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# New hema-based polymeric microbeads for drug delivery systems

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The polymeric containers (micro or nano) reached a great influence after the development of new information referring to a new concept in the solid tumour biology: EPR effect - enhanced permeability and retention. Taking into account this concept, based on clinical and biochemical results, the solid tumours (including cancer) grow faster than the adjacent tissues, because of a better vascularization and an enhanced permeability, and, as a result, the vascularization architecture presents deficiencies. The enhanced vascular permeability is also observed in granuloma and inflammatory and infected tissues. A better retention for the nano and microparticles is observed. A very disturbing aspect, but very useful in anti-tumour treatment, is the retention tendency of the polymeric beads emphasized in the tumour against other tissues or organs. The aim of the present study was to obtain some microbeads based on functionalised HEMA polymers using the dispersive polymerisation. We expect that conjugation with some specific drugs will increase the accumulation of these microbeads within the tumours and will decrease the drug toxicity for other organs. We characterised the microbeads obtained by FTIR, SEM and FOM. We studied their *in vivo* distribution. We found an equal distribution into the healthy organs.

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***Keywords:*** Enhanced permeability and retention, Microbeads, Drug delivery systems, 2-hydroxyethyl methacrylate

## 1. Introduction

The biopolymers involvement in the cellular metabolism and the possibility of a certain structural, electronic and sterical physiological effect achievement between the drug and the macromolecular structure explain the use of polymeric compounds in therapeutics.

Macromolecular compounds, characterised by a functional optimal combination meant to satisfy the enormous amount of biocompatibility, solubility, biological pH, catalytically stimulation or low toxicity requests, might offer valuable solutions.

Without elucidating all the biological active polymers mechanisms and improved technologies, without a great number of substances disposals, clinical experimental results show the great value of some natural and synthetic macromolecular compounds as drugs and as drug conditioning additives. In this way, polymer utilisation in pharmacology leads to decreased toxicity, better physiological effects, controlled position effects etc. [1].

There were also recorded some remarkable results in the polymer synthesis with biopolymer-like structure, as polypeptides, polynucleotide, thiol- and imidazol-containing polymers, showing different physiological effects [2, 3].

Micro and nanotechnologies and micro and nanostructured materials develop applications in different areas of interest, the most important being in medicine and biology, the drug delivery systems in the damaged tissue or organ.

It is known that the smallest diameter of a capillary is between 4 and 10 μm, that of the cells is between 10 and 30 μm and that of the intracellular particles (e.g., the liposomes, having the decomposition function for the particles leaded by endocytosis to the cell) are in the nanometric domain.

We proposed and realised some micropolymeric beads as containers for the physically or chemically bound drugs, in order to obtain the drug orientation to certain organs. When these particles reach the damaged tissue or organ, the drugs are slowly released at a certain rate by diffusion or splitting (enzymatically or chemically).

An area of interest is that of the ligatures functionalised nanocontainers for the cellular receptors [4, 5]. A polymeric particle containing a drug will be surface functionalised by a ligature attached to a specific surface receptor, followed by endocytosis. The proof of the nanocontainers endocytosis is given by the introduction of a fluorescent dye into the polymeric particle and the observation of the cell fluorescence after the nanocontainers endocytosis [5].

In the experimental part of this work, we present the polymeric microbeads synthesis by dispersive polymerisation from functionalised biocompatible polymers. 2-hydroxyethyl methacrylate-based compounds are both biocompatible and biodegradable (very well tolerated by the human organism) [6]. We expect that the conjugation with some specific drugs will increase the accumulation of these microbeads into the tumours and will decrease the drug toxicity for the other organs.

## 2. Experimental

**Materials.** We employed 2-hydroxyethyl methacrylate (HEMA), methacryloyl oxyethyl-phosphate (MOEP), azo-bis-isobutyronitrile (AIBN), 2-butanol, ethylenglycol dimethacrylate (EGDMA), ethyl-eosin, all these reagents being purchased from Sigma-Aldrich (St. Quentin Fallavier, France), 2-methacrylic acid 3-guanidinopropyl ester (GuaMA) (obtained cf. [7, 8]), toluene and ethylic ether from Chimopar. Polybutadiene was supplied from ICECHIM.

