

Polymeric biocompatible structures for controlled drug release

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The purpose of using polymers in drug delivery systems is to be able to create a membrane-like barrier that would control the delivery of the active agent for an extended period. Poly (2-hydroxyethyl methacrylate) (pHEMA), when cross-linked, swells to a significant extent. Hence, it was thought necessary to determine its degree of swelling when incorporating methyl methacrylate (MMA), which is a hydrophobic monomer. The polymeric micro-beads of p(HEMA) and functionalised biopolymers of p(HEMA-co-MMA), p(HEMA-co-AA) and p(HEMA-co-MMA-co-AA) were prepared by precipitant polymerisation method. There were obtained different types of micro-polymeric beads, which were characterised by elemental analysis, measure of the degree of swelling, scanning electron microscopy (SEM), and *in vitro* tests. All these tests gave satisfactory results.

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1. Introduction

The goal of controlled drug delivery is to provide a specified drug concentration within the body for an extended period of time. A device that provides a sustained release of drug can maintain desired drug concentrations in the blood with reduced number of doses, while also minimising the concern of undesirable, sometimes toxic, side effects [1-4]. Controlled release is, also, a more cost-effective way of delivering expensive medication. With less drug wasted, costs can be reduced. The first design concepts for controlled release were passive delivery systems.

In passive delivery, unassisted diffusion of solvent and solute is the only means of modulating the rate of drug delivery. Typically, there is a depot of drug contained within a polymer matrix which releases over time. However, this is just a subset of the actual goal of controlled release. The primary aim of controlled drug delivery is complete optimisation therapeutic delivery; that is the ability to deliver to the desired location, a precise dose for a finite period of time [5-6]. With this ideal system, one could achieve high bioavailability with minimal side effects and drug exposure. To achieve this idealisation, systems must be responsive to fluctuations in the patient's needs [7]. The advantage of implantable drug delivery devices is that they can be designed to meet these aims by providing a means of continually monitored and administered drug delivery [5, 8].

The purpose of the present study was to obtain new copolymers in order to create a membrane-like barrier that would control the delivery of the active agent for an extended period [9]. Poly(2-hydroxyethyl methacrylate)

(pHEMA), when cross-linked, swells to a significant extent [11-13]. Hence, it was thought necessary to determine its degree of swelling when incorporating methyl methacrylate (MMA), which is a hydrophobic monomer that is expected to restrain the swelling of pHEMA [10]. In order to be able to bind drugs, we need functionalised polymers, this being the reason for using the acrylic acid (AA) [14-16].

2. Materials and methods

Materials

MMA (Merck) was purified by *in vacuo* distillation ($T = 47^{\circ}\text{C}$, $p = 100 \text{ mmHg}$). Its comonomers, HEMA and AA (Aldrich), were used as such without further purification. Azo-bis isobutyronitrile (AIBN) was purchased from Merck. Toluene was provided by Chimopar. The micro-beads were obtained by precipitant polymerisation in the presence of AIBN as initiator.

Methods

The polymeric micro-beads of p(HEMA), p(HEMA-co-MMA), p(HEMA-co-AA) and p(HEMA-co-MMA-co-AA) were prepared by precipitant polymerisation method. There were obtained different types of micro-polymeric beads by varying the ratios of the comonomers (Table 1), while keeping the total volume of the solvent in the mixture (9 mL).

Firstly, we mixed the monomer HEMA, respectively the comonomers (HEMA and MMA), (HEMA and AA), (HEMA, MMA and AA), with the initiator AIBN 0.5 % (w/v). The mixtures were then poured into 50 mL three-neck reactors, in order to mix them with the solvent,

toluene, just before the reaction (1/5 v/v ratio comonomers/solvent). Nitrogen was bubbled through the solutions, in order to remove the oxygen. A rich nitrogen atmosphere was enabled for polymerisation to occur.

Table 1. Compositions of the micro-beads.

