

## Hydrogel microspheres from crosslinked poly(methyl methacrylate): synthesis and biocompatibility studies

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**Abstract.** Smooth, perfectly spherical, highly hydrophilic microspheres have been prepared from crosslinked poly(methyl methacrylate) microspheres by alkaline hydrolysis in ethylene glycol at elevated temperatures. These microspheres absorb varying quantities of water depending upon the extent of hydrolysis. Subcutaneous implantation studies on rabbits demonstrated that the microspheres are biocompatible. Implantation studies in the renal arteries of dogs demonstrated the occlusion effect produced by the microspheres. Microspheres could be made radiopaque by the incorporation of barium sulphate. Potential uses envisaged for these microspheres in the biomedical area are that of artificial emboli for endovascular embolization and as microcarriers for the growth and propagation of anchorage dependent mammalian cells.

**Keywords.** Microspheres; artificial emboli; embolization; biocompatibility; hydrolysed PMMA beads; endovascular occlusion.

### 1. Introduction

Hydrogels have been described as biocompatible materials since their physical properties resemble that of living tissue because of their high water content, softness and flexibility. Hydrogels have been found to be more biocompatible than many other class of synthetic high polymers (Ratner and Hoffman 1976). Since Wichterle and Lim (1960) first reported on the use of poly(2-hydroxyethyl methacrylate)/ (PHEMA) hydrogels for biomedical applications, considerable amount of research has been done on the development of new hydrogel materials for medical applications (Andrade 1976; Goldberg and Nakajima 1980; Peppas 1987). Functional hydrogel microspheres find extensive applications in controlled release, in immunology, for the immobilization of enzymes, in immunochemical studies, in the culture and propagation of mammalian cells and in gel permeation chromatography (Rembaum *et al* 1976; Allen *et al* 1980; Palacky *et al* 1981; Feder and Tolbert 1983; Sugii *et al* 1986; Vetvicka and Fornusek 1987). Recently hydrogel microspheres based on PHEMA have been used as artificial emboli for the occlusion of blood vessels (Horak *et al* 1986a, b, 1987).

Suspension polymerization is the usual technique to produce polymeric microspheres of large size. The method works very well in the case of hydrophobic monomers such as methyl methacrylate (MMA) which are fairly insoluble in aqueous media. With hydrophilic monomers such as methacrylic acid (MA) or 2-hydroxy-

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ethyl methacrylate (HEMA), which are highly soluble in water, an inverse phase suspension polymerization has to be employed using organic solvents which are immiscible with the monomer. Special surfactants are to be employed in such cases and it often necessitates the development of suitable surfactant systems to effectively stabilize the monomer droplets (Horak *et al* 1986). Suspension polymerization of hydrophilic monomers such as HEMA has also been attempted in highly concentrated solutions of sodium chloride or magnesium chloride using inorganic stabilizing agents (Munzer and Trommsdorff 1977). Beads obtained using the above techniques are usually not perfect spheres, are often sandy, and sometimes agglomerate and cake during the reaction. In this paper we report a unique procedure to prepare smooth, perfectly spherical hydrogel microspheres from crosslinked PMMA beads by alkaline hydrolysis in ethylene glycol (EG) at elevated temperatures. Polyacrylates have been subjected to alkaline hydrolysis by previous workers (Sandler and Karo 1977). Polyacrylates undergo the hydrolysis with relative ease whereas the methacrylates are hydrolysed with difficulty. The hydrolysis of PMMA using 30–50% alkali metal hydroxide in aqueous mixtures of cyclohexanol is the subject of a recent German patent (John *et al* 1986). Till recently all attempts to hydrolyse PMMA into poly(methacrylic acid) or its alkaline salt were usually confined to the uncrosslinked polymer resulting in low degrees of hydrolysis even with prolonged reaction times. No attempt has so far been made on crosslinked PMMA with the idea of generating hydrophilic microspheres containing carboxyl functions with a very high degree of swelling.

