

Studies on Inherently Radiopaque Acrylate Copolymers for Biomedical Applications

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ABSTRACT: Poly(glycidyl methacrylate-co-ethyl methacrylate) and poly(glycidyl methacrylate-co-butyl methacrylate) random copolymers (with 50–50 mol % of monomers) were made radiopaque by grafting iodine moieties through the ring opening reaction of the epoxy groups. The percentage weight of grafted iodine in the copolymers was found to be as high as 19%. The iodinated copolymers showed higher glass transition temperature and thermal stability in comparison with the parent copolymers. Iodinated copolymer of poly(glycidyl methacrylate-co-ethyl methacrylate) has improved glass transition temperature

than iodinated poly(glycidyl methacrylate-co-butyl methacrylate). Radiographic analysis of these iodinated copolymers showed excellent radiopacity. The *in vitro* cytotoxicity tests revealed cytocompatibility with cells. These radiopaque copolymers are expected to find application as dental and orthopedic cements. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 125: 2252–2261, 2012

Key words: iodinated glycidyl methacrylate copolymers; radiopacity; X-ray analysis; *in vitro* cytotoxicity

INTRODUCTION

Acrylic copolymers are widely used in medicine as orthopedic bone cements and in dentistry as denture base materials.^{1,2} Modifications of polymers to impart radiopacity would be advantageous as it enables the non-invasive evaluation of the polymers, when used for medical and dental applications. Conventional polymers are radiolucent due to the absence of high electron density elements in their polymeric backbone. Various techniques have been reported for preparing radiopaque polymers. Radiopaque polymer blends have been prepared by the incorporation of radiopacifying additives such as barium, bismuth, tungsten, or tantalum salts.^{3–8} Limitation of additive incorporated radiopaque polymeric system includes the creation of heterogenous mixtures, resulting in the deterioration of the end product. Homogenous radiopaque polymer-salt complexes have been produced by the incorporation of radiopaque heavy-metal salts into an appropriate polymer ligand by chelation. For example, radiopaque miscible polymer coordination complex of poly(methyl methacrylate) and uranyl nitrate have been investigated.⁹ However, the physico-mechanical properties and biocompatibility of the

base polymer were often adversely affected due to leaching of the noncovalently bound radiopacifying agents. Polymeric biomaterials with inherent radiopacity were developed by either covalently binding the radiopacifying elements with the monomers prior to their polymerization^{10–14} or carrying out a post-polymerization grafting of iodine-containing molecules on reactive groups.^{15–18} Permanent radiopacity was obtained by this method as the radiopacifying atoms were bound to the polymeric backbone and physico-mechanical properties of the polymer were not compromised.

Acrylic copolymers that contain epoxy groups are an interesting class of functional polymers because of the high reactivity of epoxides. 2,3-Epoxypropyl methacrylate or Glycidyl methacrylate (GMA) copolymers that contain epoxide groups have become increasingly important for biomedical applications such as for binding enzymes and other biologically active species. This article deals with the synthesis and characterization of GMA-based inherently radiopaque acrylate copolymers. Radiopacity is imparted by grafting iodine atoms onto preformed polymers. The effect of iodination on the molecular weight and thermal properties of the copolymers have been elucidated. The radiopacity of the copolymers has been demonstrated with X-radiography.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA), ethyl methacrylate (EMA) and butyl methacrylate (BMA) (Sigma-

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Aldrich) were washed first with sodium hydroxide solution, followed by water, dried over anhydrous sodium sulfate, and vacuum distilled before polymerization. Iodine (Merck) and catalyst *o*-phenylenediamine (Merck) were used as such. Initiator 2,2'-azobis(isobutyronitrile) (AIBN) (Merck) was purified through recrystallization from methanol before use. Tetrahydrofuran (THF) (Merck) was refluxed over sodium and distilled prior to use. Methanol (Merck) and Dichloromethane (Merck) were used as supplied without any further purification.

Methods

Synthesis of glycidyl methacrylate-based acrylate copolymers

GMA-based acrylate copolymers were synthesized using GMA and acrylic monomers such as EMA and BMA. In an RB flask, a mixture of 0.02 mol of GMA and 0.0004 mol of AIBN were dissolved in 15 mL of THF. Then 0.02 mol of acrylic comonomer (EMA or BMA) was added to the RB flask and the mixture was flushed with nitrogen for 30 min. Each flask was then kept in an oil bath maintained at 70°C for 24 h with constant stirring. The copolymers were precipitated from methanol, washed with methanol several times and dried under vacuum at room temperature for 24 h. The GMA-based acrylate copolymers with EMA and BMA are coded as P(GMA-*co*-EMA) and P(GMA-*co*-BMA), respectively.

Iodination of glycidyl methacrylate-based acrylate copolymers

GMA-based acrylate copolymers were converted to inherently radiopaque acrylate copolymers by regioselective ring opening of epoxide groups of GMA units and subsequent iodination. In a 100 mL RB flask, 0.01 mol of GMA-based acrylate copolymer [P(GMA-*co*-EMA) or P(GMA-*co*-BMA)] was dissolved in 15 mL of dichloromethane. To this, 0.001 mol of *o*-phenylenediamine was added as the catalyst and 0.01 mol of iodine dissolved in 25 mL of dichloromethane was then added through a pressure equalized addition funnel drop by drop. The reaction mixture was then kept at room temperature overnight with constant stirring. After the reaction, iodinated copolymer was precipitated out from methanol. It was washed again with methanol, dried under vacuum at room temperature for 24 h, and yield noted. The iodinated copolymer was then dissolved in dichloromethane and cast to form films of desired dimensions for further characterization. The radiopaque copolymers based on EMA and BMA are represented as IP(GMA-*co*-EMA) and IP(GMA-*co*-BMA), respectively.