Polymer	Monomer(s)	Ratio (v/v)
pHEMA	HEMA	-
p(HEMA-co-AA)	HEMA/AA	95/5, 90/10, 85/15, 80/20
p(HEMA-co-MMA)	HEMA/MMA	95/5, 90/10, 85/15, 80/20, 70/30, 60/40
p(HEMA-co-MMA-co-AA)	HEMA/MMA/AA	75/20/5, 70/20/10, 65/20/15, 60/20/20

The reaction was performed in a water-bath at 60-65°C, during 4-5 hours, under mechanical stirring at 400 rpm. The resulting solutions were washed to remove the residual monomer(s) three times with toluene; afterwards, the organic residues were extracted with ethylic ether. The solutions were sieved in the oven at 40°C for 3-4 hours, and then they were exposed to UV light (long wave UV, 360 nm, 12W) for about 5 hours.

The swelling test was carried out in saline solution (9% NaCl (g/L) in distilled water) for three days.

Biocompatibility tests

Polymers biocompatibility was verified by testing the *in vitro* cytotoxicity. It was used a murine fibroblasts L929 cell line, which was cultivated in culture medium (DMEM), supplemented with calf foetal serum and antibiotics. We used two methods: a) direct testing method; b) elution method.

In the first method, the polymers took contact with an adhered culture monolayer of 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 37 °C, in 5% CO₂ atmosphere. The culture medium where the testing material was maintained took contact with cells monolayer, replacing the medium where the cells were raised until that moment. Thus, the cells culture took contact with fresh medium containing all the substances extracted from testing material. The cells culture was afterwards incubated in the oven at 37°C in the presence of 5% CO₂ and was examined daily during three days. In both of the methods, the cells were microscopically examined for detecting cytotoxicity visible signs, cellular lysis or cellular components dimensions and conformation.

For cytotoxicity evaluation, besides microscopically examination, it was also used the cellular viability. For this purpose, after incubation the cells were incubated with a tetrazolium soluble salt (MTT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. This one is converted by dehydrogenases presence in viable cells

mitochondria in insoluble formazan which appears as violet crystals. We added 10 µl of MTT (5 mg/ml in buffer saline phosphate) sterile solution over 100 µl in every microplate sample where cells with testing material were incubated. The microplates were incubated (37°C, 5% CO₂) for 3-4 hours until violet crystals of formazan appeared. For their solubility it was used a buffer prepared as following: for 12.5 ml SDS solution 5% in 50% dimethylformamide there were added 12.5 ml of 80% acetic acid solution containing 2.5% HCl 1N. There were added in every sample 100 µl of lysis buffer, the microplates were incubated for 24 hours at 37°C. Using a microplates' automatic reader (ELISA), the optical density was read at 570 nm. Optical density at 570 nm is proportional with viability. Viable cells percent contained by incubated samples with testing material was evaluated versus a blank test where only cells are incubated.

3. Results and discussion

The different micro-beads compositions prepared with this procedure were characterised by elemental analysis, measure of the degree of swelling, in addition to examination of their topography in terms of homogeneity and distribution of the polymers with scanning electron microscopy (SEM).

Elemental analysis

The p(HEMA-co-MMA-co-AA) terpolymers structures were confirmed by the elemental analysis. The results are given in Table 2. The amount of nitrogen is due to the initiator radicals of AIBN.

Table 2. Elemental analysis results.

Comonomers ratios (v/v)	N (%)	C(%)	H (%)
75:20:5	0.36	54.02	7.21
70:20:10	0.16	54.91	4.51
65:20:15	0.00	52.12	7.20
60:20:20	0.36	53.41	6.96

The formulas of the polymers obtained are given below, in Fig. 1:

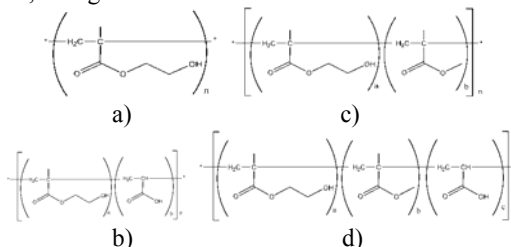


Fig. 1. Polymers obtained: a) p(HEMA); b) p(HEMA-co-AA); c) p(HEMA-co-MMA); d) p(HEMA-co-MMA-co-AA).

Swelling tests

The swelling tests were made on samples containing HEMA and MMA (the ratios given in Table 1), in order to

choose the appropriate ratio of hydrophobic comonomer (Fig. 2).