The microspheres have been prepared with the immediate objective of using them as artificial emboli in endovascular embolization by the Department of Radiology of this institute. Embolization is a therapeutic method to block blood vessels supplying a given pathological area (Benoit and Puisieux 1986). The method usually employed is the femoral pathway enabling hyperselective catheterization. Various types of emboli have been used. These include autologous blood clots, fragments of muscle and tissue, collagen sponges, oxidized cellulose, poly(vinyl alcohol), barium sulphate suspensions, alkyl cyanoacrylates, silicone prepolymers and sometimes even lead and stainless steel balls (Benoit and Puisieux 1986). Each kind of embolus has its own advantages and disadvantages. Nevertheless, it has been observed that in the case of particulate emboli a smooth spherical shape, high hydrophilicity and excellent particle size control are most desirable for the transcatheteral introduction of the emboli (Horak *et al* 1986). A porous structure contributes to the anchoring of the particles to the vascular lumen more permanently. The investigations reported in this paper demonstrate that microspheres derived from crosslinked PMMA by alkaline hydrolysis satisfy most of the requirements of particulate emboli.

## 2. Experimental

### 2.1 Preparation of microspheres

Crosslinked PMMA beads were prepared by a modified suspension polymerization (Hart 1977). Ethylene glycol dimethacrylate/(EGDM) (Aldrich Chemical Co., USA) was used as the crosslinking agent. Beads of various sizes were separated by using test sieves. The microspheres were then subjected to alkaline hydrolysis by treating them with KOH in ethylene glycol (EG) at 180–183°C. After hydrolysis the beads

were washed for several days in distilled water free of impurities and air dried. The equilibrium water contents (EWC) of the beads were estimated from the weights of the swollen and dry beads using the relation,

$$\text{EWC(\%)} = 100 \times \frac{(\text{Wt. of swollen beads}) - (\text{Wt. of dry beads})}{(\text{Wt. of swollen beads})}$$

## 2.2 *In vitro* haemolytic potential of the hydrogel microspheres

The *in vitro* haemolytic potential of the hydrogel beads was determined by a modified procedure (O'Leary and Guess 1968). The objective of this was to assess whether the microspheres contained some leachable ingredients that could cause damage to rabbit red blood cells. 100 mg in triplicate of the sample was added to 25.0 ml of phosphate-buffered saline (PBS, pH 7.4) in 50 ml test tubes with glass stoppers. 0.4 ml of fresh rabbit blood in acid citrate dextrose solution was added to each tube. Blood with PBS alone was taken in a similar tube to serve as the negative control. Positive control was prepared by addition of 0.4 ml of the blood to 25 ml of 0.1% sodium carbonate solution. All the tubes were incubated at  $37 \pm 1^\circ\text{C}$  in an incubator for one hour. At the end of incubation, the tubes were removed and the solutions were centrifuged at 2000 rpm in a laboratory centrifuge for 3 min and the supernatant was assayed spectrophotometrically at 545 nm in a Beckman model 35 UV-VIS spectrophotometer. Haemolytic percentages of the materials were calculated using the following relation:

$$\text{Percent haemolysis} = 100 \cdot \frac{(\text{OD of test}) - (\text{OD of negative control})}{(\text{OD of positive control})}$$

## 2 *Sterilization of hydrogel microspheres*

Microspheres were steam sterilized in stainless steel autoclaves (NAT, India) for 10 min at 20 psi. The material was found to be stable after steam sterilization. No visible damage to the particles was noticed.

## 2.4 *Subcutaneous implantation studies*

White albino rabbits were chosen as the model. Anaesthesia was administered using 45.0 mg/kg of sodium pentobarbitone intravenously. On either side of the vertebral column on the upper half of the back, 3 cm skin incisions were made. With blunt dissection subcutaneous pockets were created to accommodate 100 mg of the material. One more subcutaneous pocket was made on the lower left side of the back lateral to the vertebral column. Thus each animal received three subcutaneous implants of the material. The material was duplicated in two animals. Post implantation animals were sacrificed at the end of 1, 6 and 12 weeks. Their skins were reflected alongwith the implants and scrutinized for any macroscopic evidence of tissue reactions such as inflammation, necrosis, haemorrhage, exudation and encapsulation. Materials explanted along with the tissue were fixed in 10% buffered formalin for further histological evaluation.