Characterization of copolymers

The ¹H-NMR spectra were recorded using a Bruker 300 MHz instrument (Bruker AC-300, USA) with TMS as the internal standard and CDCl₃ as the solvent. The IR spectra of the copolymers were determined using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet, Impact 410, USA) as KBr pellets. The molecular weights of the copolymers were determined by gel permeation chromatography (GPC) analysis using Waters HPLC system with Styragel HR columns, and R401 Differential refractometer. THF was used as the mobile phase at a flow rate of 1 mL/min. Elemental iodine in the copolymers were qualitatively determined using an energy dispersive X-ray analysis (EDX) fitted to an Environmental Scanning Electron Microscope (FEI, Quanta 200, The Netherlands). Elemental analysis of iodine was performed at Service Central d'Analyse, Centre National de la Recherche Scientifique, Solaise, France. The thermal stability was determined by thermogravimetric analyzer having specifications SDT-2960, Instruments, USA. Evaluations were done in nitrogen atmosphere at a heating rate of 10°C/min. DSC traces of the polyurethanes were obtained with DSC-2960, TA Instruments Inc. Experiments were done in nitrogen atmosphere at a heating rate of 10°C/min. X-ray opacity of the iodinated copolymers was evaluated using a standard clinical X-ray machine (General Electric, USA). The copolymer films having 2 mm thickness were radiographed and the resulting images were compared with the opacity of 2 mm thick aluminium wedge, a widely used radiographic standard.¹⁹ The grayness of the films in the resulting image was measured using Photoshop CS (Adobe Inc software). The radiopacity of the samples was then quantitatively determined as the ratio of their grayness to that of aluminium (thickness 2 mm) as reported elsewhere.²⁰

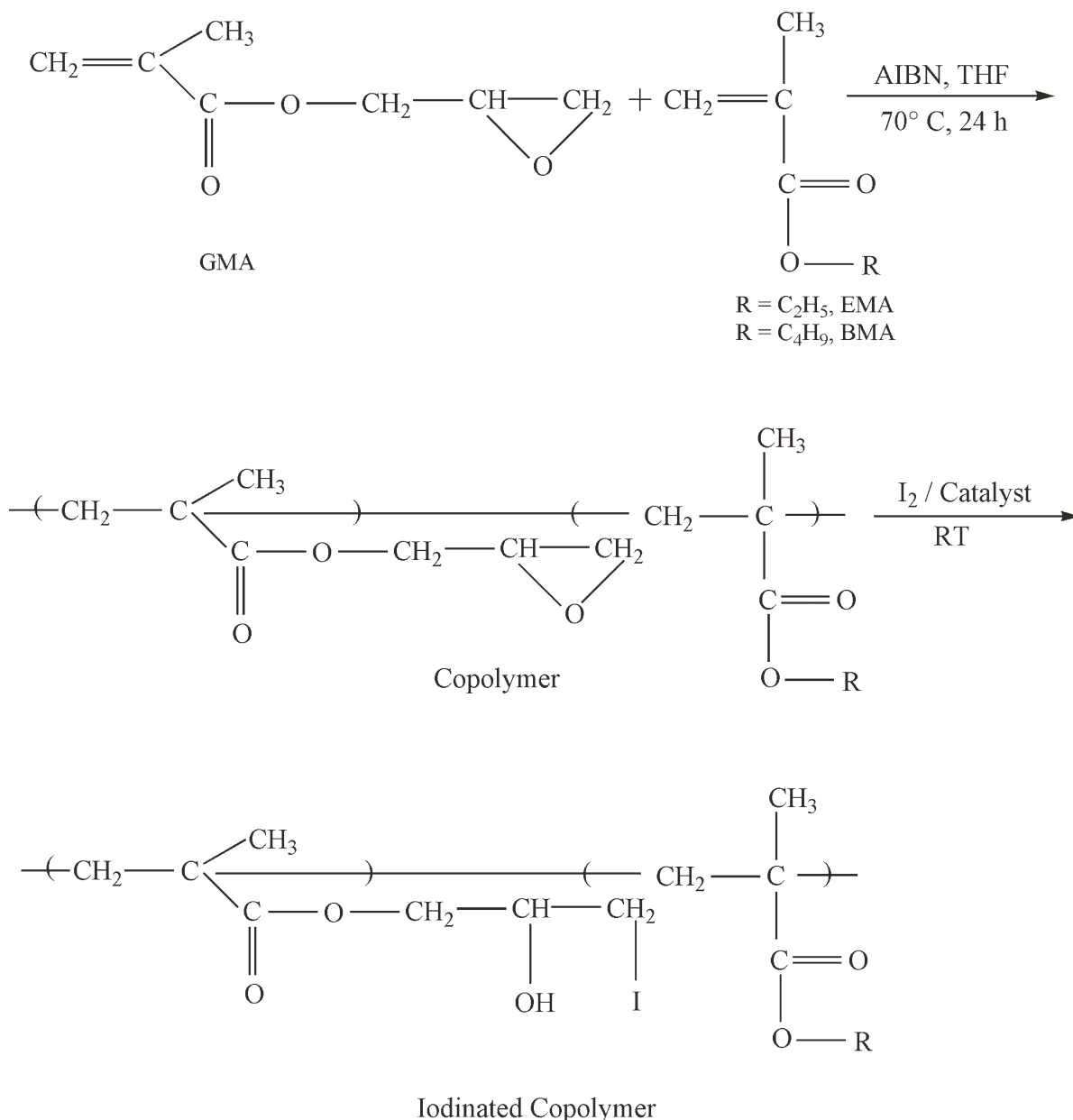
Cytocompatibility using *in vitro* culture

In vitro cytotoxicity testing of iodinated copolymers was done by the direct contact method with L929 mouse fibroblast cells as reported. MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay was also done with copolymer extracts to evaluate cytotoxicity quantitatively.²¹

RESULTS AND DISCUSSION

Synthesis of copolymers

2,3-Epoxypropyl methacrylate (GMA) is a versatile monomer having a polymerizable methacrylic group and a highly reactive epoxy functional group. GMA has been subjected to homopolymerization and copolymerization reactions using free radical initiators



