International Journal of Pharmaceutics, 55 (1989) 31–37
Elsevier

IJP 01847

Release characteristics of bleomycin mitomycin C and 5-fluorouracil from gelatin microspheres

R. Jeyanthi and K. Panduranga Rao

Polymer Division, Central Leather Research Institute, Adyar, Madras (India)

(Received 3 March 1989)
(Accepted 23 March 1989)

Key words: Gelatin; Microsphere; Controlled release; Bleomycin; Mitomycin C; 5-Fluorouracil

Summary

Gelatin microspheres containing the anticancer drugs, bleomycin (BLM), mitomycin C (MMC) and 5-fluorouracil (5-FU) were prepared by crosslinking with glutaraldehyde. The in vitro release of the entrapped anticancer drugs from gelatin microspheres was carried out in phosphate buffer, pH 7.4, at 37 °C. Optical and scanning electron microscopic studies of gelatin microspheres before and after drug release were also carried out. The in vitro release of BLM, MMC and 5-FU from gelatin microspheres followed a zero-order release pattern except for an initial 'burst effect' observed in the case of 5-FU. Microscopic studies clearly indicated that the spherical shape of the spheres changed gradually due to slow disintegration of the sphere and release of the drug by diffusion.

Introduction

Microparticulate drug delivery devices have recently gained attention in targeting chemotherapeutic agents (Davis and Illum, 1988; Burgess et al., 1987; Lee et al., 1988). These systems have the advantage of being able to entrap relatively large amounts of pharmacologically active agents and are easy to prepare. Various biodegradable and non-degradable carriers such as gelatin, starch, albumin, poly(akyl cyanoacrylate), poly(lactic acid), poly(acrylates and methacrylates) and polyacrylamide have been investigated as microspherical carriers (Tabata and Ikada, 1987; Fujimoto et al., 1985; Benita et al., 1986). Gelatin microspheres have been widely studied in parenteral formulations. However, most of these gelatin microspheres are prepared as oil-in-water or water-in-oil emulsions involving the use of surfactants. Recently, we have developed (Jeyanthi and Panduranga Rao, 1987) a novel method of preparation of gelatin microspheres (using concentrated solutions of PMMA) without the use of surfactants and high temperature. Smooth, uniform, spherical, hydrophilic, crosslinked microspheres in the particle size range of 10–30 μm were obtained. Three potent cancer chemotherapeutic agents, namely bleomycin, mitomycin C and 5-fluorouracil, were entrapped in these microspheres. The present study was undertaken to evaluate the release characteristics of the entrapped anticancer drugs from the gelatin microspheres in vitro.

Correspondence: K. Panduranga Rao, Polymer Division, Central Leather Research Institute, Adyar Madras 600 020, India.

Experimental

Materials

Gelatin (Oxoid, U.K.), poly(methyl methacrylate) (BDH, U.K.) and glutaraldehyde (Riedel, F.R.G.) were used as obtained. The anticancer drugs used were bleomycin (Nippon Kayaku Co., Japan), mitomycin C (Kyowa Hakko Kogyo Co., Japan) and 5-fluorouracil (Sisco Research Laboratory, India).

Methods

Preparation of gelatin microspheres. Gelatin microspheres containing the anticancer drugs, bleomycin (BLM), mitomycin C (MMC) and 5-fluorouracil (5-FU), were prepared using concentrated poly(methyl methacrylate) (PMMA) solution. A detailed procedure for the preparation of the microspheres is given in our earlier paper (Jeyanthi and Panduranga Rao, 1987). Briefly, the method is as follows: microspheres were prepared by adding PMMA in an organic medium to the aqueous gelatin medium. Glutaraldehyde in toluene was used for crosslinking. The microspheres were allowed to settle and the excess PMMA was removed by treating with toluene followed by acetone. Finally, the microspheres were washed with distilled water and dried at room temperature. A free-flowing powder was obtained. The size of the microspheres were found to be in the range of 10–30 μm. The microspheres were smooth, uniform and spherical. The drug content of the microspheres was assayed for both surface drug and entrapped drug. The procedure for analysis of drug content is given in our earlier paper (Jeyanthi and Panduranga Rao, 1989).

