JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS, Vol. 10, No. 12, December 2008, p. 3436 - 3441

Acrylic cements for dental prosthetics

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The subject of the present work is the synthesis of polymeric biocompatible materials based on methyl methacrylate (MMA) copolymers chemically modified by quaternization for use in dental prosthetics. Two copolymers were used, methyl methacrylate – diethylaminoethyl methacrylate (MMA-DEAEMA) and methyl methacrylate – chloroethyl methacrylate (MMA-CLEMA). The former presents tertiary amino groups and was quaternized using methyl iodide. Triethyl amine was used for the quaternization of the latter. For both of the copolymers, the quaternization was performed either in homogeneous media, or at the surface of the hardened cements. Cytotoxicity and cellular adherence tests were performed in order to confirm the appropriateness of the resulting products in dentistry.

(Received August 4, 2008; accepted November 27, 2008)

Keywords: MMA copolymer, Dental prosthetics, Quaternization, Kinetics, Cytotoxicity, Cellular adherence

1. Introduction

Methyl methacrylate-based products for medical applications have been studied since 1930 [1]. Such systems - used in dental reconstruction - consisted of poly(methyl methacrylate) (PMMA) beads mixed with a maximum of 30% methyl methacrylate (MMA) and polymerised at room temperature with a redox initiation system (benzoyl peroxide- tertiary amine). Further studies [2] have proposed the use of small quantities of superior methacrylic esters in order to reduce volatility and increase the impact resistance, the addition of tetrafunctional monomers in order to obtain a final network-type product and the addition of some reactive methacrylic derivatives to enhance adhesion. The acrylic cements are also used in bone surgery; the main problems that must be solved in this case are: the reduction (to ppm levels) of the residual monomer amount after hardening, the minimisation of the heat flow during hardening, the decrease of the volume contraction, and the obtaining of a product which is X-rays opaque in order to facilitate the radiological examination of *in vivo* implants. Inert, mineral fillers, such as hydroxyapathite [3] or α-calcium triphosphate [4] have been added to the system in order to obtain bone-like mechanical properties. Some studies [5-7] have proposed the use of a small amount of hydrophilic monomer (e.g. 1-hydroxy-propyl-methacrylate or ethoxyethylenglycol methacrylate) in order to compensate the volume contraction with the swelling in biological fluids; only partial compensation could be achieved though, since a too large amount of hydrophilic monomer resulted in reduced mechanical properties. Doping the cement with X-ray opaque materials such as $BaSO_4$ or ZrO_2 also caused a decrease of the mechanical indexes, and therefore iodinated monomers, such as 4-iodo-phenol-methacrylate [8], 2-(4-iodo-benzoyl)-oxo-ethylmethacrylate and 5,7-diiodokinolin-8-methacrylate [9,10] have been added to facilitate X-rays examination of the bone-prosthetics interface.

The present work addresses the synthesis of a polymeric biocompatible material for the purpose of obtaining MMA copolymers, chemically modified by quaternization, for dental prosthetics use. The polycationic structures (recognized inhibitors of microorganism proliferation) could - if applied as a protective film - serve as a superficial layer with antibacterial properties.

2. Materials and methods

Materials

Monomers: methyl methacrylate (MMA) – Merck, purified by vacuum distillation (63°C, 200 mmHg); diethylaminoethyl methacrylate (DEAEMA) – Aldrich; chloroethyl methacrylate (CLEMA) – Merck, purified by vacuum distillation (90°C, 5 mm Hg). Initiators: benzoyl peroxide (BP) – Merck, recrystallised from methanol; N,N'-dimethyl-p-toluidine (DMPT) – Fluka, purified by vacuum distillation (74°C, 5 mmHg). Iodination agents: sodium iodide (NaI) - Merck, methyl-iodine (MI) – Merck. Quaternization agent: triethyl amine (TEA) – Merck. Solvents: benzene (B) - Chimopar, distilled (80.2°C), N,N'-dimethylformamide (DMF) – Fluka, acetone (A) – Aldrich. Precipitation agents: petroleum ether (PE) - Chimopar, ethyl-ether (EE) – Chimopar.