Scheme 1

known to selectively react with methacrylic group. Free-radical random copolymerizations of GMA with conventional monomers have been widely investigated and the interest in GMA-based copolymers is mainly due to the high reactivity of the epoxy group. In our previous work, we have reported the study on radiopaque copolymer based on GMA and methyl methacrylate in the weight ratio 30 : 70.²¹ In this investigation, the synthesis and characterization of GMA-based inherently radiopaque EMA and BMA copolymers have been described. Random copolymers of GMA with EMA and BMA were synthesized by free radical polymerizations at 70°C using AIBN as an initiator to give the copolymers P(GMA-co-EMA) and P(GMA-co-BMA) in good

yields. The copolymers were modified to their radiopaque counter parts by the regioselective ring opening of the pendant epoxy groups using elemental iodine in the presence of a catalytic amount of o-phenylenediamine as shown in Scheme 1. Copolymers having higher iodine content and possessing excellent radiopacity than previously investigated copolymer were obtained.

Characterization of copolymers

Nuclear magnetic resonance spectroscopy

Incorporation of the monomers in the copolymers was confirmed by $^1\text{H-NMR}$. The $^1\text{H-NMR}$ spectra of the copolymers are given in Figure 1. The actual

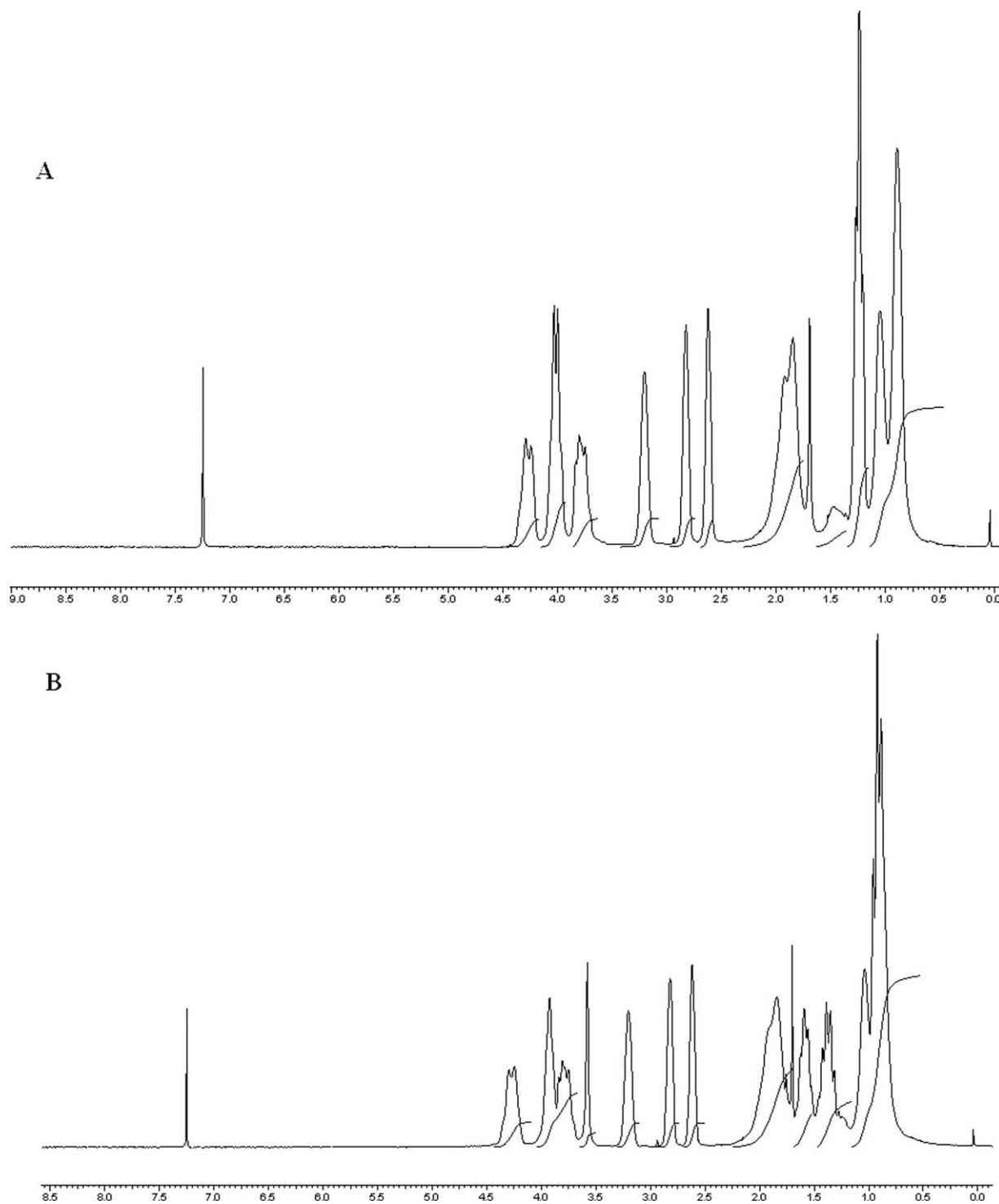


Figure 1 $^1\text{H-NMR}$ spectra of the copolymers P(GMA-co-EMA) (A) and P(GMA-co-BMA) (B).

composition of monomers in the copolymers was determined from the nuclear magnetic resonance (NMR) spectra and the composition of copolymers more or less matched the original ratio of the monomers used (Table I). The $^1\text{H-NMR}$ spectra of the copolymers showed two signals at 4.3 and 3.7 ppm due to splitting of methylene protons in the CH_2O group attached to the carbonyl group of the GMA

unit by the methyne proton of the epoxy group. The peak at 3.2 ppm corresponded to the methyne proton of epoxy group. The peaks at 2.6 and 2.8 ppm were assigned to the methylene protons of the epoxy group. The resonance signals at 4.03 and 3.92 ppm were attributed to two methylene protons of $-\text{COOCH}_2$ in copolymers P(GMA-co-EMA) and P(GMA-co-BMA), respectively. The peaks at 0.9–2.6