We used as emulsifiers Brij 35, Tween 60, Tween 80 and sodium dodecyl sulfate (DDSNa), all being purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

The initiator (AIBN) was purified by recrystallisation from a mixture methanol-chloroform (1/1 v/v) at 40 $^{o}$C.

**Synthesis of the polymers.** We introduced into the reactor a 0.5% (w/v) solution of polybutadiene (5% (w/w) vs. monomer) in toluene. At 40 $^{o}$C under stirring, we slowly introduced the 2-butanol (we chose ratio variations around 55/45 vs. toluene [9]) (solvent non-solvent solution). Separately, we prepared a solution containing the monomer/s (MOEP and GuaMA, respectively, 5 and 10% (w/w) vs. HEMA), the initiator (AIBN) (0.4/20 (w/w) vs. monomer), the cross-linking agent (EGDMA) (4% (w/w) vs. monomer), the fluorescent dye (ethyl-eosin) (0.25% (w/w) vs. monomer). We added dropwise this second solution to the first solution, magnetically stirred, increasing the temperature to 60-65 $^{o}$C and the stirring rate to 1000 rpm. Polymerisations were performed under nitrogen atmosphere. An optimal result of the reaction was noticed after 6.5-7 hours.

The homopolymer and the copolymers obtained were washed twice with toluene and with ethylic ether, in order to remove any traces of the unreacted monomer or other organic residue with low molecular weight. The microbeads were then dried at 37-38 $^{o}$C for 24 hours and sieved. For the dispersion of the microbeads in water, we tried several emulsifiers: Brij 35, Tween 60, Tween 80 and DDSNa.

**Characterisation of the microbeads.** In order to characterise the microbeads obtained by dispersive polymerisation, we used Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Fluorescence Optic Microscopy (FOM). FTIR analysis was performed with a Schimadzu spectrophotometer. All samples were mixed and ground with spectroscopic grade potassium bromide prior to being placed in the sample cell, and the diffuse reflectance spectra were scanned over the range of 400-4000 cm$^{-1}$. SEM was performed on a Philips XL30 - ESEM Turbo Molecular Pump (TMP). The samples were first carbon-coated. Fluorescence imaging was carried with a Zeiss fluorescence microscope.

***In vivo* tests.** The research was approved by the University Animal Care Committee. We used Wistar rats (18-19 weeks old, from Charles River, Cléon, France, 300 ± 52 g weight), conditioned to local vivarium for two weeks (24 $^{O}$C and 12h/12h light/dark cycles). The animals received standard test food (UAR, Villemoison sur Orge, France) and water *ad libitum*.

For the injection into rats, we used a 0.005 g/ml emulsifying agent concentration. We followed the recipe: carboxymethyl-cellulose 1.25%, mannitol 4%, normal saline solution (9 g/l NaCl diluted in distilled water) 93.75%. For an optimal use, we diluted 10 times this solution. All the reagents used had pharmaceutical purity.

## 3. Results and discussion

Microdishes are drug containers with larger dimension (1-500 μm) and consist in a polymeric membrane containing the drug. These materials are used in several fields, such as pharmaceutics, agricultural science, agricultural chemistry and textiles. There are three micropackage methods [10, 11, 12]: chemical, physical-chemical and mechanical methods.

In the experimental part of this work, the polymeric microbeads were obtained using the chemical method.

These micropolymeric containers reached a great importance after obtaining new information concerning a new concept on the solid tumour biology: EPR effect - enhanced permeability and retention [13, 14, 15]. This concept is based on clinical and biochemical results; the solid tumours (including the cancerous ones) develop faster than the adjacent tissues because of a thicker vascularization with higher permeability. We are also able to see a diminished lymphatic drainage and an important permeability generation: bradykinin, nitric oxide (NO), peroxynitrite ($ONOO^{-}$), prostaglandins, metalloproteinase etc.