Methods

The first step of the study was the synthesis of two MMA copolymers, MMA-DEAEMA and respectively MMA-CLEMA, both of them being capable of forming quaternary ammonium salts through polymer-analogue reactions. Two different copolymer compositions have been obtained in each case. The MMA-DEAEMA copolymer has pre-existent tertiary amino groups on the polymer chain and has been quaternized using MI. For MMA-CLEMA copolymer, TEA was used as quaternization agent. The quaternization reactions have been performed both in the copolymer bulk (in homogeneous media) and at the surface of the hardened cements. A scheme of the operations is presented in fig. 1.

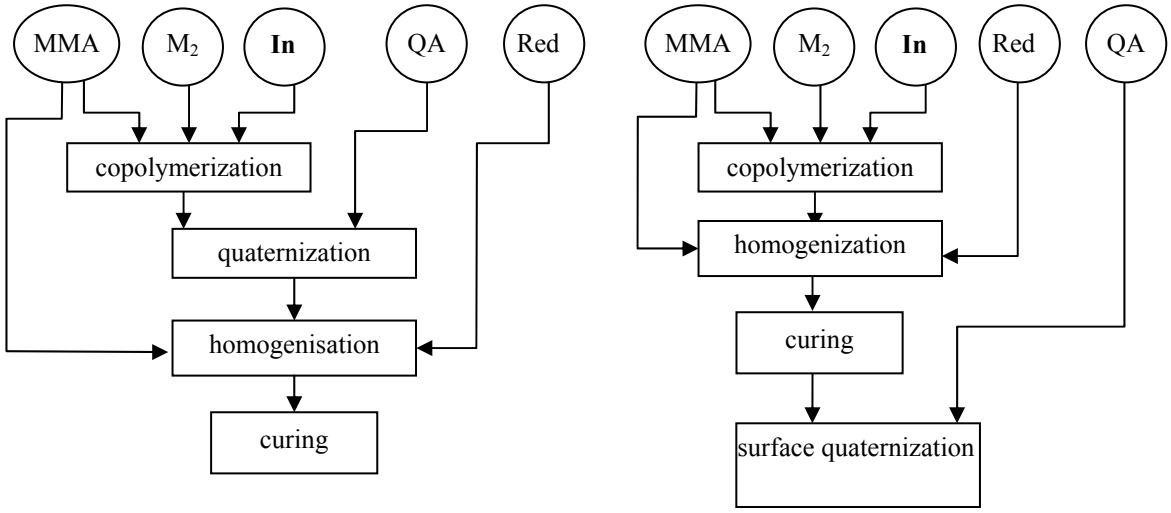


Fig 1. Reactions scheme: M_2 = comonomer of MMA (DEAEMA/CLEMA), In = initiator (BP), Red = reducer in a redox initiation system (DMPT), QA = quaternization agent (TEA).

a. MMA-CLEMA copolymerization

MMA (1 mole/l), CLEMA (0.2 mole/l) for composition I and respectively MMA (1 mole/l), CLEMA (0.1 mole/l) for composition II were copolymerized using BP ($5 \cdot 10^{-2}$ mole/l) as initiator in a B solution, under a nitrogen atmosphere, at 70°C, for 2.3 hours. The copolymer was precipitated with PE, filtered, dried under vacuum until constant weight. The final conversion was 70% for both compositions.

b. Iodination of MMA-CLEMA copolymer

4.5 g of the 10:1 mol. MMA-CLEMA copolymer was dissolved in 50 ml A and mixed with a solution of 1 g NaI in 20 ml A [11]. The mixture was maintained at 50°C for 5 hrs. The resulting sodium chloride was separated by centrifugation and (after washing with A and drying), weighed for evaluating the reaction conversion. The iodinated copolymer was precipitated in distilled water, filtered, washed and dried under vacuum. A similar procedure was performed using the 5:1 molar MMA-CLEMA copolymer. The molar ratios used for the synthesis of the copolymers refer to the initial monomer compositions; the overall composition of the copolymer is to be determined.

c. Quaternization of the iodinated CLEMA-MMA copolymer

0.1281 g of iodinated MMA-CLEMA polymer were dissolved in 5 ml DMF to which a 5:1 volumetric excess of TEA has been added (0.2 ml) [12]. After maintaining the mixture at room temperature, for 24 hrs, the quaternized copolymer was precipitated in an aqueous solution of calcium chloride, separated by filtering, washed with distilled water and vacuum dried. The reaction succession is presented in figure 2.