TABLE I
Composition, Molecular Weights, and Yield of Copolymers

Polymer	P(GMA-co-EMA)	P(GMA-co-BMA)	IP(GMA-co-EMA)	IP(GMA-co-BMA)
Mol ratio of GMA and comonomer (%)	55: 45	48: 52	45: 45	40: 52
M_w	51,767	70,391	65,522	86,515
M_n	33,186	47,093	42,598	64,864
M_w/M_n	1.5	1.4	1.5	1.3
Yield (%)	73	70	71	69

ppm were due to the methylene groups in the polymeric chain and other alkyl groups. Absence of signals around 5.00 and 5.30 ppm indicated the absence of protons corresponding to the methacrylic unsaturation. The average composition of monomeric units in the copolymers was obtained from the resonance peaks in the $^1\text{H-NMR}$ spectra as reported elsewhere.²²

FTIR spectra

The radiopaque copolymers were characterized and the physico-chemical properties were compared with that of noniodinated copolymers. The FTIR spectra of the noniodinated and iodinated copolymers are given in Figures 2 and 3, respectively. IR absorption bands at 2991 cm^{-1} (P(GMA-co-EMA)) and 2962 cm^{-1} (P(GMA-co-BMA)) corresponded to asymmetrical and symmetrical stretching of the methyl and methylene groups in the copolymers. The peaks at 1718 cm^{-1} (P(GMA-co-EMA)) and 1737 cm^{-1} (P(GMA-co-BMA)) in the spectra were attributed to

the ester carbonyl stretching of GMA and other comonomer units. The peaks at 907 cm^{-1} (P(GMA-co-EMA)) and 908 cm^{-1} (P(GMA-co-BMA)) suggested the presence of epoxy functional group in the copolymers.

The FTIR spectra of the copolymers after iodination showed marked reduction in intensity of absorbance of the epoxy groups at 907 cm^{-1} for iodinated copolymer IP(GMA-co-EMA) in comparison to that of uniodinated copolymer P(GMA-co-EMA). A similar change in the intensity of peak 908 cm^{-1} was also observed with IP(GMA-co-BMA) in comparison to that of uniodinated copolymer P(GMA-co-BMA). The appearance of peak at 907 cm^{-1} with reduced intensity in iodinated copolymers suggested the presence of residual epoxy groups and the partial iodination of the copolymers could be due to the close packing of the voluminous iodine moiety around the polymeric backbone resulting in a diminished reaction rate at higher graft ratios.²³ A broad band appeared at 3500 cm^{-1} for hydroxyl functional groups in iodinated copolymers (IP(GMA-co-EMA)

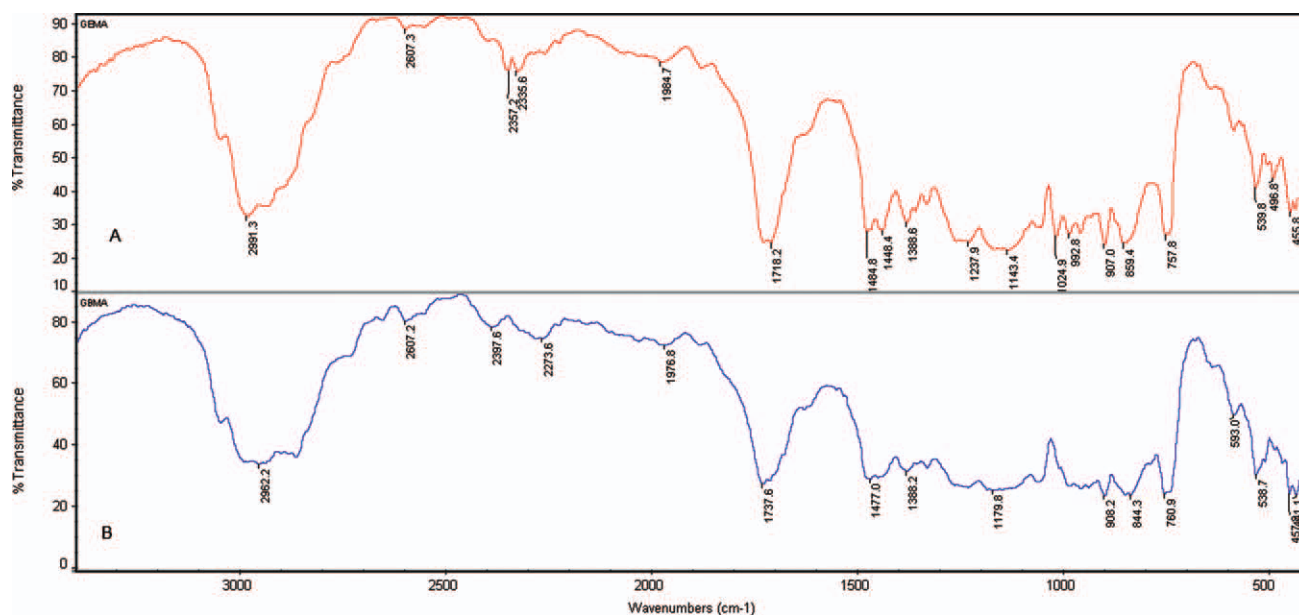


Figure 2 IR spectra of copolymers P(GMA-co-EMA) (A) and P(GMA-co-BMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