A very surprising aspect, and so very useful in anti-tumour treatment, is the tumour higher retention tendency than other tissues or organs [16].

These phenomena lead to polymeric drugs or polymeric drug containers controlled release and tumour higher retention, slowly released [17]. In this way, we avoid the general toxic effect of drugs, especially that of the anticancer.

It has been observed the EPR effect not only in solid tumours, but also in the case of granuloma and different tissue inflammation. In this case, macromolecules are slowly released through the lymphatic system [13].

The tumour or inflammatory specific polymer retention can be used for diagnose. Binding nano or microbeads with radio-opaque elements (barium, iodine etc.), scintigraphic elements (gallium) or magnetic resonance analysis allow finding better diagnose methods [15].

Nano or micropolymeric beads are usually not soluble in the living bodies' plasma. They can swell and liberate drugs diffusively or by enzymatic splitting.

The polymeric microbeads were synthesised by dispersive polymerisation from functionalised biocompatible polymers.

We know that, in the case of the dispersive polymerisation, the solvent influences the dimension and polydispersity degree of the beads. Even a very small

variation of this ratio changes significantly the diameter of the beads. We were able to control them varying the solvent/non-solvent ratio.

We present the results obtained in the following graphics, as polymers diameter distribution (Fig. 1).

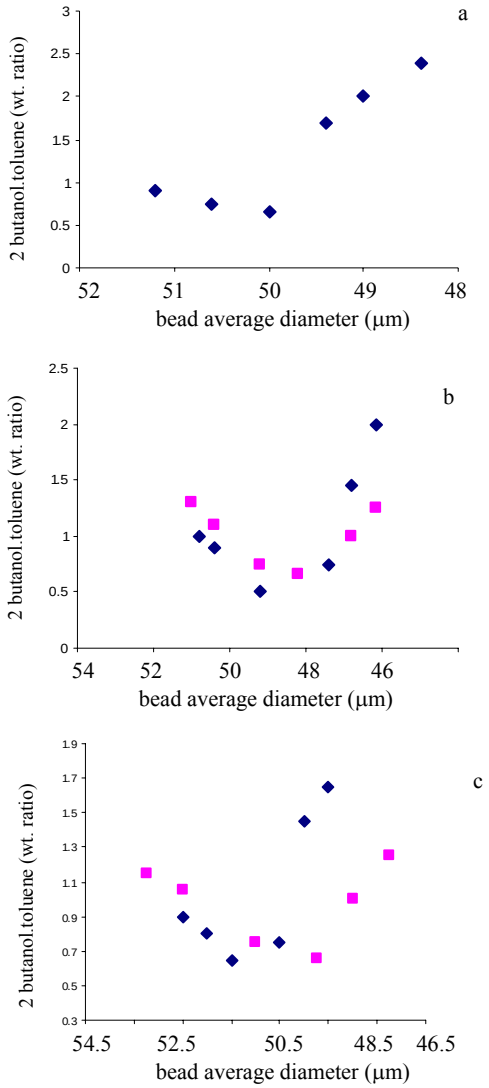



*Fig. 1. 2-butanol/toluene weight ratio versus beads average diameter (μm) for: a) pHEMA; b) p(HEMA-co-MOEP), ♦ - 5% MOEP, ■ - 10% MOEP; c) p(HEMA-co-GuaMA), ♦ - 5% GuaMA, ■ - 10% GuaMA. ( 2-butanol ratio grows ← and toluene ratio, →).*

Concerning the fact seen from experience that the beads diameter grows with the 2-butanol ratio, we found an optimal ratio in order to obtain uniform microbeads.

We obtained polymeric microbeads of pHEMA, p(HEMA-co-MOEP) and p(HEMA-co-GuaMA), in accordance with the FTIR spectra obtained.

FTIR spectra show specific absorption bands of polymer functions: pHEMA (3435, 2989, 1733, 1267, 1164 $cm^{-1}$), p(HEMA-co-MOEP) (3488, 3100, 2924, 2864, 1742, 1374, 1168, 802, 685 $cm^{-1}$), p(HEMA-co-GuaMA) (3410, 3161, 2947, 2885, 1728, 1666, 1454, 1280, 1159, 1074, 748 $cm^{-1}$) (spectra not shown).

