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Polylactic acid nanoparticles, a colloidal drug delivery system for lipophilic drugs

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Summary

Nanoparticles of polylactic acid (PLA) loaded with triamcinolone acetonide were prepared by a modified solvent evaporation process. The products were characterized concerning their interior structure, size distribution, drug content, drug release and in vivo distribution. Furthermore, the influence of the starting polymer concentration (range from 0.5 to 1.5 g of polymer/100 ml of emulsion) was studied. The procedure yielded particles with a mean diameter below 1 μm, showing a broader size distribution for nanoparticles prepared with the highest studied polymer concentration. Having a drug content from 8% w/w to 2.9% w/w the particles retarded drug release in comparison to microcrystalline trimacinolone acetonide in all cases. After removing surface adherent drug, drug release was further slowed down, being most sustained for nanoparticles prepared with 1.5 g PLA/100 ml of emulsion. After intravenous injection of ^{99m}Tc-labeled PLA nanoparticles into rats they accumulated predominantly in liver, kidney and bone marrow, a distribution pattern usually found for colloidal particles.

Introduction

Injectable, colloidal drug delivery systems have gained great interest in the last years due to their enhanced efficiency against tumours (Brasseur et al., 1980; Widder

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et al., 1981), their sustained release action (Widder et al., 1980; Couvreur et al., 1979a) and due to the possibility of influencing the drug distribution in the body after intravenous injection (Kreuter et al., 1979; Yoshioka et al., 1981; Oppenheim et al., 1978).

One prerequisite for a successful application of these carriers is the biodegradability of the wall material because the carrier cannot be removed after injection. For this reason most investigators used albumins (Kramer et al., 1974; Widder et al., 1981), gelatin (Oppenheim et al., 1979; Yoshioka et al., 1981) or polyalkylcyanoacrylates (Couvreur et al., 1979b; Kreuter et al., 1983).

Polylactic acid (PLA) was suggested as a matrix for drug delivery for the first time in 1971 (Kulkarni et al., 1971). Beck and coworkers developed a procedure which allowed the preparation of microspheres in a size range too large (20–70 μm diameter) for intravenous injection and evaluated the potential use of these particles as a depot contraceptive for local administration (Beck et al., 1980a).

Gurny and coworkers reported the production of a pseudolatex of PLA consisting of particles with a size of 0.45 μm and showed the good histocompatibility of a subcutaneously injected suspension (Gurny et al., 1981). Scope of the present investigation was the preparation and characterization of PLA nanoparticles as a drug carrier prepared by a modification of the procedure of Beck (Beck et al., 1980a). Triamcinolone acetonide was chosen as a model drug. Furthermore, the influence of different polymer concentrations on size distribution, drug content and drug release of the particles was examined. In order to see whether the drug distribution in the body can be altered by an intravenous injection of nanoparticles the biodistribution was also investigated.

Materials and Methods

Materials

L-(−)-lactide was purchased from Boehringer Ingelheim (Ingelheim, F.R.G.). ^{3}[H]Triamcinolone acetonide (spec. act. 32.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Cold triamcinolone acetonide was kindly provided by von Heyden GmbH (München, F.R.G.).

All other reagents had analytical reagent grade and were used without further purification.

Ultrasonic equipment consisted of a Branson Sonifier Cell Disrupter B 15 (Branson Schallkraft, Heusenstamm, F.R.G.), fitted with a booster horn gold.

Electron microscopic investigations were performed with a SEM S 450 scanning electron microscope (Hitachi Deshi, Rodgau, F.R.G.) and with a Zeiss transmission electron microscope EM 9 (Zeiss, Oberkochen, F.R.G.).

A Packard tricarb scintillation counter (Packard Tricarb 300, Packard Instruments, Downers Grove, U.S.A.) was used for measurement of radioactivity.

The size of the nanoparticles was determined with an automatic picture analyzer (MOP AM 03, Kontron Messgeräte, Eching, F.R.G.).

Preparation of polylactic acid

The lactide was polymerized accoring to a previously reported method by Kleine (Kleine and Kleine, 1959). After purification the polylactic acid was identified by melting point and IR analysis (Rak et al., 1983).