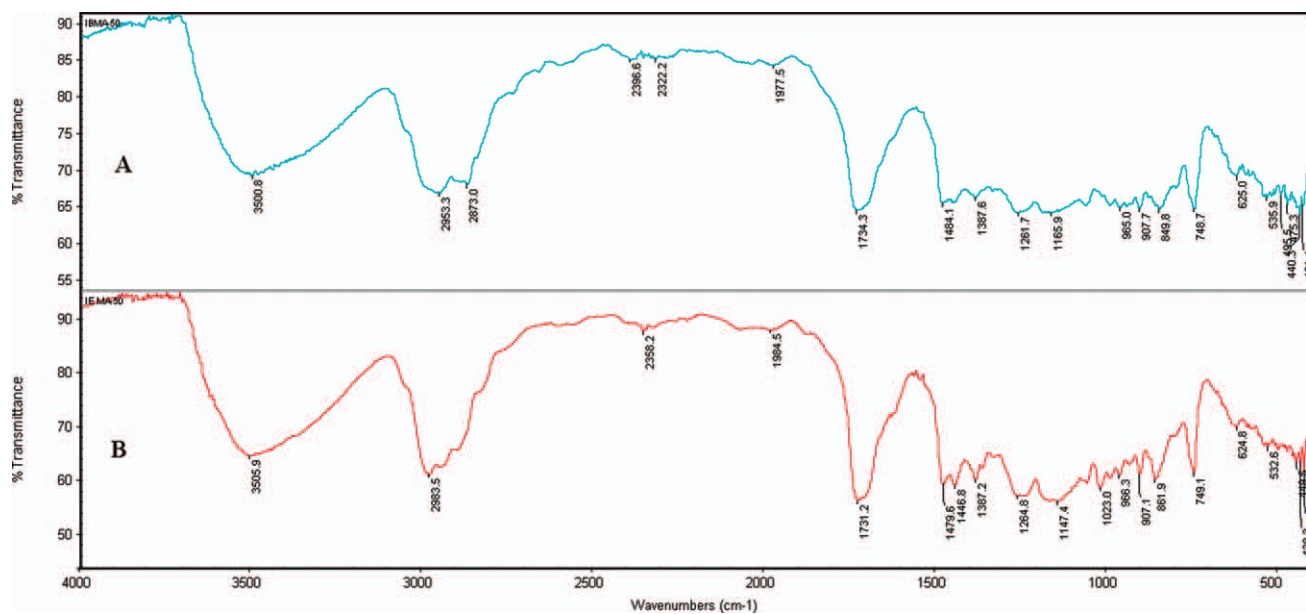


Figure 3 IR Spectra of iodinated copolymers IP(GMA-co-BMA) (A) and IP(GMA-co-EMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

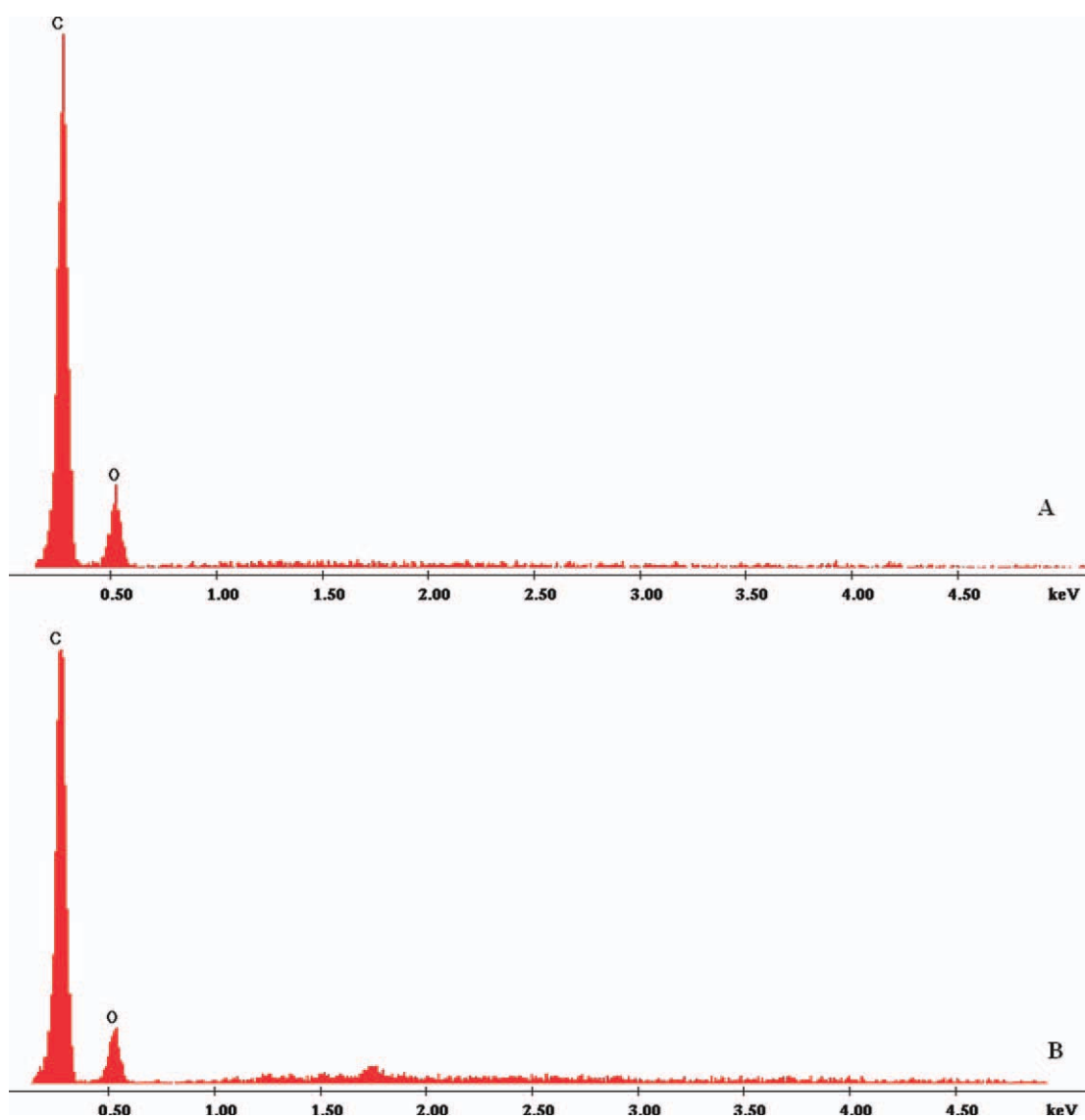


Figure 4 EDX spectra of copolymers P(GMA-co-EMA) (A) and P(GMA-co-BMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

