that MGA is one of the more readily prepared N-methacryloyl substituted amino acids. Further, MGA is readily copolymerized with acrylic acid, itaconic acids and other monomers, using traditional free-radical polymerization techniques. Using MGA one may prepare a large family of polyelectrolytes or polychelatogen materials. In this effort we report only on our studies of these type polyelectrolytes to prepare

new and improved dental biomaterials.

Our studies show that poly(AA-co-IA-co-MGA) materials, Table 2, having a 7:3:3 ratio, respectively, of monomers AA, IA and MGA may be blended with a commercially used calcium-alumino-fluoro-silicate (CaAlFSi) glass, at a P/L ratio of 2.7/1, to formulate significantly improved conventional GIs, compared to the commercial control (Fuji II) system. Further, the results show that the MGA monomer, even though slightly more hydrophobic than the heretofore evaluated AGA monomer, is as useful as AGA for tethering the amino acid moiety to the backbone of the polyelectrolytes.

Table 2. Conventional AGA and MGA Based GI Mechanical Properties

System	CS, MPa (SD)	FS, MPa (SD)	FT, $Mn/m^{1.5}$ (SD)
Fuji II	184.5(8.6)	14.7(0.7)	0.48(0.08)
A	207.8(17.8)	40.6(4.7)	----------
B	175.9(11.6)	25.7(5.3)	0.49(0.05)
C	187.9(15.7)	27.7(3.2)	0.51(0.07)
D	223.6(8.4)	34.6(7.0)	0.64(0.13)

*The P/L ratio, with Fuji II glass powder used in all cases, was 2.7/1. The setting time (ST) and working time (WT) were comparable for all formulations.

The aforesaid terpolymer may also be treated with 2-isocyanatoethyl methacrylate (IEM), using patented procedures.[1] to prepare a VLC formulation. VLC formulations of this type employ 2-hydroxyethyl methacrylate (HEMA) as a reactive diluent in the aqueous solutions.[1] Here again, we are able to produce VLC GIs with improved properties, compared to commercial VLC systems (Table 3). In addition, we may use the MGA monomer to modify the HEMA / water solution of commercial VLC copolymers (Table 4) to further formulate improved VLC GIs. These promising results show we are on track for finding ways to improve GIs, with the results supporting the original hypothesis.

To show utility of monomers such as MGA for modification of existing VLC GIs, we modified Fuji II LC and Vitremer Tricure systems by addition of 5 % by wt. of MGA. After blending and placing in suitable molds for sample preparation, the VLC systems were exposed to visible light, using an Elipar Lamp (ESPE, Seefeld,Germany). Mechanical properties were evaluate, with results summarized in Table 4.

CONCLUSION

It has been demonstrated, via mechanical property studies, that tethering amino acids

to the backbone of water soluble copolymers offers a new route to formulating improved inorganic-organic composites known as glass-ionomers. Further studies will focus on using FT IR and Raman Spectroscopy to characterize / evaluate the acid-base reactions, occuring when the polyelectrolytes or polychelatogens are combined with a glass powder containing leachable Ca^{2+}, Al^{3+}, etc. cations. Results to date support the concept that these new polyelectrolytes afford greater opportunity for aluminum-carboxylate salt bridges to form in the cured matrix, thereby giving improved mechanical properties to the dental biomaterials obtained. Our results also suggest we need to more fully characterize the solution properties of these new polyelectrolytes. Further, we should evaluate these amino acid tethered poly-chelatogens with other metals such as Co(II), Zn(II), Ni(II), Cu(II), Fe(II), Pb(II), etc., in aqueous solution, using a liquid-phase polymer-based retention (LPR) type technique.

Table 3. VLC GIs Based on IEM Grafted AGA and MGA Terpolymers[1]

System	CS, MPa (SD)	FS, MPa (SD)	FT, $Mn/m^{1.5}$(SD)
[2]Fuji II LC	241.4(6.60)	74.0(5.0)	0.73(0.05)
[3]Vitremer	214.0(4.60)	63.0(11.0)	0.86(0.03)
[4]AGA LC	227.4(8.50)	87.0(14.6)	1.07(0.33)
[5]MGA LC	247.7(16.9)	81.0(9.02)	1.06(0.13)

[1]All samples (n=8) cured 1 min.with an Elipar lamp. [2]GC Corp. product with P/L=3:1. [3]3M Vitremer Tricure VLC GI with P/L=2.5/1. [4]7:3:3 poly(AA-IA-AGA) and [5]7:3:3 poly(AA-IA-MGA) blended with Vitremer powder at the 2.5/1 P/L ratios. The ST and WT values for the AGA and MGA systems were not far removed from the controls.

Table 4. MGA Modified Commercial VLC Formulations[1]

System	CS, MPa(SD)	FL, MPa(SD)	FT, $MPam^{0.5}$(SD)
Vitremer (V)	220.5 (6.6)	63.7(4.2)	0.68(0.14)
VMGA	260.2(6.3)	80.6(4.8)	1.04(0.18)
Fuji II LC(F)	238.4(5.6)	72.1(3.1)	0.74(0.04)
FMGA	266.7(6.1)	86.6(5.1)	0.85(0.07)

[1]VMGA and FMGA are Vitremer Tricure(3M) and Fuji II (GC Int.) products, respectively, modified by addition of MGA at the 5% by wt. level. Samples (n=10) were conditioned in deionized water at 37 ^{o}C for 1 week prior to testing.

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REFERENCES

1. S. B. Mitra, U. S. Pat. 5,130,347 (July 14, 1992), to 3M
2. B. M. Culbertson and E. Kao, U.S.Pat.5,369,142 (Nov. 29,1994) to Ohio State University
3. B. M. Culbertson. E. Kao, and D. Xie, J. Dent. Mater., 12, 44-51(1996)
4. S. Crisp, B. E. Kent, B. G. Lewis, A. J. Ferner and A. D. Wilson, J. Dent. Res., 59,1055-1063(1980)
5. R. E. Kovarek and C. W. Fairhurst, J. Dent. Mater., 9, 222-228 (1993)