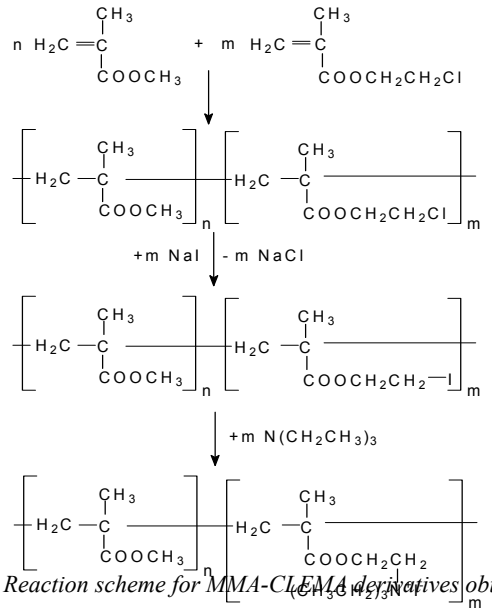


Fig. 2. Reaction scheme for MMA-CLEMA derivatives obtaining

d. Curing of the CLEMA-MMA-based cements

0.2276 g quaternized MMA-CLEMA copolymer (method A) were homogenised by dry mixing with 0.003 g BP, thus forming the "solid" component of the cement. The liquid component was a mixture of MMA and DMPT (0.002 ml). The "solid"-liquid components ratio used was 2:1 (g/g). The powder was placed in a mould and then the liquid was poured in. The curing occurred after 5-10 min, at room temperature. The curing process consists in the free-radical polymerization of MMA with the redox system BP (oxidizing agent) – DMPT (reducing agent). The redox system was chosen in order to allow a low-

temperature-polymerization while ensuring a significant reaction rate.

A similar procedure was used for the iodinated MMA-CLEMA copolymer (non quaternized) (method B). After curing, the cements were immersed in TEA for 24 hrs in order to induce surface quaternization.

e. MMA-DEAEMA copolymerization

MMA (1 mole/l), DEAEMA (0.2 mole/l) for composition I and respectively MMA (1 mole/l), DEAEMA (0.1 mole/l) for composition II were copolymerised using BP ($5 \cdot 10^{-2}$ mole/l) as initiator, in a benzene solution, under nitrogen atmosphere, at 70°C for 2.3 hours. The copolymer was precipitated in PE, filtered, and dried under vacuum until constant weight.

f. Quaternization of MMA-DEAEMA copolymer

3.0 g of MMA-DEAEMA copolymer were dissolved in 40 ml DMF to which a 5:1 volumetric excess of MI was added (1.7 ml). After maintaining the mixture at room temperature, for 24 hrs, the quaternized copolymer was precipitated in EE, separated by filtration, washed and vacuum dried. The reaction succession is presented in figure 3.

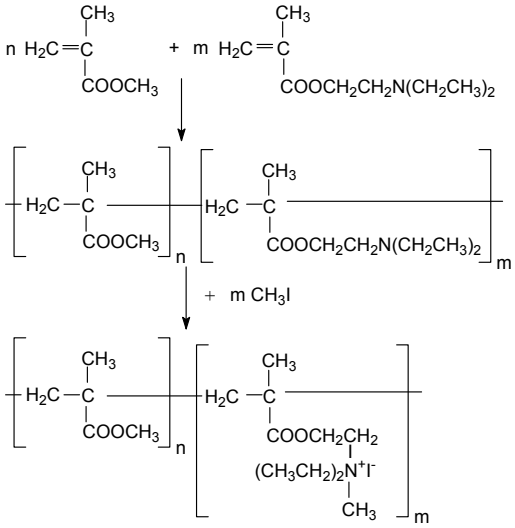


Fig. 3. Reaction scheme for MMA-DEAEMA derivatives obtaining

g. Curing of the DEAEMA-MMA-based cements

0.4046 g quaternized MMA-DEAEMA copolymer (method A) were homogenized by dry mixing with 0.0054 g BP, thus forming the "solid" component of the cement. The liquid component was a mixture of MMA and DMPT (0.004 ml). The "solid"-liquid components ratio was 2:1 (g/g). The powder was placed in a mould and then the liquid was poured in. The curing occurred after 5-10 min, at room temperature.