### 2.5 *Implantation in the renal arteries of dogs*

Studies were conducted in preconditioned mongrel dogs of either sex and of 15 to 20 kg weight. Kidney function of the animal was ascertained preoperatively by checking the haematological and biochemical parameters. The animals were premedicated with atropine sulphate (0.04 mg/kg) and chlorpromazine (1 mg/kg) intramuscularly. Induction of anaesthesia was done with thiopentone sodium (2.5% solution) 10 mg/kg body weight and was maintained with N<sub>2</sub>O/O<sub>2</sub> mixture. Through the midline incision in the abdomen, the left renal artery was isolated after careful dissection. The artery was cannulated using an 18 G indwelling catheter, and 1 to 1.5 ml of the hydrogel microspheres as suspension in saline were injected in the direction of blood flow. The catheter was withdrawn and bleeding through the puncture wound was controlled by pressure. The abdominal wound was closed in layers in standard fashion. The animals remained on the table for 1½ to 2 h and when fully conscious were returned to the kennel. At the end of 6 and 12 weeks, the animals were autopsied and the organs examined macroscopically. Tissues were fixed in 10% buffered formalin for histopathological studies.

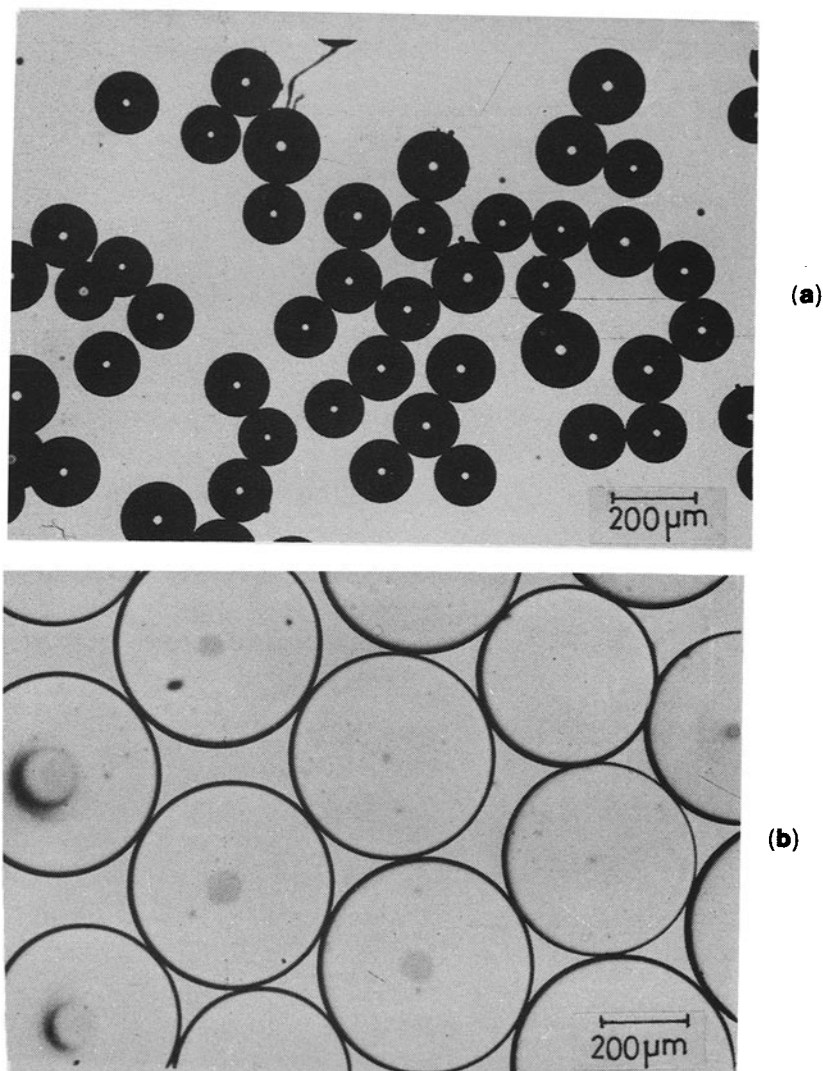
### 2.6 *Histopathological evaluation*

Tissues were fixed in 10% buffered formalin and processed for paraffin embedding. 5 µ thick sections were stained with haematoxylin and eosin and examined under the microscope.