In vitro release studies. The in vitro release profiles of the anticancer drugs entrapped in gelatin microspheres were obtained by carrying out the release studies in 0.01 M phosphate buffer, pH 7.4, at 37 °C in a water-bath shaker with mild agitation. A known weight of gelatin microspheres was placed in a known volume of buffer. At periodic intervals of time, aliquots were pipetted out, filtered through 0.45 μm Millipore filters and assayed for drug content in a Beckman Model 26 UV/vis spectrophotometer (λ_{max} for BLM: 290 nm; MMC: 363 nm; and 5-FU: 266 nm).

Optical and scanning electron microscopy. The size, shape and surface characteristics of placebo and drug-loaded gelatin microspheres were studied by optical and scanning electron microscopy (SEM). The samples for SEM analysis were prepared by sprinkling the dried microsphere powder onto one side of a double adhesive tape which was stuck to an aluminium stub. The stubs were then coated with gold using an Edwards E-306 sputter coater to a thickness of 20–30 nm. The samples were then examined using a Cambridge Stereoscan S-150 Scanning Electron Microscope.

Gelatin microspheres containing bleomycin and 5-fluorouracil were used as representative samples for optical and SEM studies, respectively.

Results and Discussion

Preparation of gelatin microspheres containing anticancer drugs

Chemically crosslinked gelatin microspheres were prepared by a novel polymer dispersion technique and smooth, spherical particles in the size range of 10–30 μm were obtained. The amounts of anticancer drugs associated with the microspheres were analysed for both surface drug and the entrapped drug. The mean particle size range and the percent free drug and entrapment efficiency of gelatin microspheres are given in Table 1. It can be seen from these results that BLM had the highest encapsulation efficiency followed by 5-FU and MMC.

In vitro release of anticancer drugs from gelatin microspheres

The in vitro release profiles give an indication of the efficacy of the delivery system for the

TABLE 1

Particle size and drug content of gelatin microspheres

Anticancer drug	Particle size range (μm)	Surface drug (%)	Entrapped drug (%)
Bleomycin	20–30	28.76	71.24
Mitomycin C	15–30	43.36	56.64
5-Fluorouracil	10–20	32.72	67.28
Placebo	15–20	–	–

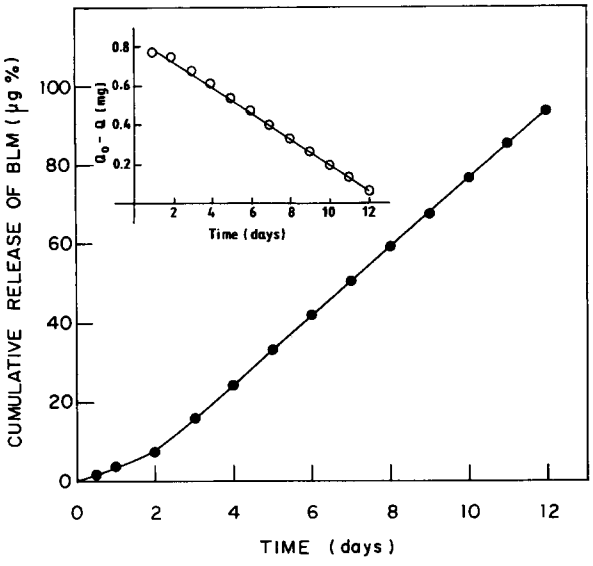


Fig. 1. In vitro release of bleomycin from gelatin microspheres. The inset is a plot of the residual values versus time. Q_0 is the original amount of drug present in the microspheres. Q is the amount of drug released.

controlled release of anticancer drugs. The in vitro drug release study is a prerequisite for testing the in vivo performance of the controlled drug delivery system. Hence the release of the anticancer drugs, 5-fluorouracil, mitomycin C and bleomycin, from the gelatin microspheres in vitro was studied.

Figs. 1–3 show plots of the data expressed as the cumulative amounts of the drugs released from the microspheres versus time. In the case of BLM, the percent drug release followed zero-order release kinetics as shown in Fig. 1. The rate of release of about 70 µg/day was maintained up to 12 days. It was also found that about 94% of the entrapped drug was released during that time. The in vitro release profile of MMC from gelatin microspheres is shown in Fig. 2. The rate of MMC release also followed the zero-order release pattern. The release rate was about 200 µg/day up to a period of 8 days. It was found that about 100% of the entrapped drug was released during that time period. Two phases of drug release from the gelatin microspheres was observed in the case of 5-FU: (i) an initial period of rapid release of about 12.5% in 6 h termed the 'burst effect' due to the release of the drug dispersed in the peripheral domains of the microsphere matrix; and (ii) a period when release was approximately linear with respect to time. The release profile of 5-FU from the gelatin microspheres is given in Fig. 3. The