Preparation of PLA nanoparticles

Each batch was prepared in triplicate. 200, 400 or 600 mg of PLA and 20 mg of triamcinolone acetonide, mixed with 100 μl of ^{3}[H]triamcinolone acetonide, were dissolved in 2 ml of chloroform. This solution was subsequently emulsified in 40 ml 0.5% w/w gelatin solution by sonication for 45 min. During this emulsification the temperature of the mixture was maintained below 15°C with the aid of an ice-bath. Then, the temperature was raised to 40°C allowing a slow evaporation of the chloroform. After another 45 min the solvent had evaporated and the sonication was finished. The resulting suspension was centrifuged (19,500 $\times$ g, 30 min) and the pellets were washed three times with water. After the last washing the nanoparticles were suspended in water and freeze-dried for 2 days.

Size distributions

A suspension of the nanoparticles was sprayed onto an aluminium foil with the aid of an atomizer. The fine drops were dried overnight, sputtered with an about 10 nm thick gold layer and examined with a SEM. For each distribution between 500 and 1000 particles were measured from enlarged negatives with an automatic picture analyzer. The photos (usually 10) were taken at different magnifications to assure that all sizes of nanoparticles were detected.

Determination of the drug content

After dissolving a known amount of nanoparticles in chloroform the activity was determined by liquid scintillation counting. Quench correction was performed by the external standard method, using [^{14}C]-standards. To eliminate chemiluminiscence, samples were counted after standing in the dark overnight. Counting efficiency was generally greater than 30%.

Drug release

The release pattern was investigated by two different approaches. In order to examine drug release during the first 15 min, nanoparticles were suspended in 10 ml of phosphate buffer (pH 7.35, 37°C) by sonication, agitated (25 rpm) and after 3, 6, 9, 12 and 15 min 1-ml samples were removed. The samples were filtered through 0.1 μm Nucleopore membranes and the radioactivity in an aliquot of the filtrate was measured. During the whole release experiments sink conditions (amount of dissolved drug generally $< 10\%$ of saturation solubility) were maintained (Mauger et al., 1983).

The investigation of drug release from nanoparticles without surface-adherent drug was performed by a previously reported dialysis procedure (Souder and Ellenbogen, 1958). 1 ml of a nanoparticle suspension was filled into dialysis bags, put into centrifuge tubes and rotated at 25 rpm. After certain time intervals the

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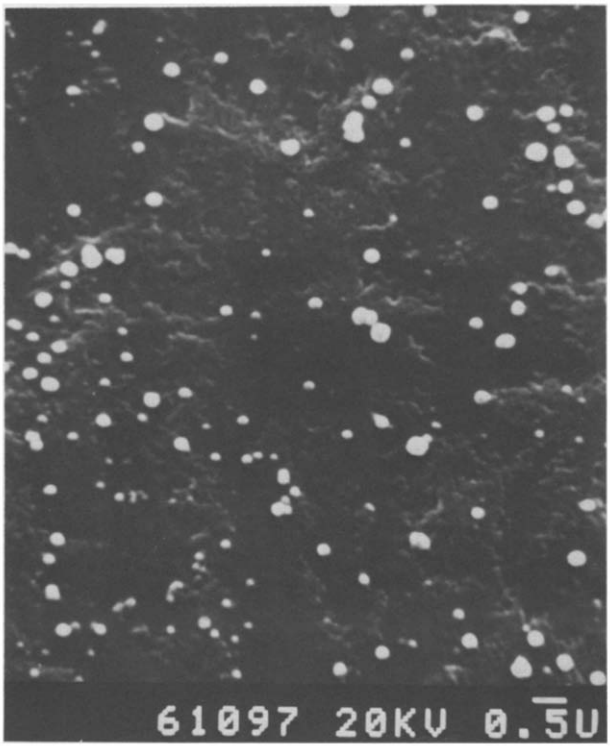

Fig. 1. SEM picture of PLA nanoparticles. Magnification: 10,000×; bar represents 0.5 μm.