A similar procedure was used for the MMA-DEAEMA copolymer (non-quaternized) (method B). After curing, the cements were immersed in 0.01 ml MI for 24 hrs in order to induce surface quaternization. After the reaction, the cement was washed with DMF in order to remove the unreacted MI, and then dried.

h. Testing of the cytotoxicity of cured cements

Cements biocompatibility was assessed through cytotoxicity tests according to standards (ISO 10993-5, "Tests for Cytotoxicity - *In vitro* methods"). In this respect, murine fibroblast L929 cell line was used. Cells were cultivated in monolayer, and then they were placed in contact with an extract material within the culture medium. In this way, the cement was kept in the culture medium in standard conditions (37°C, 5% CO_2, 24–48 h). The culture medium was then put into contact with the cells monolayer, thus replacing the medium where the cells had grown. The cell culture was kept in permanent contact with fresh medium, which contained all the substances eluted from the tested material. Cell culture was then incubated in a thermostat at 37°C, in the presence of 5% CO_2, and examined periodically for 3 days. After incubation, the presence of cytotoxicity effects around and beneath the material was microscopically examined with a fluorescence inversion microscope (60%) (Zeiss, Axiovert 135, Germany).

i. Testing of the cellular adherence of the cured cements

The samples were sterilized in 70% ethanol for 30 min. and then dried in the clean cabinet. Fresh overnight culture of *Staphylococcus aureus* was diluted to 1000 or 10000 colony forming units (c.f.u.)/ml. The polymers were immersed separately in the solution thus prepared *S. aureus* suspension (200 ml). After 2 h incubation at room temperature, one aliquot of bacteria suspension was spread onto LB plate to determine the bacteria number. The reduction rate in bacteria number was calculated by dividing the bacteria number of the treated sample by that of non-treated one.

3. Results and discussion

a. Copolymer composition for the MMA-CLEMA systems

The reactivity ratios for the MMA (1) – CLEMA (2) system are [13]: r_1=0.51, r_2=0.48. Based on these values, the PROCOP [14] software was used to compute the overall copolymer composition for a final conversion of 70%. For composition I (10:1 molar ratio MMA-CLEMA) the final copolymer composition is 10.99% CLEMA and 89.01% MMA (molar ratios), almost identical with initial composition of the monomer mixture. Moreover, since the two reactivity ratios have very close values, the copolymer composition does not vary significantly with the conversion (which permits the use of the above composition in further computations even allowing for a certain level of experimental error in measuring the final conversion of the copolymerisation process).

For the second composition (5:1 molar ratio MMA-CLEMA) the cumulative composition of the copolymer is 19.92% CLEMA and 80.08% MMA, again having only a slight variation versus the conversion.

b. Iodination conversion for the CLEMA-MMA copolymers

This intermediate stage was performed in order to substitute the chlorine atom in the CLEMA monomer units with iodide, which is more reactive in the quaternization process. The conversion was measured by gravimetric method, based on the weighting of the modified copolymer and of the reaction product (sodium chloride). For both copolymer compositions, the iodination degree was found to be between 95 and 97%.

c. Quaternization kinetics for the iodinated CLEMA-MMA copolymers

The unreacted TEA was titrated with a 0.2N HCl solution in 1:1 vol. isopropanol/ethylenglycol mixture, using a 0.05 % methyl red in ethanol solution as indicator. Before titration, the sample was diluted in a chloroform:propyleneglycol (1:1 vol.) mixture. The variation of the amino groups' concentration versus time is illustrated in figure 4.

