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Methylmethacrylate-Iodothiophene copolymers for the obtaining of bone and dental cements

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This paperwork deals with the obtaining of a new radio-opaque biomaterial that can be used in the manufacture of bone or dental cements. We have obtained some radio-opaque copolymers based on methyl methacrylate (MMA) and 2-iodothiophene (ITf). The copolymers were characterised by Gel Permeation Chromatography and their molecular weights were obtained. Next, they were subjected to viscosity analysis to evaluate the K and α constants from Kuhn-Mark-Houwink-Sakurada equation. The values for the two constants showed an increase of the rigidity of the macromolecular chains, approaching as shape the model of the rigid rod. The biocompatibility of the copolymers was tested *in vitro* on an L929 cell line of murine fibroblasts and these materials revealed no cytotoxic effect upon the cells. These results combined with the excellent radio-opacity of the materials lead to the suggestion that this type of polymers could be used as dental or bone cements for the replacement of barium or zirconium particles usually added to provide X-ray opacity.

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1. Introduction

In many clinical applications, polymeric biomaterials require radio-opacity in order to be properly implanted in tissues. They are often made radio-opaque through addition of an X-ray absorbing agent, such as barium sulphate or zirconium dioxide [1, 2] and used as bone cements [1, 3], denture base [4], radio-opaque microspheres [5], to obtain catheters for epidural anaesthesia [1] or in intravascular neurosurgery [6].

The major drawback associated with the use of radio-opaque additives is that the physico-mechanical properties of the polymeric matrix are altered. In this respect the phase-boundaries between filler and polymer may become over time places where the mechanical failure (especially fatigue) occurs.

For methacrylic bone cements, there has been reported that the barium sulphate particles tend to form clumps, which influence the rate of formation and propagation of the mechanical defects [7, 8].

For polyurethane catheters, it is known [1] that the addition of barium sulphate has a negative influence on the smoothness and lubricity of the inner and outer surfaces. Usually, 40-60% barium sulphate is required to impart sufficient contrast in catheters, since the wall thickness is only of the order of several tenths of a millimetre.

The presence of these inorganic particles ($BaSO_4$, ZrO_2) can favour the aseptic loosening of the prosthesis [8].

From biological point of view it is proven that $BaSO_4$ wear particles may enhance the differentiation of macrophage into bone resorbing osteoclasts [9].

In the view of the negative influence of the inorganic particles on both the mechanical and biological behaviour of the radio-opaque polymers, it is desirable to find an alternative for the use of radio-opaque additives [10-15].

A promising approach is the use of (co)polymers having covalently bound heavy atoms, for example iodine. The synthesis of such biomaterials with good X-ray visibility has been reported starting with several years ago [16-23].

This work proposes a new polymeric material with X-ray opacity that can replace the inorganic salts from the orthopaedic or dental cements and presents some solution properties of this polymer related to its mechanical behaviour. Our team has already achieved very good results in the synthesis and characterisation of some cement-based materials, with X-ray visibility, good mechanical properties, and low cytotoxic effects [13-14, 18].

Methyl methacrylate could be copolymerised with an iodine-containing monomer such as 2-iodothiophene. Iodothiophene is a convenient partner for methyl methacrylate as it is commercially available and the copolymers are easy to obtain. The material proposed was tested from physico-chemical and biological point of view and it proved to be a good candidate for replacing the classical radio-opacifying inorganic agents from dental or bone cements.

2. Materials and methods

2.1 Polymer preparation

MMA (Merck) was used after a preliminary purification by distillation *in vacuum* (63°C and 200 mmHg). The iodine-containing monomer namely 2-iodothiophene (ITf) (Aldrich) was used as such. Ethylene glycol dimethacrylate (EGDMA) (Aldrich) was used as such without any further purification. Benzoyle peroxide (BPO) was employed from Merck and purified through recrystallisation from methanol at 40 °C. Toluene (S.C. Reagent) was distilled (110 °C, 1 bar pressure) and stored at +8 °C. Tetrahydrofurane (THF, Merck) was purified by distillation in the following manner: first, it was distilled on cuprous chloride ($CuCl_2$), then the medium fraction was deposited on potassium hydroxide pellet (KOH) overnight and finally it was rectified over metallic sodium (66.8°C).

2.1.1 Preparation of the copolymers MMA-ITf.

There were obtained by bulk polymerisation copolymers MMA/ITf with the initial molar composition 90/10 and varying the molar concentration of the initiator (BPO): 0.1%, 0.4%, 1% and 2%. The polymerisation reactions were performed in test tubes, under inert atmosphere at 80ºC for 6-8 hours.

After synthesis, the copolymers were purified by extraction with methanol for 2 days. The presence of residual monomer was determined chromatographically (Gel Permeation Chromatography, GPC, analysis).

There were also obtained some pellets of MMA-ITf also through bulk copolymerization. The monomers, initiator ([BPO]=10^{-2} mole/mole monomers) and the cross-linking agent EGDMA (3% molar with respect to monomers mixture) were mixed together by vortexing at 30 Hz, then they were poured into polyethylene moulds (10 mm in diameter and 3 mm in height).