1972) because intense ultrasonic waves are known to induce chemical reactions or to degrade substances (Weissler, 1953). After dissolving particles in chloroform no degradation products of triamcinolone acetonide could be detected.

Table 1 lists yield, drug content, mean diameter and encapsulation efficiency of the products. The yield is similar to the one usually found for products prepared by solvent evaporation (Beck et al., 1980b; Wakiyama et al., 1981). About 80% of the starting material can be recovered as nanoparticles. For all preparations the yield and the encapsulation efficiency are independent of the amount of polymer. Concerning the drug content it is obvious that by choosing a certain drug-to-polymer ratio the resulting drug content can be adjusted. To our knowledge there exists only one report (Gurny et al., 1981) in which polylactic acid particles were prepared in a similar size range. These authors used testosterone as a model compound, but the amount of encapsulated drug on a w/w basis cannot be calculated from their data.

The influence of polymer concentration on the size distribution is shown in Fig. 4 and Table 1. Higher amounts of polymer caused a broader size distribution due to larger drops formed during emulsification. Using increasing PLA concentrations the viscosity of the inner phase increased. Consequently this solution was more difficult to emulsify than an inner phase with a lower viscosity for constant emulsification time and energy.

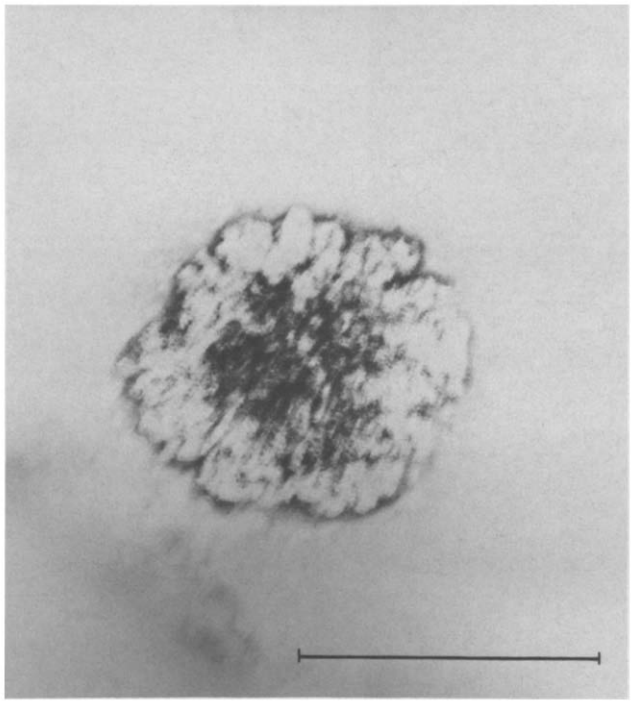

Fig. 2. TEM picture of sectioned PLA nanoparticles. Note highly porous interior structure. Magnification: 20,000×; bar represents 1.1 μm.

The results of the drug release experiments are shown in Fig. 5. All nanoparticle batches released triamcinolone acetonide slower than micronized drug crystals (mean diameter 1.9 μm) dissolved. The identical release pattern for all batches and

TABLE 1

FEATURES OF POLYLACTIC ACID NANOPARTICLES

Nanoparticle batch	Yield [a] % w/w ± rel. S.D.	Drug content [b] % w/w ± rel. S.D.	Encapsualtion [c] efficiency % ± rel. S.D.	Mean diameter [d] (nm) ± S.D.
PLA 200	82.6 ± 5	7.8 ± 5.4	64.5 ± 10	500 ± 397
PLA 400	79.2 ± 4.7	4.5 ± 2.4	71 ± 6.4	476 ± 410
PLA 600	85.3 ± 10	2.9 ± 19	75.4 ± 15	710 ± 406

[a] $\text{Yield} = \frac{\text{amount of employed polymer and drug}}{\text{amount of recovered nanoparticles}} \times 100$, n = 3.

[b] n = 9.

[c] $\text{Encapsulation efficiency} = \frac{\text{mg of drug bound by total amount of nanoparticles}}{\text{total amount of applied drug [mg]}} \times 100$, n = 3.

[d] n = 500–000 measured particles.