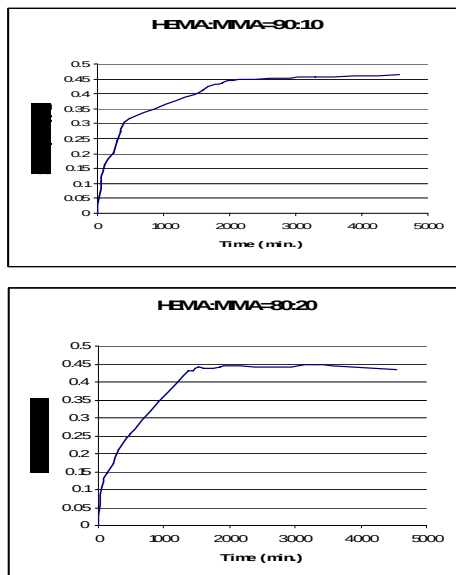


Fig. 2. Swelling rate (g/g) versus time (min.) for *p*(HEMA-co-MMA): a) 90:10; b) 80:20.

After testing the first series of MMA-containing samples, it was concluded that a higher content of MMA would decrease micro-beads swelling. It was decided that choosing a higher MMA:HEMA ratio would be the best approach in the construction of a controlled drug release system because with a lower swelling degree the release rate is expected to be lower.

Scanning electron microscopy

Moreover, from scanning electron microscopy it can be observed that a higher percent of MMA comparative with HEMA implies the micro-beads agglomeration. This is the reason for using a 20% ratio of the MMA in the terpolymer (Fig. 3, Fig. 4, and Fig. 5).

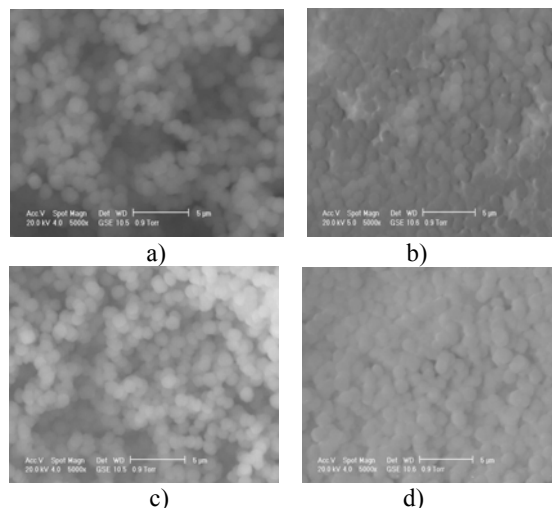


Fig. 3. Micro-beads distribution of: *p*(HEMA) and *p*(HEMA-co-AA): b) HEMA:AA=95:5; c) HEMA:AA=90:10; d) HEMA:AA=85:15.