## 3. Results and discussion

### 3.1 *Properties of microspheres*

PMMA microspheres of 125–180 µm size obtained by suspension polymerization are shown in figure 1a. These microspheres were hydrolysed to produce an EWC of 95%. The water-swollen microspheres are shown in figure 1b. The smooth, spherical shape of the microspheres is retained even after the hydrolysis which involved drastic reaction conditions. Even with 95% water, the microspheres were found to be mechanically strong presumably due to the presence of unhydrolysed methacrylate ester functions randomly present in the microspheres. PMMA microspheres (1 g) were hydrolysed using 5 wt% KOH in 50 ml of EG for 60 min. Longer reaction times produced microspheres with larger water contents but inferior mechanical properties. The swelling ratio of these microspheres (defined as volume of swollen beads/volume of dry beads) was estimated to be approximately 28. Such large volume expansion of the microspheres in contact with an aqueous medium provides considerable advantage for their use in the occlusion of blood vessels. It is apparent that the high swelling ability of these microspheres is due to the presence of potassium methacrylate produced on the microspheres upon hydrolysis (Gregonis *et al* 1976). Acidification of the microspheres results in the conversion of the alkali metal salt into the free acid as evidenced by the decreased swelling characteristics of the microspheres. For example, hydrolysed PMMA microspheres having an EWC of 95% on acidification using sulphuric acid imbibe only 75% water. However,



**Figure 1.** 1 mol% EGDM crosslinked poly(methyl methacrylate) microspheres of 125–180 μm size obtained by suspension polymerization, (a) and microspheres after alkaline hydrolysis and swelling in water to an EWC of 95% (b).

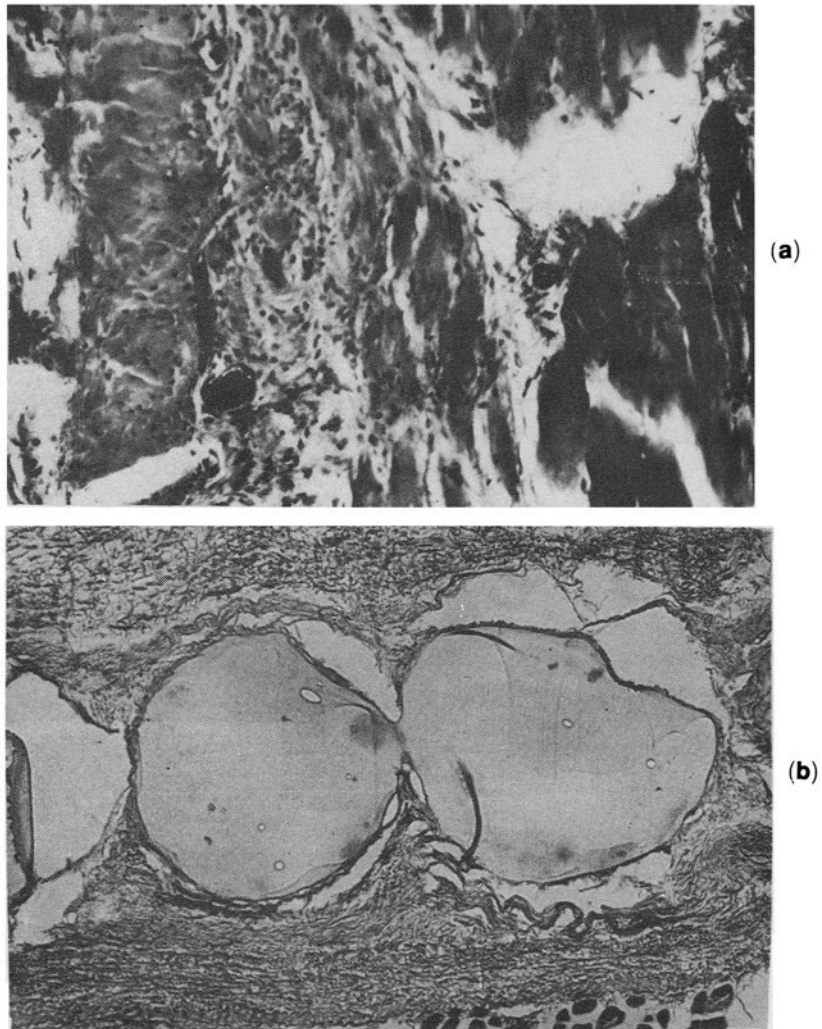
these microspheres on equilibration in 0.15 M phosphate buffered saline (PBS, pH 7.4) undergo progressive ionization of their carboxyl functions to regain their original EWC of 95%. Similar behaviour was observed in blood, serum and plasma. This phenomenon offers an additional advantage in embolization. Microspheres in their acid form can be delivered transcatheterilly in saline to the area to be embolized, progressive ionization and swelling of the microspheres in contact with blood would effect the occlusion of blood vessels firmly.