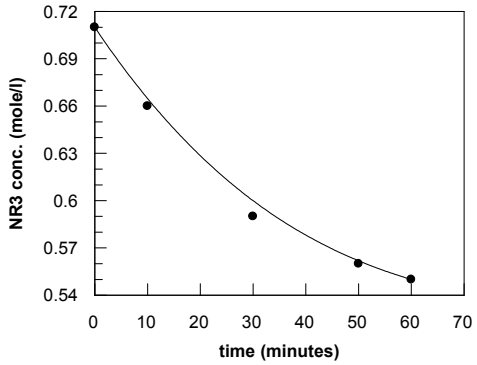


Fig. 4. Variation of the concentration of the amino groups with time during the reaction of quaternization of the iodinated CLEMA-MMA copolymers.

The results were used in order to compute the concentration of the reacted amino groups. Considering a first order kinetics:

$$\frac{dx}{dt} = k(a-x)(b-x) \qquad (1)$$

where ***x*** is the concentration of the reacted amino groups, ***a*** and ***b*** are the initial concentrations of the amino, respectively iodine groups, and ***k*** is the reaction rate constant.

By integration for a reaction time *t*, (1) becomes:

$$\frac{1}{a-b}\ln\frac{b(a-x)}{a(b-x)} = kt = B \qquad (2)$$

From the ***B*** versus time dependency (figure 5) the value of the rate constant was obtained, k = 0.0082 l/mole·min; for the quaternization of the 5:1 MMA-CLEMA iodinated copolymer. Since the experimental results tend to be disposed along a curve and not a straight line, the above constant should be considered as an average value.

d. Copolymer composition for the MMA-DEAEMA systems

The reactivity ratios for the MMA (1) - DEAEMA (2) system are [15]: r_1=1.1, r_2=0.907. The values, very close to each other and approaching the unit, indicate that the copolymerisation can be considered as ideal and azeotropic, having a copolymer composition practically identical with the composition of the monomer mixture and independent of the conversion. Runs of the PROCOP [14] software confirmed the assumption.

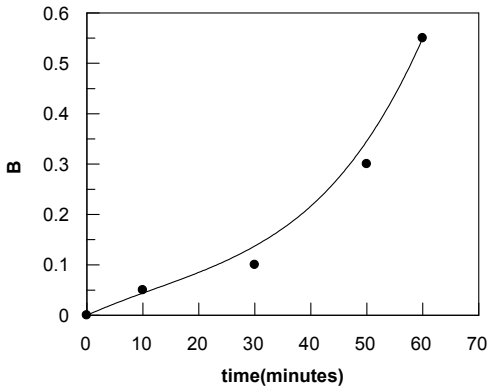


Fig. 5. Data used for computing the reaction rate constant for the quaternization of the CLEMA-MMA iodinated copolymer.

e. Kinetics of the quaternization reaction for the DEAEMA-MMA copolymers

The unreacted amino groups in the DEAEMA-MMA copolymers were titrated with an HCl solution, following a similar procedure with the one used for CLEMA-MMA products. The variation of the amino groups concentration versus time is given in figure 6.

The data were used to compute the kinetic rate constants, according to relation (2). The dependency *B=f(t)* for the 10:1 and respectively 5:1 molar MMA-DEAEMA copolymers are given in figure 7.

The rate constants obtained were:

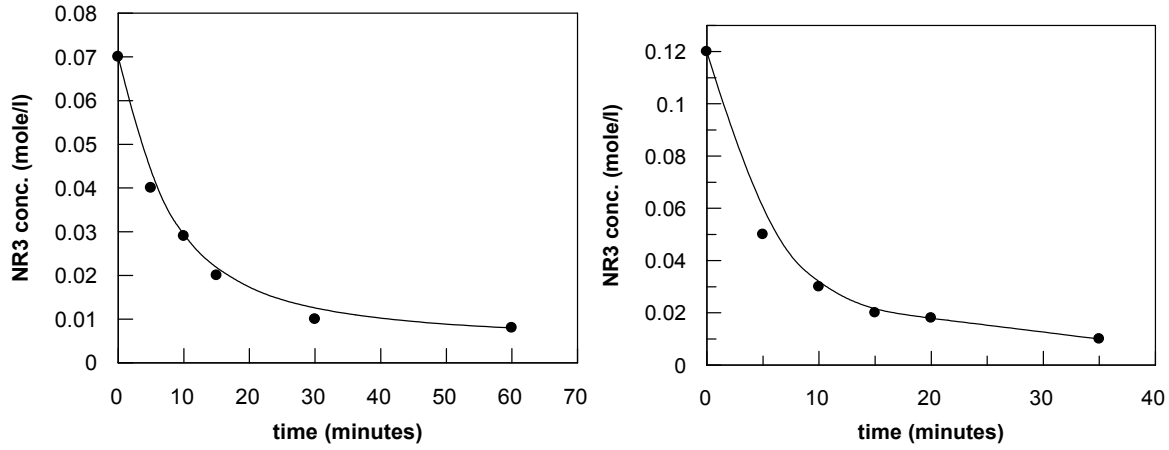
k_1 = 0.303 l/mol·min for the 10:1 mol MMA-DEAEMA copolymer;