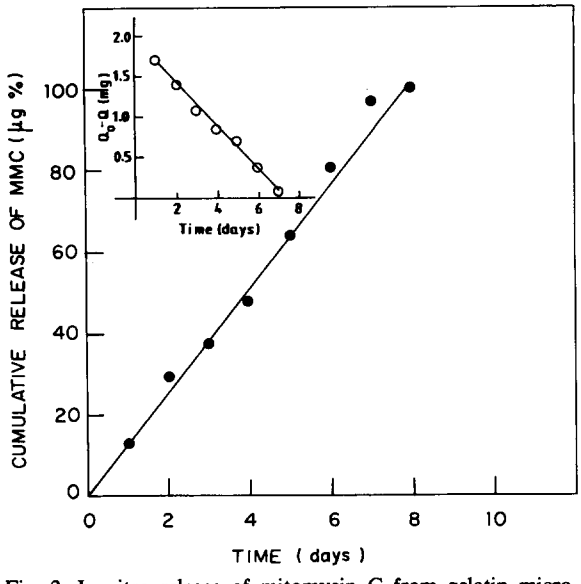


Fig. 2. In vitro release of mitomycin C from gelatin microspheres. The inset is a plot of the residual values versus time. Q_0 is the original amount of drug present in the microspheres. Q is the amount of drug released.

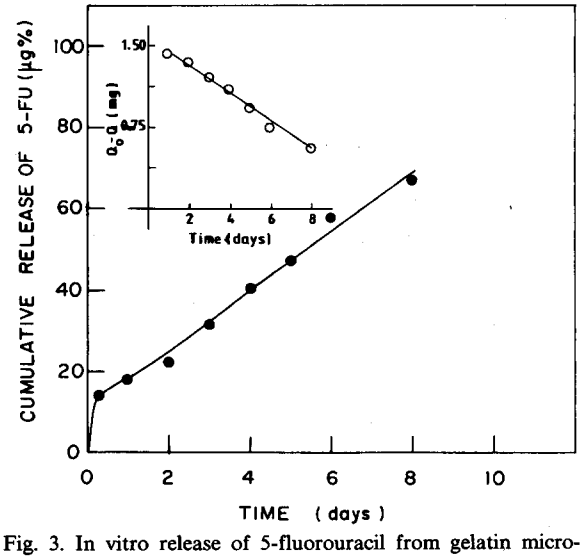


Fig. 3. In vitro release of 5-fluorouracil from gelatin microspheres. The inset is a plot of the residual values versus time. Q_0 is the original amount of drug present in the microspheres. Q is the amount of drug released.

rate of release of 5-FU was found to be 150 μg/day for a period of 8 days. About 67% of the drug was released during this period. Since the rate of release declined after this time period, release study was not continued further.

The results of the in vitro release studies of the anticancer drugs, BLM, MMC and 5-FU, from gelatin microspheres indicated that all 3 drugs entrapped gave zero-order release profiles except for the initial 'burst effect' observed in the case of 5-FU. It was clearly evident from the release profiles that BLM was released for a longer period as compared to MMC and 5-FU.

Mechanism of drug release

Release from a microsphere can take place via a number of routes (Tomlinson and McVie, 1983) including surface tension, total sphere disintegration, microsphere hydration (swelling), drug diffusion and desorption with attack by enzymes mainly effecting in vivo microsphere breakdown. A balance has to be achieved between the microsphere stability and microsphere biodegradability to permit a steady and controlled release of drug from the matrix. Crosslinking of the matrix material to varying extents is a flexible means of achieving this balance. In the present study, since gelatin itself is biodegradable, chemical stabilization of the microspheres has been carried out to achieve microsphere stability as well as controlled degradation. Glutaraldehyde is widely used in protein chemistry as a crosslinking agent (Nimni et al., 1987) and it reacts primarily with the amino groups of proteins in biological systems. It has been used in the manufacture of medical devices, tissue bioprostheses and as a sterilizing agent. Glutaraldehyde is also being used for improving the mechanical strength of collagen which will be controlled by the degree and type of crosslinks introduced. Hence, gelatin, which is a degraded form of collagen, was crosslinked with glutaraldehyde to stabilize the microspheres.