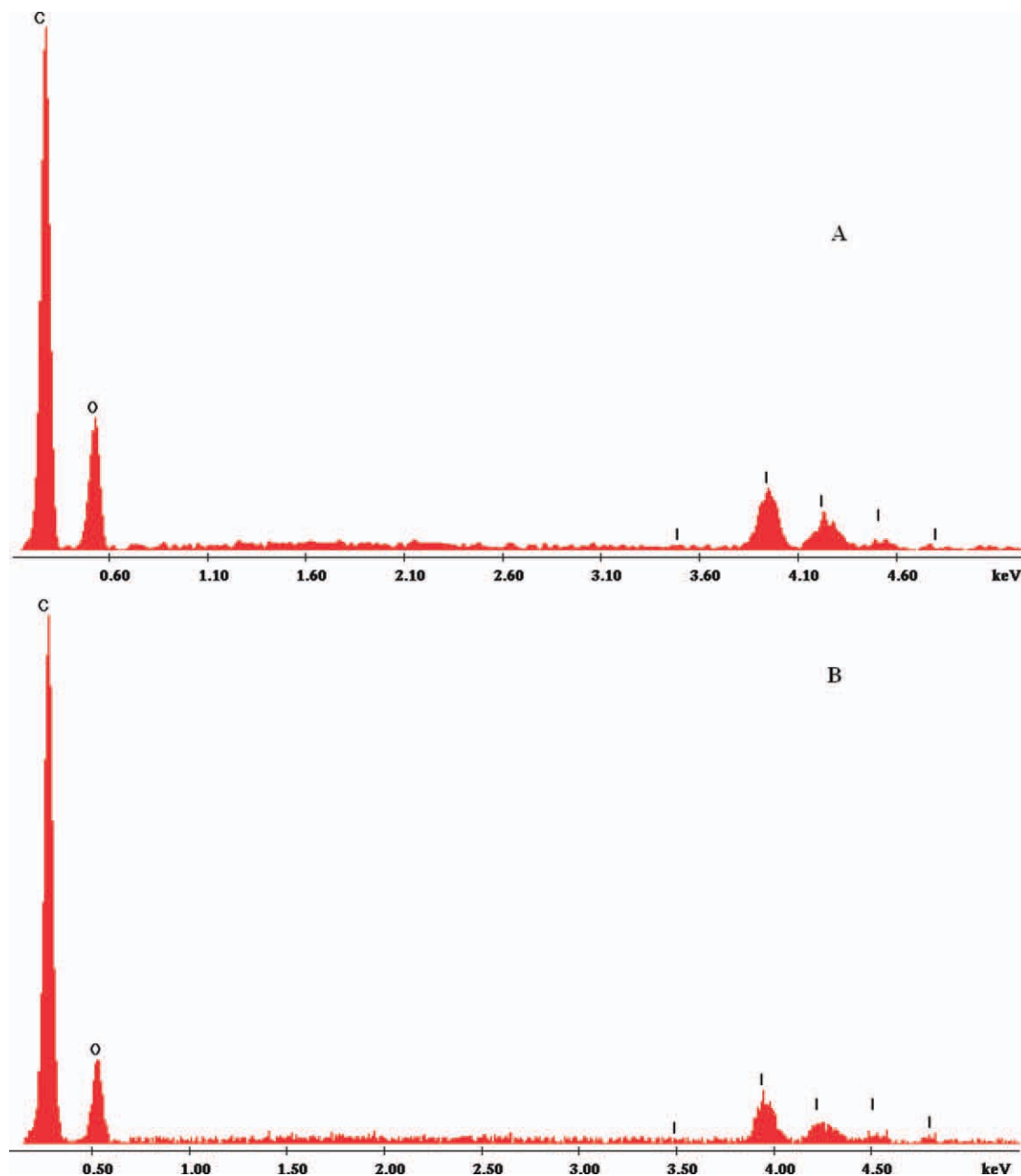


Figure 5 EDX spectra of iodinated copolymers IP(GMA-co-EMA) (A) and IP(GMA-co-BMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and IP(GMA-co-BMA) in comparison to that of uniodinated copolymers indicating the formation of iodo alcohols.

Gel permeation chromatography

The number and weight average molecular weights of the synthesized copolymers were determined by GPC and are presented in Table I. The molecular weights were found to be higher than the analogous non iodinated copolymers. The GPC analysis showed that high molecular weight copolymers could be produced by grafting the prepolymer with iodine atoms.

Energy dispersive X-ray analysis

Iodination of copolymers were further confirmed by energy dispersive X-ray analysis. The peaks around 3.5–5 keV in EDX images were assigned to the presence of iodine atoms in the iodinated copolymeric chains. EDX carried out on noniodinated copolymers gave peaks for carbon and oxygen atoms only. The EDX spectra of the copolymers are given in Figures 4 and 5. The percentage iodine in the copolymers was estimated quantitatively by elemental analysis. The percentage weight of iodine in the present iodinated copolymers was found to be as high as 19% (IP(GMA-co-EMA)) and 17% (IP(GMA-co-BMA))