Blood haemolysis studies conducted using these microspheres both in the potassium salt form and in the acid form showed that they are non-haemolytic in character.

### 3.2 Subcutaneous implantation results

Seven days post-implantation, gross examination of each implant site revealed well-demarcated implants which were loosely bunched together within the subcutaneous tissue, with transparent connective tissue overgrowths over them. By the 6th and 12th week, the implants were firmly enclosed in the subcutaneous tissue. There was no gross evidence of necrosis or inflammation at any of the time periods.

Microscopically, at 7 days, mild necrosis, haemorrhage and inflammation were noted. The inflammatory infiltrate was composed of lymphocytes, histiocytes, fibroblasts, fibrocytes and a few muscle giant cells. Fibrous connective tissue was seen partially condensed around the periphery of a few microspheres (figure 2a). At

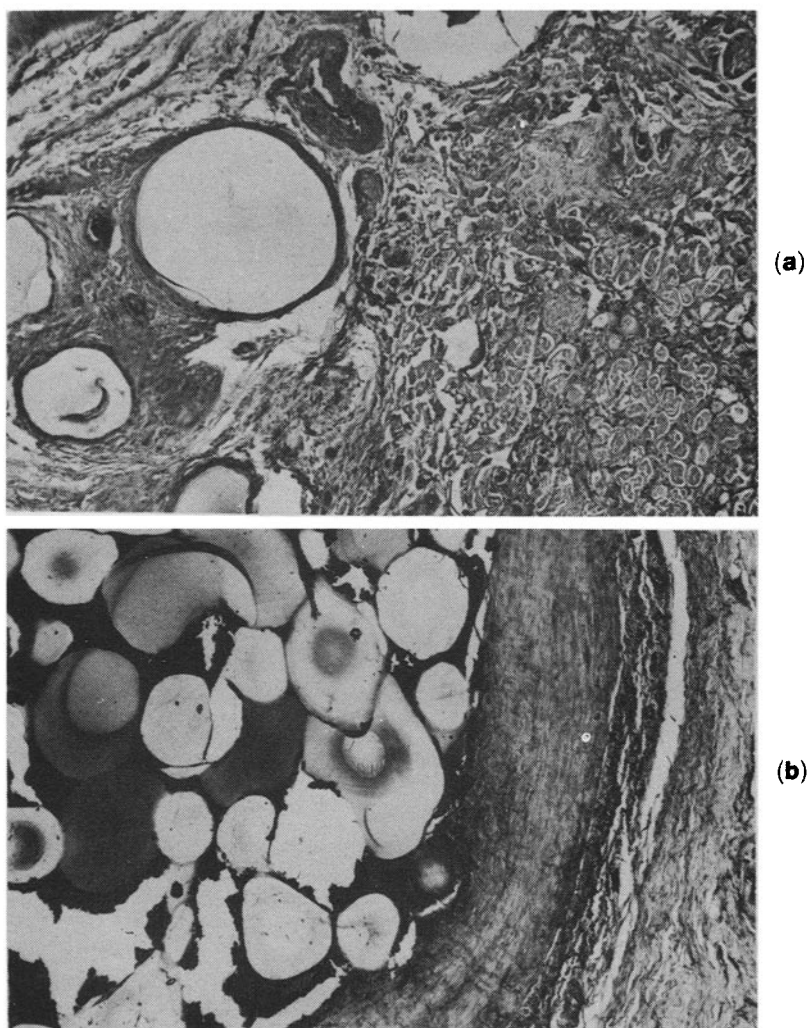


**Figure 2.** Light microscopic picture of tissue reaction around microspheres having an EWC of 95% in the subcutaneous tissue, at 7 days post-implantation (a) (magnification 30 $\times$ ), and at 12 weeks post-implantation (b) (magnification 60 $\times$ ).

12 weeks, there was no evidence of inflammation or necrosis and fibrous connective tissue was seen encircling individual microspheres. The response at 3 months post-implantation was essentially the same (figure 2b).