From the optical and scanning electron microscopic studies it is clearly evident that drug release from the gelatin microspheres occurred by a slow and gradual degradation of the microsphere matrix and diffusion of drug through the crosslinked gelatin matrix.

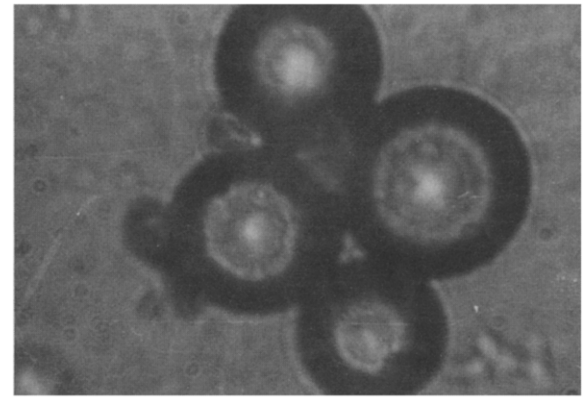


Fig. 4. Optical photomicrograph of gelatin microspheres containing bleomycin. Mean particle size 20–30 μm (before drug release).

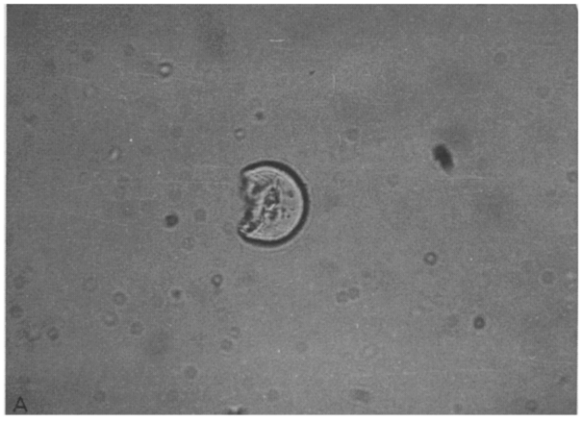
Optical microscopic studies

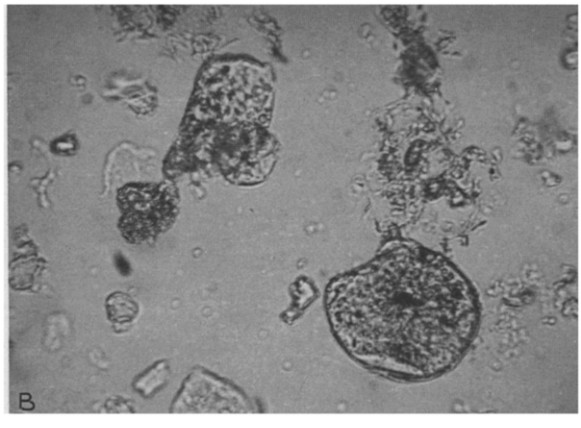
The optical photomicrograph of gelatin microspheres (Fig. 4) shows the polymer coating surrounding the gelatin microsphere dispersion. Microspheres were invariably spherical and well-dispersed. BLM-containing gelatin microspheres were studied by optical microscopy after dissolution for various time intervals. It can be observed from Figs. 5A, B and C that the microspheres disintegrated after release of drug and became porous. However, the rate of disintegration depended on the release period. After two days of dissolution, the microspheres showed slight disintegration losing their spherical shape (Fig. 5A). Some of the microspheres tended to be non-spherical and appeared to release their contents by slow disintegration after 6 days as shown in Fig. 5B. A near-complete disintegration of the microspheres occurred after dissolution for 12 days, forming an amorphous mass (Fig. 5C). It seems likely that the release mechanism of anticancer drugs from microspheres involves both disintegration of matrix and diffusion of drug.