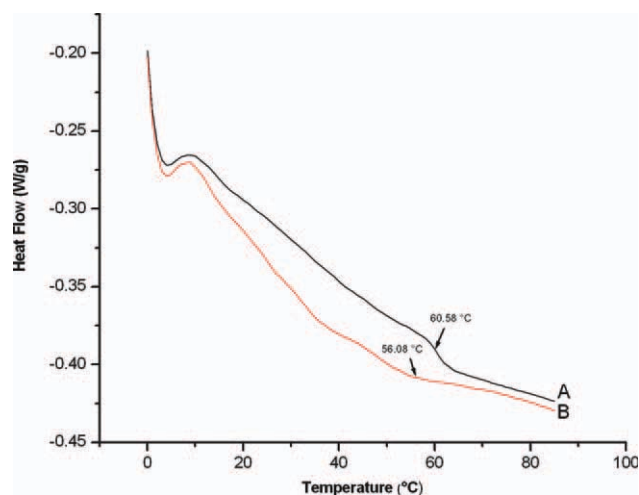


Figure 6 DSC scans of copolymers P(GMA-*co*-EMA) (A) and P(GMA-*co*-BMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with respect to total mass. High iodine content in a material would result in effective X-ray absorption. From the iodine content value, the composition of monomers in copolymers, IP(GMA-*co*-EMA) and IP(GMA-*co*-BMA) were theoretically calculated as 45 : 45 and 40 : 52, respectively. The partial iodination of the copolymer could be due to the presence of bulky iodine atoms in the polymers and the resultant decrease in reaction rate at higher graft ratios.

Thermal analysis

Thermal characteristics of the copolymers were evaluated by DSC and TGA analysis. The DSC traces are shown in Figures 6 and 7 and thermal parameters

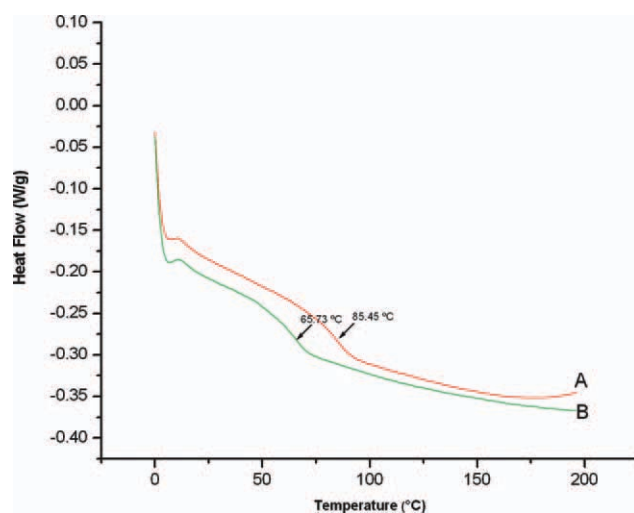


Figure 7 DSC scans of iodinated copolymers IP(GMA-*co*-EMA) (A) and IP(GMA-*co*-BMA) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
Thermal Characteristics of Copolymers

Polymer	T_g (°C)	T_{d1} (°C)	Weight remained at T_{d1} (%)	$T_{d1/2}$ (°C)
P(GMA- <i>co</i> -EMA)	60	113	98	346
P(GMA- <i>co</i> -BMA)	56	180	95	316
IP(GMA- <i>co</i> -EMA)	85	231	98	335
IP(GMA- <i>co</i> -BMA)	65	246	98	320

T_{d1} , initial decomposition temperature; $T_{d1/2}$, temperature at 50% decomposition.

are given in Table II. Iodinated copolymers possessed higher glass transition temperature (T_g) and thermal stability than their noniodinated counterparts. The T_g value of copolymers with EMA was found to be higher than BMA based copolymers. As the size of monomer substituent in the polymeric chain increases, the physical separation between the chains is increased, producing a corresponding increase in mobility between adjacent chains. The DSC scans also showed that the T_g of the modified copolymers increased significantly after iodination. The incorporation of iodine moieties into the copolymeric chains are believed to have increased the rigidity of the chains resulting in higher T_g values. TGA analyses have shown that the noniodinated copolymers were stable only up to 180°C. However, the iodinated copolymers showed higher thermal stability than their noniodinated counterparts. The thermal stability of copolymers with monomer EMA was lower than BMA-based copolymers suggesting that, as the bulk size of the substituent increases, the thermal stability increases. The iodinated copolymers had high initial decomposition temperature and were stable up to 250°C. Grafting with highly sterically hindered iodine atoms would have decreased the mobility of the polymeric chains thereby reducing the thermal decomposition.²² These thermal characteristics enable the copolymers to be processed by any fabrication techniques that depend upon plastic flow at high temperature, thereby making them potential candidates for biomedical applications.

Radiopacity

X-radiographic analysis of iodinated copolymeric films showed excellent radiopacity when compared with standard aluminum wedge. The X-ray visibility of the iodinated copolymer films were compared with an aluminum step wedge and noniodinated copolymers (Fig. 8). Noniodinated copolymers were not radiographically visible due to the absence of heavy elements in their structure. Quantitative evaluation of radiopacity has shown that the radiopacity of iodinated copolymers films having 2 mm

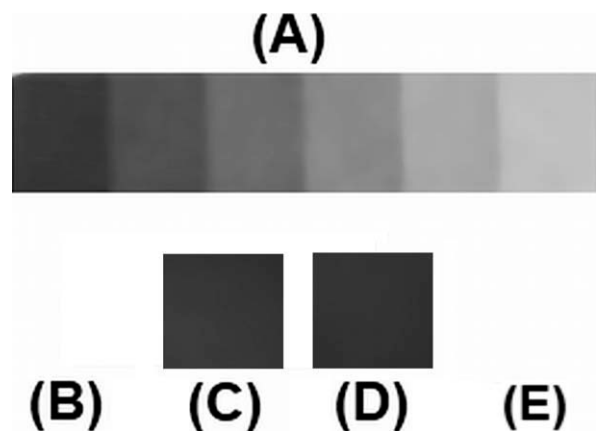


Figure 8 Positive print of a radiograph showing: an aluminium step wedge 0.5–3 mm thick in 0.5 mm steps (right to left) (A), P(GMA-*co*-EMA) (not visible in print) (B) IP(GMA-*co*-EMA) (C), IP(GMA-*co*-BMA) (D), P(GMA-*co*-BMA) (not visible in print) (E). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

thickness were higher than that of aluminum wedge having same dimensions (Fig. 9). The X-ray opacity of these copolymers is sufficient for clinical monitoring when used as biomedical implants.