SEM microphotographs give the beads dimensions (0.5 - 2 μm) and lead to the conclusion that they are homogeneously distributed (Figs. 2 to 4).

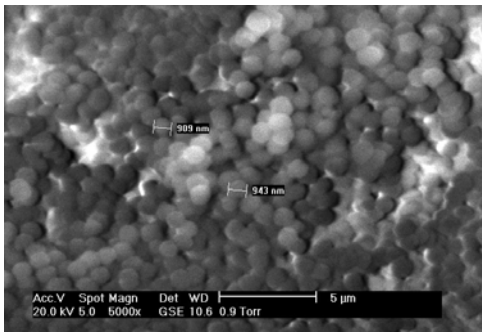



*Fig. 2. SEM for pHEMA microbeads obtained using 54.5/45.5 2-butanol/toluene ratio.*

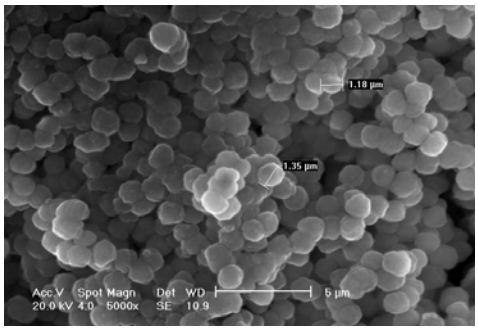



*Fig. 3. SEM for p(HEMA-co-MOEP) microbeads obtained using 54.5/45.5 2-butanol/toluene ratio.*

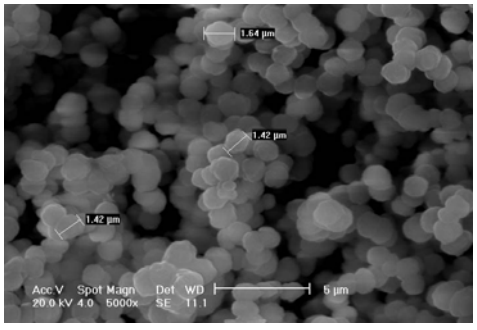



*Fig. 4. SEM for p(HEMA-co-GuaMA) microbeads obtained using 54.5/45.5 2-butanol/toluene ratio.*

*In vivo* tests results. Organs distribution analysis

Among the emulsifiers used for the dispersion of the microbeads in water, in order to inject them into rats, only Tween 80 performed satisfactorily.

The FOM gives the microbeads localisation and the distribution into the main organs. The analysis leaded to

the conclusion that the polymeric particles are homogeneously distributed into the injected damaged organs: brain, lung and spleen (Figs. 5 to 8).

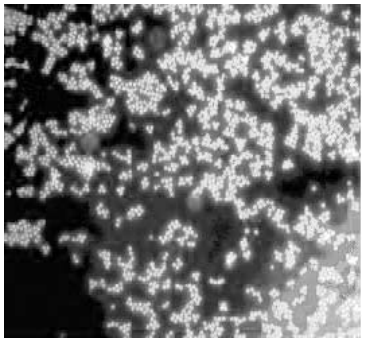


*Fig. 5. FOM for pHEMA beads containing ethyl-eosin.*

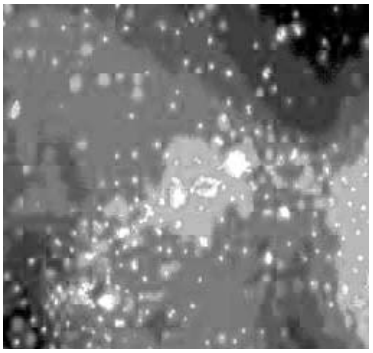
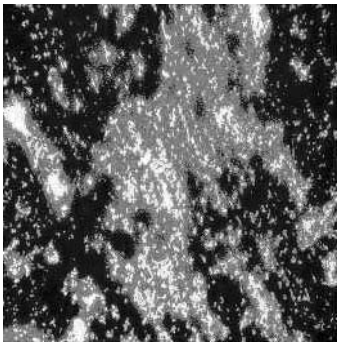