Scanning electron microscopy

Fig. 6 shows the uniform, solid, smooth and spherical geometry of gelatin microspheres as examined by scanning electron microscopy (SEM). Longo et al. (1982) have also reported solid, spherical particles of bovine serum albumin prepared using PMMA as dispersant. Fig. 7 shows

the SEM picture of 5-FU-containing gelatin microspheres after release of entrapped drug. It can be seen from the figure that dramatic changes in the surface characteristics of microspheres occurred with time. The surface of the microspheres which was smooth and spherical in shape before drug release, became rough and non-spherical and were completely vacuolated after release of the drug. This is probably caused by the dissolution of the entrapped drug. Gupta et al. (1986) and Lee et al. (1988) have also observed the formation of cavities on the surface of albumin microspheres which was attributed to the dissolution of en-





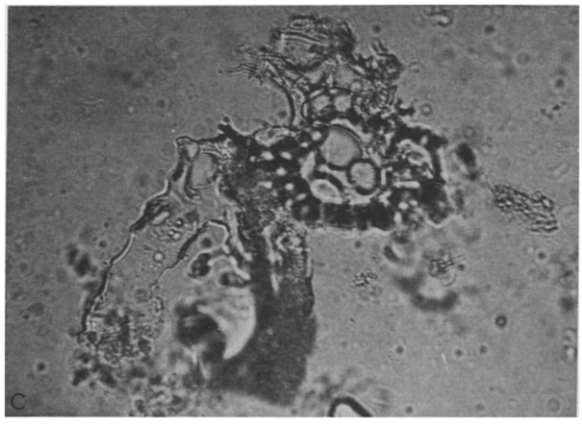


Fig. 5. A: optical photomicrograph of gelatin microspheres after partial release of incorporated bleomycin in vitro for 2 days. Microspheres show partial disintegration of gelatin matrix. B: optical photomicrograph of gelatin microspheres after release of incorporated bleomycin in vitro for 6 days, showing the ruptured spherical surface. C: optical photomicrograph of gelatin microspheres after complete release of incorporated bleomycin in vitro for 12 days forming an amorphous mass.

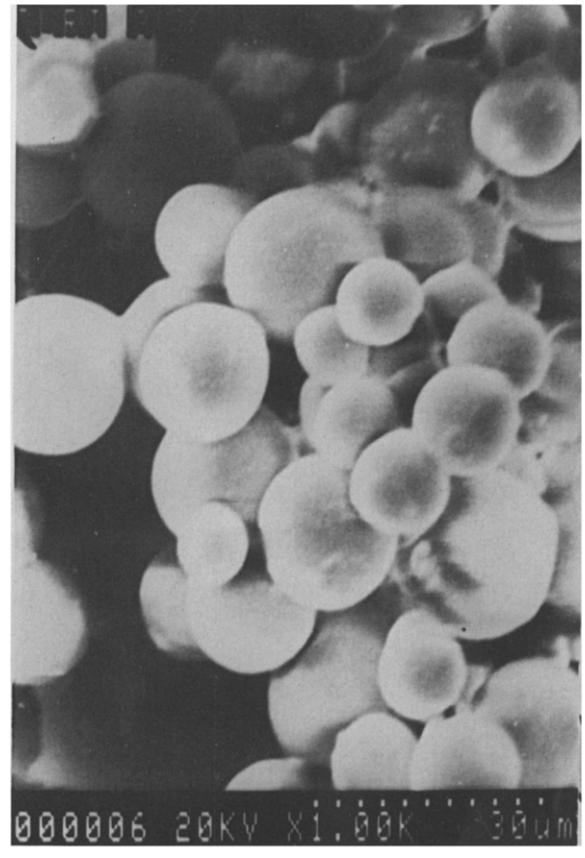


Fig. 6. Scanning electron photomicrograph of gelatin microspheres containing 5-fluorouracil (before drug release).

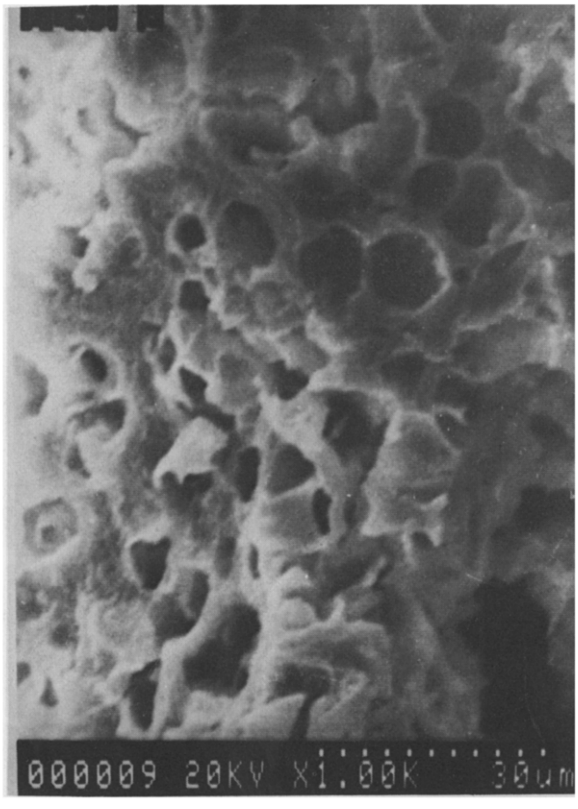


Fig. 7. Scanning electron photomicrograph of gelatin microspheres after in vitro release of incorporated 5-fluorouracil.