Polymerisations were carried out in inert atmosphere, at 75 °C for 5 hours, followed by post-polymerisation at 110 °C, for 3 hours. The obtained pellets were immersed in methanol for 2 days to extract the residual monomers.

2.2 Viscosity analysis

The study of the viscosity of macromolecular solutions is one of the most used methods to determine the average molecular weight of the polymers. In this study, we propose the determination of the molecular weight by GPC and then the computation of the constants K and α from Kuhn – Mark – Houwink – Sakurada equation.

The study of the viscosity of macromolecular solutions in toluene was performed with a capillary viscometer Ubbelohde and an automatic AVS350 apparatus at 30 ºC and the molecular weight was determined by using an HPLC Waters510 apparatus.

2.3 Cytocompatibility using *in vitro* culture

Polymers biocompatibility can be verified by testing *in vitro* cytotoxicity. It was used cell line L929 of murine fibroblasts, which was cultivated in culture medium (DMEM or RPMI 1640), supplemented with calf foetal serum and antibiotics. Two methods were used: direct testing and elution method.

In the first method, the tested polymers were put into contact with a culture adherent monolayer cells in microplates of different cultures. In the second method, the testing material was maintained in culture medium in standard conditions for 24-48 hours, at 37ºC, in 5% CO_2 atmosphere.

Finally, the cells were microscopically examined for detecting cytotoxicity visible signs, cellular lysis or cellular components dimensions and conformation (optical microscopy, ZEISS - Axiovert 135 Microscope). A cellular viability test (MTT) was also employed.

3. Results and discussion

3.1 GPC characterisation

After extraction with methanol, all the copolymers were analysed by GPC to check the presence of the residual monomers. GPC analysis showed pure copolymers that could be further analysed against macrophage cells.

By GPC, we have also determined the molecular weight of the copolymers, an important parameter that was useful in the determination of K and α constants. The results are presented in Table 1.

Table 1. Molecular weights of the copolymers MMA-ITf

Copolymer	MW(g/mole)
MMA-ITf with 0.1% BPO	159000
MMA-ITf with 0.4% BPO	95700
MMA-ITf with 1% BPO	74600
MMA-ITf with 2% BPO	28000

3.2 Viscosity analysis

10% molar MMA-ITf solutions of copolymer in toluene were obtained in the following concentrations (c): 0.8 g/dl, 0.65 g/dl, 0.5 g/dl, 0.35 g/dl and 0.2 g/dl.

Then the flowing times for the solvent and for the solutions, the relative viscosity (η_r) and specific viscosity (η_{sp}) were computed. The graphs η_{sp}/c versus c were plotted for each copolymer and by extrapolation we obtained the intrinsic viscosities (Figs. 1-4).

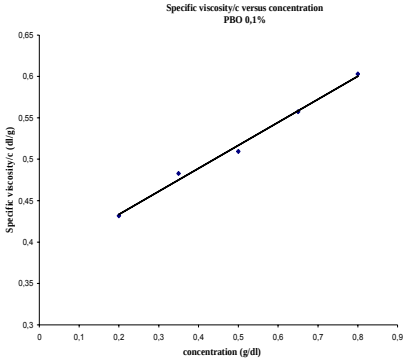

Fig. 1 Specific viscosity/c vs. c for MMA-ITf with 0.1% BPO

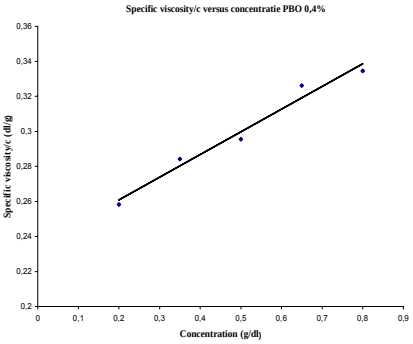

Fig. 2 Specific viscosity/c vs. c for MMA-ITf with 0.4% BPO

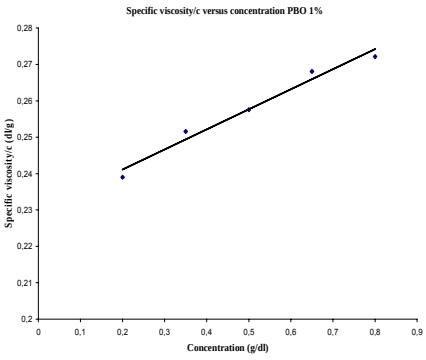

Fig. 3. Specific viscosity/c vs. c for MMA-ITf with 1% BPO

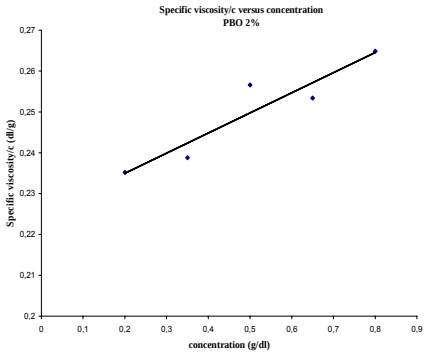

Fig. 4. Specific viscosity/c vs. c for MMA-ITf with 2% BPO

From these graphs, the values of the intrinsic viscosities were computed (Table 2).