### 3.3 Arterial implantation results

Dogs were sacrificed 12 weeks after implantation. The left kidneys in both dogs were shrunken and lobulated. The cut surface revealed an infarct in the upper pole of one and cortical necrosis in the other. The contralateral kidneys were grossly normal. On cutting open the embolized vessels, the microspheres were found impacted in the vessels with blood.

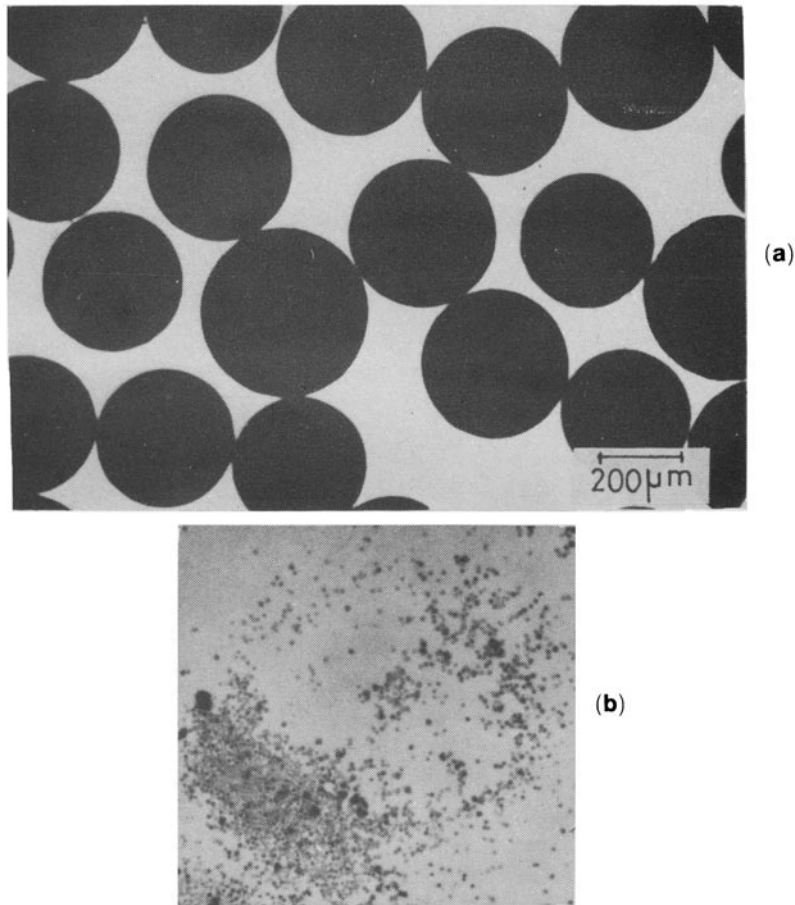


**Figure 3.** Section of the kidney showing impacted beads with adjoining infarct at 12 weeks post-implantation (a) (magnification  $60\times$ ), and cross-section of renal vessel showing embolized beads at 12 weeks post-implantation (b) (magnification  $60\times$ ).

Multiple sections of the kidneys were examined. Healed and healing infarcts were seen with cortical and medullary congestion in the rest of the kidney. The microspheres were seen in partially thrombosed vessels with an occasional adjacent giant cell (figure 3a). Chronic interstitial inflammation was noticed. Cut sections of the implanted vessels contained microspheres with partial thrombosis (figure 3b). A 6-month evaluation of implantation is expected to be carried out in the near future.

#### 3.4 Radiopaque hydrogel microspheres

Hydrolysed PMMA microspheres having higher water contents (>90%) could easily be made radiopaque by the incorporation of  $\text{BaSO}_4$ . Microspheres were swollen in a 25 wt% solution of  $\text{Na}_2\text{SO}_4$  and the  $\text{BaSO}_4$  was precipitated inside the microspheres by the addition of a concentrated solution of  $\text{BaCl}_2$ . Figure 4a shows the photomicrograph of microspheres loaded with 53 wt%  $\text{BaSO}_4$  in the dry state. The X-ray image of the microspheres is shown in figure 4b. In comparison with non-radiopaque microspheres, the  $\text{BaSO}_4$ -loaded radiopaque microspheres would



**Figure 4.** Photomicrograph of hydrogel microspheres loaded with 53 wt%  $\text{BaSO}_4$ , in the dry state (a), and an X-ray image of the same (b).



possess the advantage that post-operative status of embolization could be assessed by X-ray images without the aid of angiography immediately following embolization and for a long period of time thereafter. Such radiopaque emboli are awaiting further biocompatibility studies.