trapped adriamycin in the microspheres. Further, the SEM figure clearly indicated that the average size and surface area of the gelatin microspheres increased during the dissolution study. This may have led to an increase in the drug diffusion layer thickness and hence a decrease in drug release from the carrier, a phenomenon observed in the case of 5-FU release from the microspheres.

Drug release from microspheres can also be affected by other factors (Tabata and Ikada, 1987; Tomlinson and McVie, 1983; Davis et al., 1987) such as its position in a microsphere, the type and amount of matrix material, the size and density of the sphere, the extent and nature of any crosslinking, denaturation or polymerization, the physicochemical type, molecular weight and concentration of the drug, the presence of adjuvants in the microsphere, surface area ad charge, surface coating, physicochemical interaction between the drug and the matrix material and the release environment.

The results of the in vitro release profiles of the entrapped anticancer drugs from gelatin microspheres showed zero-order release behavior except for an initial boost release in the case of 5-FU. The surface morphological studies of the gelatin microspheres by optical and scanning electron microscopy showed that the drugs were released by a combination of diffusion and slow and gradual erosion of the spheres. Gelatin microspheres thus offer excellent potential for the controlled release of anticancer drugs.

References

Benita, S., Zouai, O. and Benoit, J.P., 5-Fluorouracil-carnauba wax microspheres for chemoembolization: an in vitro evaluation. *J. Pharm. Sci.*, 75 (1986) 847–851.

Burgess, D.J., Davis, S.S. and Tomlinson, E., Potential use of albumin microspheres as a drug delivery system. I. Preparation and in vitro release of steroids. *Int. J. Pharm.*, 39 (1987) 129–136.

Davis, S.S. and Illum, L., Polymeric microspheres as drug carriers. *Biomaterials* 9 (1988) 111–115.

Davis, S.S., Mills, S.N. and Tomlinson, E., Chemically crosslinked albumin microspheres for the controlled release of incorporated Rose Bengal after intramuscular injection into rabbits. *J. Controlled Rel.*, 4 (1987) 293–302.

Fujimoto, S., Miyazaki, M., Endoh, F., Takahashi, O., Shrestha, R.D., Okui, K., Morimoto, Y. and Terao, K., Effects of intraarterially infused biodegradable microspheres containing mitomycin C. *Cancer*, 55 (1985) 522–526.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres I. Release characteristics of adriamycin. *Int. J. Pharm.* 33 (1986) 137–147.

Jeyanthi, R. and Panduranga Rao, K., Preparation of gelatin microspheres of bleomycin. *Int. J. Pharm.*, 35 (1987) 177–179.

Jeyanthi, R. and Panduranga Rao, K. A novel method of preparation of gelatin microspheres for the controlled release of anticancer drugs. *Int. J. Pharm.*, (1989) in press.

Lee, K.C., Koh, I.B., Kim, W.B. and Lee, Y.J., Size and morphological analysis of albumin microspheres in the lung and liver of mice. *Int. J. Pharm.*, 44 (1988) 49–55.

Longo, W.E., Iwata, H., Lindheimer, T.A. and Goldberg, E.P., Preparation of hydrophilic albumin microspheres using polymeric dispersing agents. *J. Pharm Sci.*, 71 (1982) 1323–1328.

Nimni, M.E., Cheung, D., Strates, B., Kodama, M. and Sheikh, K., Chemically modified collagen: A natural biomaterial for tissue replacement. *J. Biomed. Mater. Res.*, 21 (1987) 747–777.

Tabata, Y. and Ikada, Y., Macrophage activation through phagocytosis of muramyl dipeptide encapsulated in gelatin microspheres. *J. Pharm. Pharmacol.*, 39 (1987) 698–704.

Tomlinson, E. and McVie, J.G., New directions in cancer chemotherapy. 2. Targeting with microspheres. *Pharmacy Int.*, (1983) 281–284.