Table 2. Values of the intrinsic viscosity

Copolymer	Intrinsic viscosity (dl/g)
MMA-ITf with 0.1% BPO	0.378
MMA-ITf with 0.4% BPO	0.235
MMA-ITf with 1% BPO	0.230
MMA-ITf with 2% BPO	0.225

Finally, taking into account Sakurada equation, the values of intrinsic viscosities and the molecular weights of the polymers, the two important constants K and α were computed and the values were:

$$K= 0.518x10^{-3} \text{ ml/g and } \alpha = 0.9$$

It is known that the value of α parameter lies between 0.5 and 1. The high value of the constant α from Kuhn – Mark – Houwink – Sakurada equation shows an increase of the rigidity of the macromolecular chains, approaching as shape the model of the rigid rod.

3.3 Cytocompatibility using cell cultures

The results from the cytotoxicity tests, as expressed by microphotographs, are presented in figures 5 and 6. As it can be observed, the cells are alive and they maintain their characteristics after the culture on the surface of the MMA-ITf copolymers.

Numerous cells were encountered at the surface of the polymer disks after a three day period.

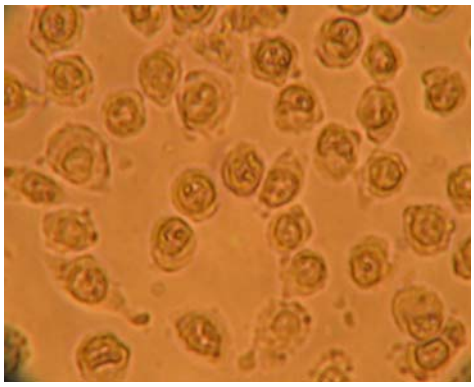

Fig.5. Cytotoxicity assay on macrophage murine cells (L929). The cells have retained their characteristic appearance

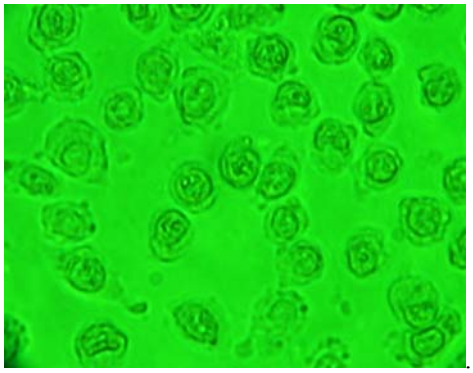

Fig.6. Cytotoxicity assay on macrophage murine cells (L929)

Murine cells were mainly found in a round shape, but sometimes, they exhibited an elongated shape. They exhibited numerous thin phyllopods, allowing the anchorage to the polymeric pellets, and sometimes, thicker extensions were encountered, allowing communication with cells.

MTT photographs showed also a good biocompatibility of the copolymer against macrophage cells (figures 7 and 8).

4. Conclusions

The use of iodine-based monomers provides a convenient way to prepare polymeric biomaterials that combine uniquely good chemical stability, X-ray visibility, cellular non-cytotoxicity and improved mechanical properties.

The physico-chemical properties influence the mechanical and biological behaviour of the material. *In vitro* assay against murine L929 fibroblast cells onto MMA-ITf copolymers revealed a good biocompatibility of the tested material.

The replacement of barium sulphate and zirconium dioxide from the bone or dental cements with radio-opaque polymers could improve the mechanical properties of the final cement especially upon ageing since fatigue microcracks are known to start on these material grains.

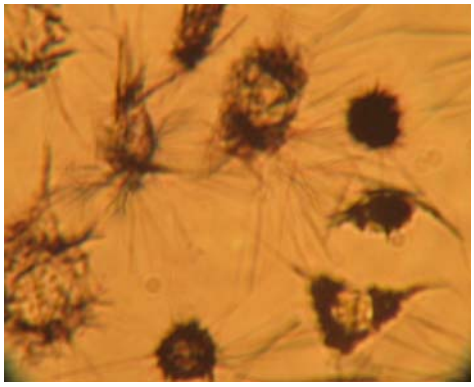

Fig. 7. L929 murine fibroblasts (without copolymer)

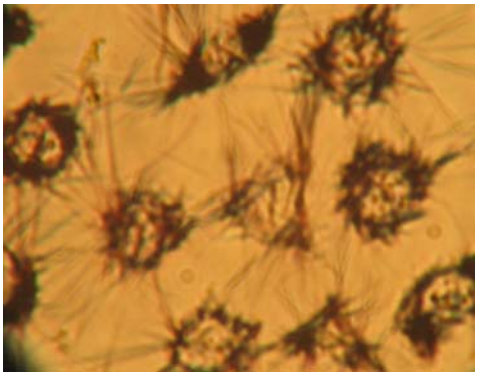

Fig. 8. L929 murine fibroblast cultured on the surface of MMA-ITf copolymers

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