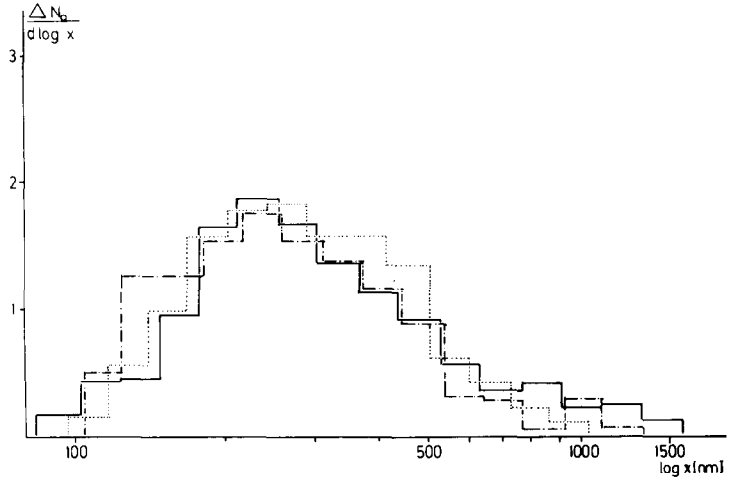

Fig. 3. Size distribution of three batches of PLA nanoparticles.

	n	$\bar{X} \pm$ S.D. [nm]
———	707	388 ± 234
·–·–·	463	313 ± 166
·····	713	315 ± 157

the quite rapid drug release was thought to be caused by drug which was located on the surface of the particles.

In an attempt to remove this drug the nanoparticles were washed with absolute ethanol. The remaining drug content was in the order of 0.1% w/w, independent of

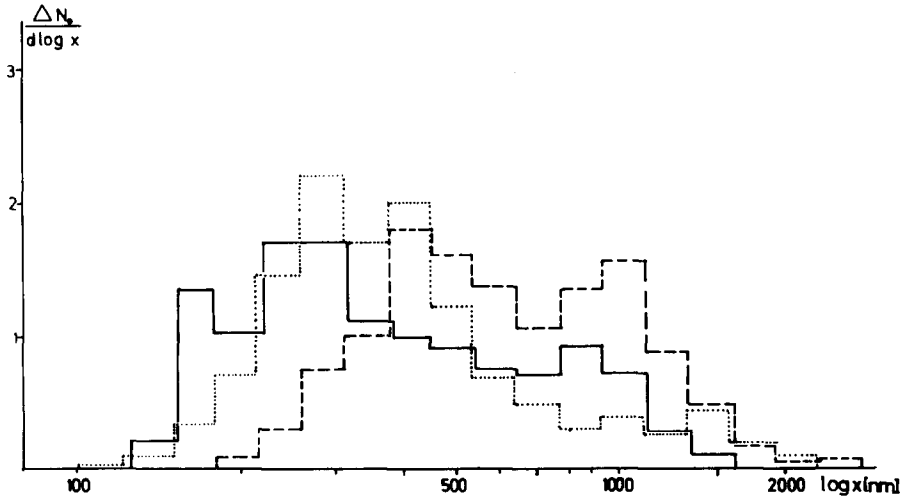

Fig. 4. Influence of polymer concentration on the size distributions of PLA nanoparticles, n = 500–1000 particles. ·····, PLA 200 mg; ———, PLA 400 mg; — — —, PLA 600 mg.

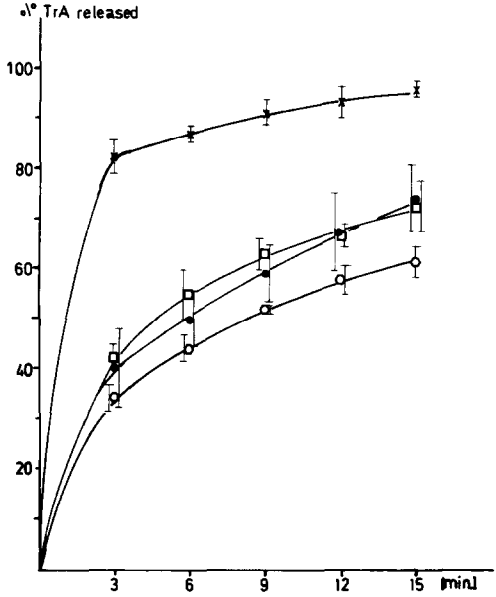

Fig. 5. Drug release from PLA nanoparticles determined by membrane filtration (mean of three experiments ± S.D.). × ——— ×, micronized triamcinolone acetonide crystals, 1.9 μm; ●———●, PLA 200; ○———○, PLA 400; □———□, PLA 600.