*Fig. 6. FOM for p(HEMA-co-MOEP) beads containing ethyl-eosin.*



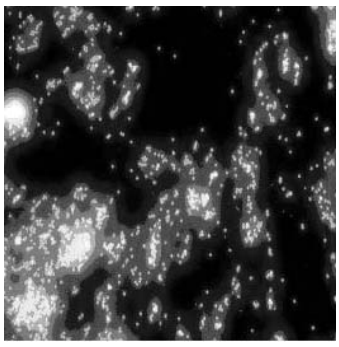
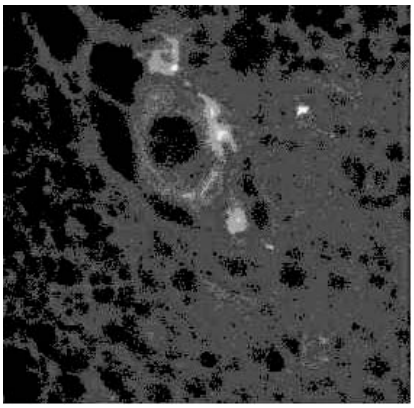
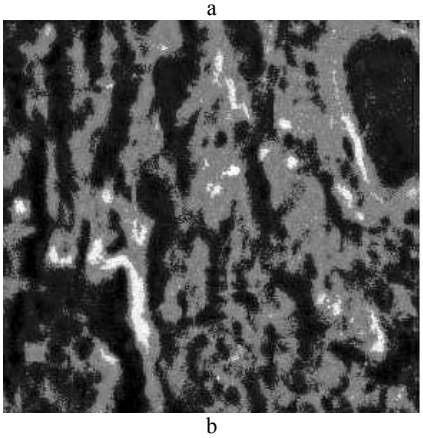


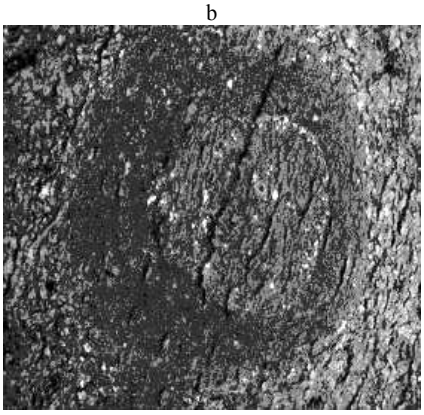
*Fig. 7. FOM for p(HEMA-co-GuaMA) beads containing ethyl-eosin.*



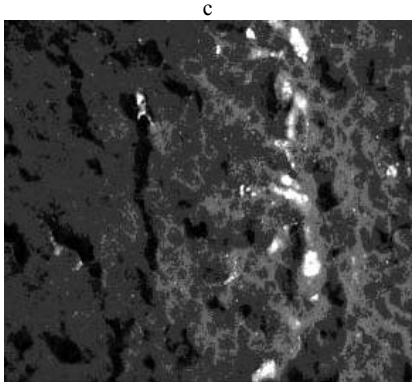
a



b



c



d

*Fig. 8. FOM for microbeads injected in: a) brain; b) lung; c), d) spleen.*

## 4. Conclusions

Several conclusions can be drawn based on the obtained experimental data:

1. We obtained beads of pHEMA and new copolymers, p(HEMA-co-MOEP) and p(HEMA-co-GuaMA), 0.5 - 2 μm, by dispersive polymerisation.

2. The optimal polymerisation conditions were established for the synthesized copolymers.

3. *In vivo* tests shown that the bead distribution approaches the main organs, do not block the capillary ($d_{capillary}$ = 5 - 7 μm) and do not affect other cells or tissues. These microbeads are expected to be used as injectable controlled drug release (see EPR effect) after drug binding to microbeads and afterwards traceable agents. This expectation is based on the combined physical properties of the copolymers, their non-cytotoxicity and their cell sufficient anchoring in the soft tissues of the implantation site.

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