k_2 = 0.332 l/mol·min for the 5:1 mol MMA-DEAEMA copolymer.

The differences between the above values and the slight deviation of the points from a straight line in figure 7 are due to experimental errors. The rate constants' values obtained for the quaternization of the MMA-DEAEMA copolymer are two orders of magnitude higher as compared to the quaternization of the MMA-CLEMA products. The difference in the reactivity can be explained by the better penetration of the reacting agent, with a smaller volume, inside the molecular coil, in the case of the MMA-DEAEMA quaternization.

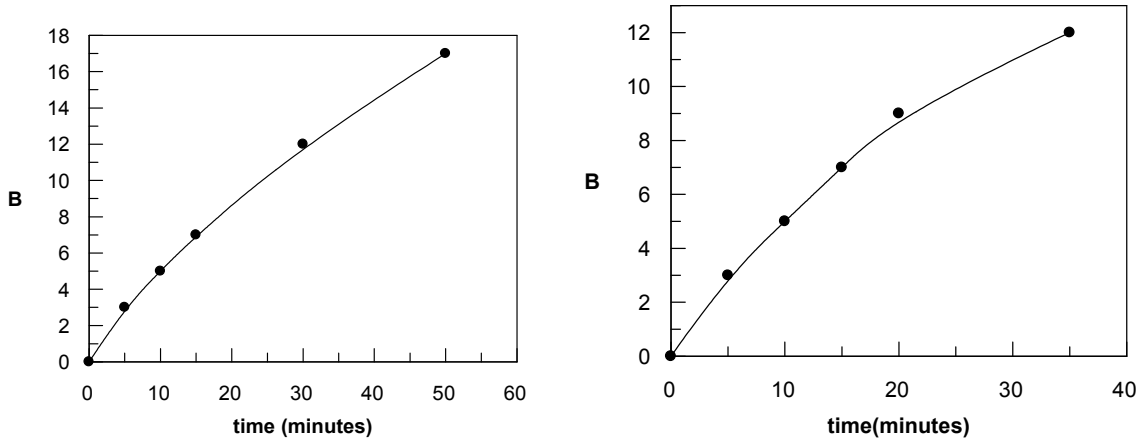
d. Results of the cytotoxicity tests

After incubation, the cells where microscopically examined for detecting visible signs of cytotoxicity such as shape changes, membrane disruption (cellular lysis) or modification of the aspects and dimensions of the cell's components. No such changes were noticed (figure 8) and therefore the synthesized compounds (regardless of the synthesis route followed) can be considered non-toxic.