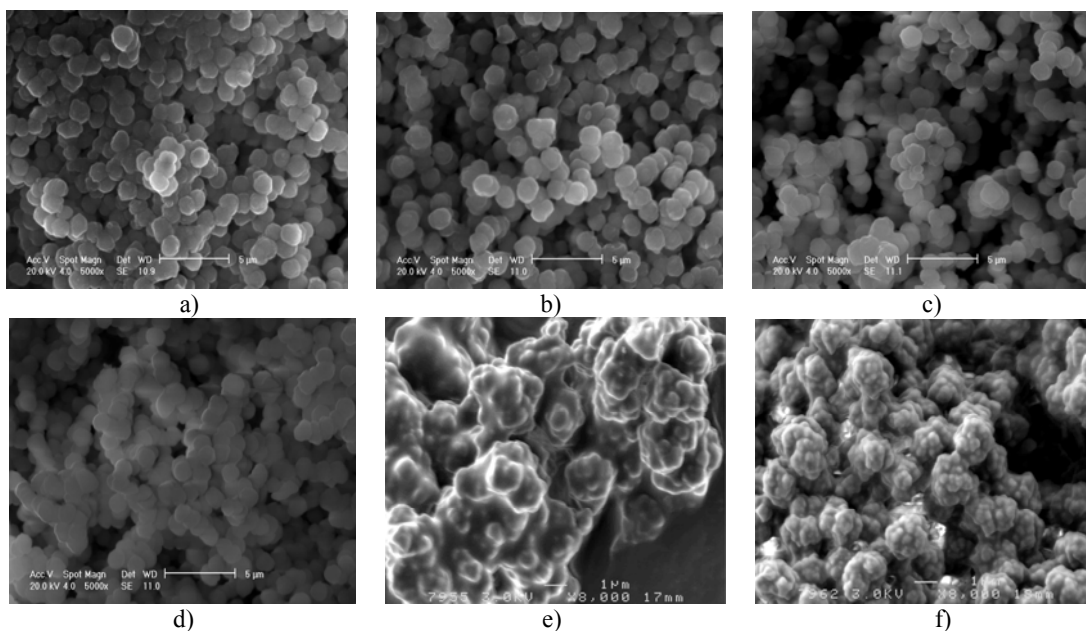


Fig. 4. Micro-beads distribution of *p*(HEMA-co-MMA): a) HEMA:MMA=95:5; b) HEMA:MMA=90:10; c) HEMA:MMA=85:15; d) HEMA:MMA=80:20; e) HEMA:MMA=70:30; f) HEMA:MMA=60:40.

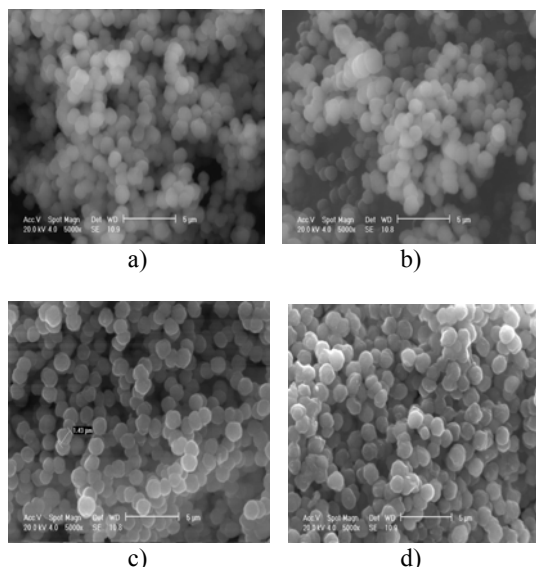


Fig. 5. Micro-beads distribution of *p(HEMA-co-MMA-co-AA)*:

a) HEMA:MMA:AA=75:20:5;

b) HEMA:MMA:AA=70:20:10;

c) HEMA:MMA:AA=65:20:15;

d) HEMA:MMA:AA=60:20:20.

SEM microphotographs gave adequately results for all final compositions obtained (Fig. 5) and relevant from dimensionally point of view (1.3-1.5 μm).

In vitro tests

The *in vitro* tests of cytotoxicity and viability made on fibroblasts murine L929 line cell gave adequately results for all compositions obtained (Fig. 6).

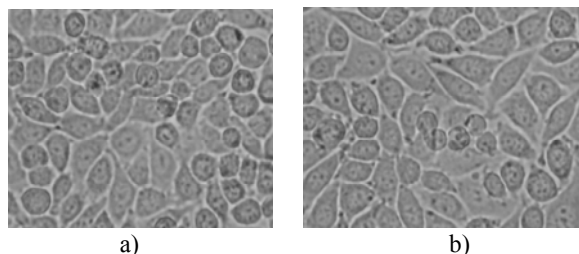


Fig. 6. Murine fibroblast L929 cell line in *p(HEMA-co-MMA-co-AA)*: a) 70:20:10; b) 60:20:20.

4. Conclusions

Swelling of micro-beads is a highly important property. It was concluded that choosing a higher MMA:HEMA ratio would be the best approach in the construction of a controlled drug release system. From the swelling tests we decided that a high content of MMA

decreases the micro-bead swelling rate, and also it decreases more, but in adequate parameters for the use in drug delivery systems, when we used both MMA and AA.

From the SEM spectra, we obtained homogeneously distributed micro-beads with the diameter of 1.3-1.5 μm . The *in vitro* tests performed gave satisfactorily results.

In vitro tests show that all the compositions used are biocompatible and non-toxic for the cells.

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