Evaluation of cytotoxicity *in vitro*

The cytotoxicity evaluation was done to assess the potential of the iodinated polymers for biomedical applications. The fibroblasts cells around the iodinated copolymer films retained their original spindle shaped morphology and no detectable zone of cell lysis, vacuolization, detachment, or membrane disintegration was observed around or under the specimens even after 24 h of contact. Fibroblasts cells after exposure to iodinated copolymer films for

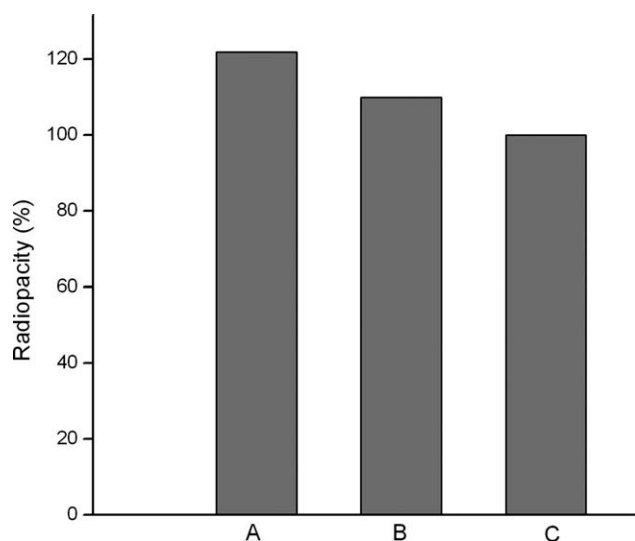


Figure 9 Radiopacity of IP(GMA-*co*-EMA) (A), IP(GMA-*co*-BMA) (B) evaluated as ratio of absorption relative to standard 2 mm thick Al wedge (C).

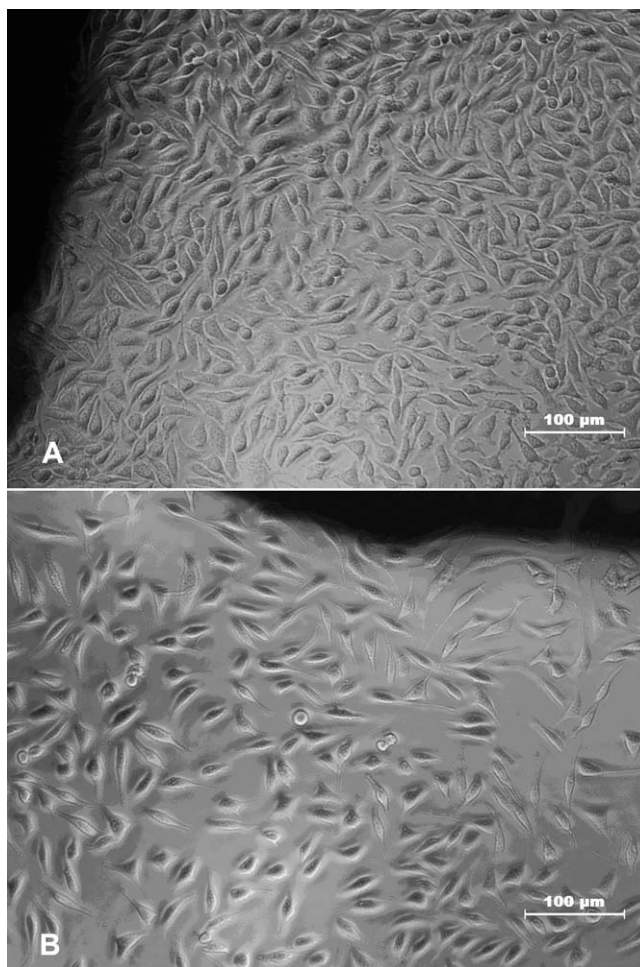


Figure 10 Representative microphotograph of L929 mouse fibroblast cells around iodinated copolymers IP(GMA-*co*-EMA) (A), IP(GMA-*co*-BMA) (B).

24 h are shown in Figure 10. The MTT assay of cells after contact with extracts of iodinated copolymer, IP(GMA-*co*-EMA) and IP(GMA-*co*-BMA) have shown that the copolymers were noncytotoxic in nature. Generally, samples showing greater than 60% cell death are considered to be toxic. The polymers were found to be noncytotoxic in nature. The MTT assay of cells after contact with extracts of iodinated copolymer IP(GMA-*co*-EMA) and IP(GMA-*co*-BMA) showed 71 and 70% metabolic activity, respectively. The studies revealed that the polymers did not possess any leachants capable of inducing cell toxicity. It is also worth noting that with preformed polymers no release of potentially toxic monomers occurs.

CONCLUSIONS

Radiopaque copolymers of GMA with EMA and BMA were synthesized by introducing iodine atoms via the regioselective ring opening reactions of epoxide groups. The percentage weight of iodine in the present copolymers was found to be as high as

17–19%. The iodinated copolymers showed higher glass transition temperature and thermal stability in comparison with unmodified polymers. The presence of bulky iodine atoms in the polymer backbone decreased the flexibility of the macromolecules and created modified polymers with novel properties. Radiographic analysis showed that the copolymers possessed excellent radiopacity. The iodinated copolymers were cytocompatible with L929 mouse fibroblast cells. Radiopaque copolymers of GMA with monomers, EMA and BMA could be used as additives of dental or bone cements in place of barium or zirconium particles, which are usually added to provide X-ray opacity. It can also be used in vertebroplasty for the augmentation of osteoporosis induced vertebral compression fractures.

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