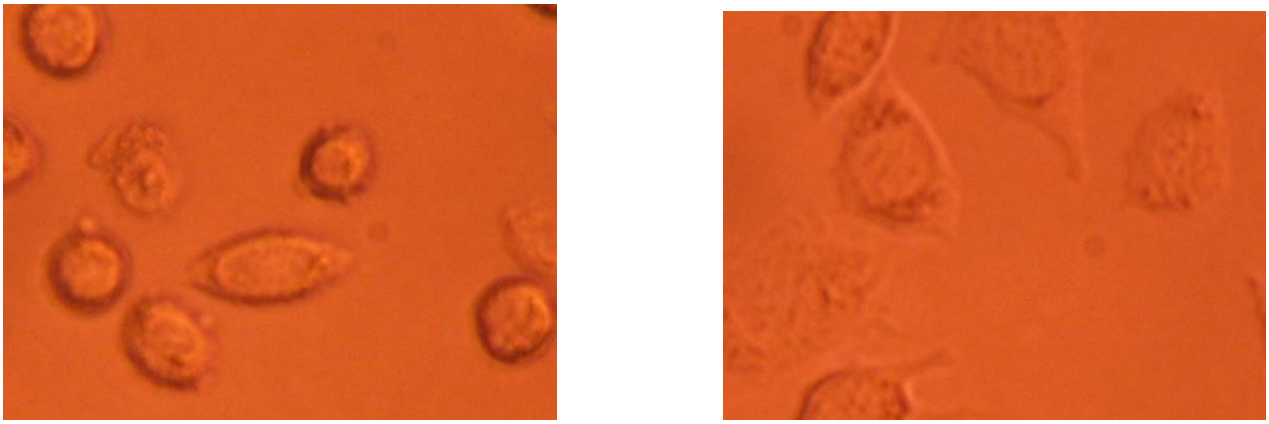
a) *b)*

Fig. 6. Variation of the amino groups' concentration versus time for the quaternization of the MMA-DEAEMA copolymers. a) 10:1 mol. MMA-DEAEMA; b) 5:1 mol. MMA-DEAEMA..

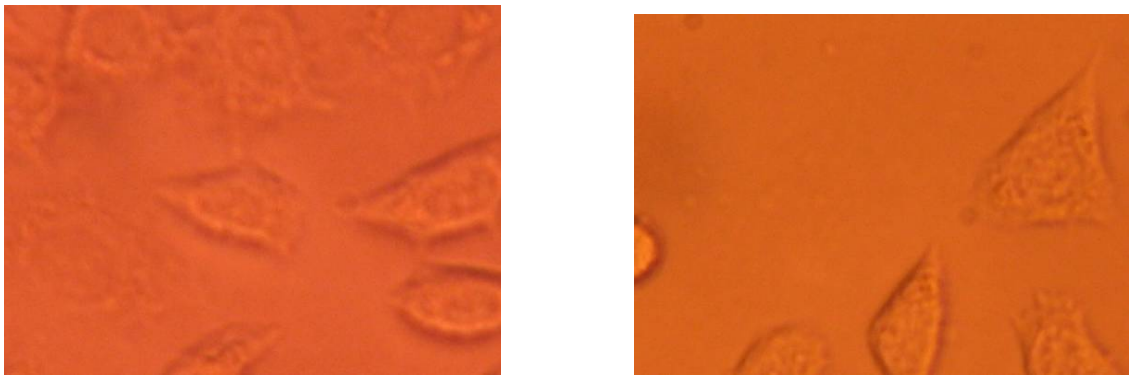


a) *b)*

Fig. 7. Data used for computing the reaction rate constants for the quaternization of the MMA - DEAEMA copolymers: a) 10:1 mol. MMA-DEAEMA; b) 5:1 mol. MMA-DEAEMA..



a) 10:1 mol. MMA-CLEMA -method A b) 10:1 mol. MMA-CLEMA - method B



c) 5:1 mol. MMA-CLEMA - method B d) 5:1 mol. MMA-DEAEMA - method B

Fig. 8. Results of the cytotoxicity tests.

e. Results of the cellular adherence tests

The copolymers were reported to inhibit the growth of bacteria through binding of their positively charged amino groups to negatively charged bacterial cell wall. Therefore, the copolymers reduced the number of bacteria attained by immersion into a bacteria suspension. The reduction rate of *S. aureus* number after 2 hours culture contact with the copolymers is shown in table 1.

Table 1. Staphylococcus aureus reduction rate

Copolymers	**Reduction rate (%)** ***Staphylococcus aureus***
MMA-CLEMA (10:1 mol.)-method A	80
MMA-CLEMA (10:1 mol.)-method B	88
MMA-CLEMA (5:1 mol.)-method B	93
MMA-DEAEMA (5:1 mol.)-method B	91

4. Conclusions

The synthesis of two polymers with cationic groups has been performed in order to obtain biocompatible materials with antimicrobial properties, which find use in dental surgery and dental prosthetics. For the polymer analogous reactions some kinetic parameters have been determined. The products have shown no cytotoxicity and low bacterial adherence.

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