#### 4. Conclusions

Hydrogel microspheres synthesized from crosslinked PMMA beads by alkaline hydrolysis are smooth and possess perfect spherical geometry. The swelling ratio of these microspheres has been found to be large and favourable for their use as artificial emboli. Microspheres in their potassium salt form have been evaluated for their biocompatibility in the subcutaneous tissue of rabbits. No adverse tissue reactions has been observed in such studies. Embolization of the renal arteries of dogs proved the occlusion effect produced by the microspheres. Microspheres could easily be made radiopaque by the impregnation of barium sulphate. The use of these microspheres as microcarriers for cell culture for the growth and propagation of mammalian cells is demonstrated in the paper of Sivakumar *et al* (1989).

#### References

- Allen G G, Beer J W, Cousin M J and Mikels R A 1980 in *Controlled release technologies: Methods, theory and applications* (ed.) A F Kydonieus (Boca Raton, FL: CRC Press) vol. 2, chap. 2
- Andrade J D (ed.) 1976 *Hydrogels for medical and related applications* (Washington, DC: Am. Chem. Soc.)
- Benoit J P and Puisieux F 1986 in *Polymeric nanoparticles and microspheres* (Boca Raton, FL: CRC Press) chap. 5
- Feder J and Tolbert W R 1983 *Sci. Am.* **248** 24
- Goldberg E P and Nakajima A (eds) 1980 *Biomedical polymers: Polymeric materials and pharmaceuticals for biomedical use* (New York: Academic Press)
- Gregonis D E, Chen C M and Andrade J D 1976 in *Hydrogels for medical and related applications* (ed.) J D Andrade (Washington, DC: Am. Chem. Soc.) p. 99
- Hart D P 1977 in *Macromolecular synthesis* (ed.) J A Moore (New York: John Wiley & Sons) Coll. vol. 1, pp. 23–25
- Horak D, Svec F, Kalal J, Gumargalieva K, Adamyan A, Skuba N, Titova M and Trostenyuk N 1986a *Biomaterials* **7** 188
- Horak D, Svec F, Kalal J, Adamyan A A, Volynsk Y D, Voronkova O S, Kokov L S and Gumargalieva K Z 1986b *Biomaterials* **7** 467
- Horak D, Metalova M, Svec F, Drobnik J, Kalal J, Borovicka M, Adamyan A A, Voronkova O S and Gumargalieva K Z 1987 *Biomaterials* **8** 142
- John W, Sinow D, Teige W, Braumann D, Bischof C, Kapelle R and Saudner B 1987 *Ger (East) Pat.* DD 237,312, *Chem. Abstr.* **107** 59737b
- Munzer M and Trommsdorff E 1977 in *Polymerization processes* (eds) C E Schildknecht and I Skeist (New York: John Wiley & Sons) p. 135
- O'Leary R K and Guess W L 1968 *J. Pharm. Sci.* **57** 12
- Palacky J, Kiss F, Viska J and Borak J 1981 *Czech Pat.* CS 188, 620, 1982 *Chem. Abstr.* **96** 36086d
- Peppas N A (ed.) 1987 *Hydrogels in medicine and pharmacy* (Boca Raton, FL: CRC Press) vols. 1, 2 and 3
- Ratner B D and Hoffman A S 1976 in *Hydrogels for medical and related applications* (ed.) J D Andrade (Washington, DC: Am. Chem. Soc.) chap. 1
- Renbaum A, Yen S P S, Cheong E, Wallace S, Molday R S, Gordon I L and Dreyer W J 1976 *Macromolecules* **9** 328
- Sandler S R and Karo W (eds) 1977 *Polymer synthesis* (New York: Academic Press) vol. 2, pp. 264–305
- Sugii A, Ogawa N and Matsumoto M 1986 *J. Appl. Polym. Sci.* **32** 4931
- Vetvicka V and Fornusek L 1987 *Biomaterials* **8** 341
- Wichterle O and Lim D 1960 *Nature (London)* **185** 117