the polymer concentration. Concerning the highly porous structure of the particles it is reasonable to assume that on the one hand drug was released from the interior of the particles during washing. On the other hand, due to the very minute diameter of the particles they have a large surface on which the greatest amount of drug was located. Unfortunately, there exist no data on drug contents of PLA or polymethylcyanoacrylate (PMCA) nanoparticles on a weight of drug to weight of wall material basis. However, comparative investigations performed with PMCA nanoparticles prepared according to a previously published method (Roland and Couvreur, 1981) revealed that the PLA nanoparticles have a 10–20 times higher drug content than the corresponding values for the PMCA nanoparticles (Krause et al., submitted for publication). The remaining drug was released very slowly as can be seen in Fig. 6. After 4 h only 8–13% of the drug leaked out of the nanoparticles. There was no marked difference in drug release between PLA 200 and PLA 400, but in the case of PLA 600 the thicker polymer wall restricted drug release to a greater extent.

During the first hour there exists a burst effect, which may be due to drug lying beneath the surface of the nanoparticles dissolving faster than drug crystals embedded in the center of the spheres. This release pattern is similar to that observed by Beck (Beck et al., 1980c). Their suggestion was that the dissolution medium invades the sphere through pores, dissolving drug crystals in them. In these channels a saturated drug solution will exist from which drug diffuses slowly into the surrounding fluid. Further drug will be provided by diffusion through the polymer

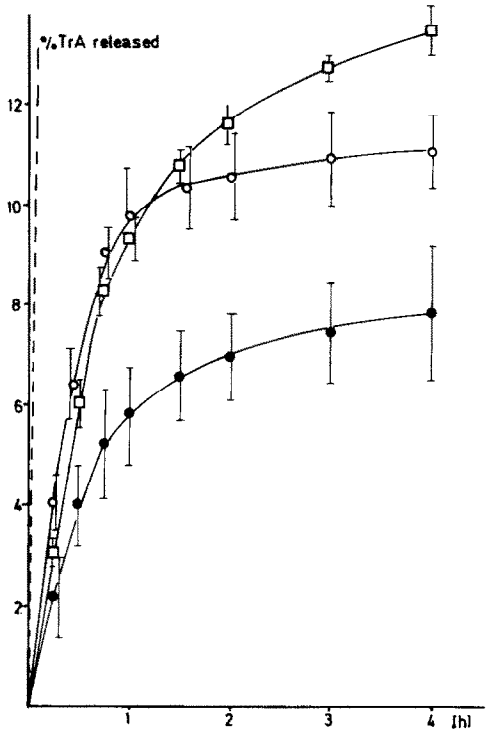

Fig. 6. Drug release from PLA nanoparticles without surface adherent triamcinolone acetonide, determined by dialysis (mean of nine experiments ± S.D.). — — —, triamcinolone acetonide, 1.9 μm diameter; ○———○, PLA 200; □———□, PLA 400; ●———●, PLA 600.

TABLE 2

ORGAN DISTRIBUTION OF ^{99m}Tc-LABELED PLA NANOPARTICLES IN RATS, 2 h AFTER INTRAVENOUS INJECTION

Organ (whole)	% of injected dose
liver	42.9
spleen	0.61
kidney	5.42
lung	1.17
thyroid glands	0.051
bone marrow [a]	1.96
blood [b]	0.99
muscle [a]	0.049
bone [a]	0.93

[a] 1 g of organ measured. [b] 1 ml of blood measured.

into the water-filled channels until no more drug is present. This process can be enhanced by the simultaneous biodegradation of the wall material, leading to the breakdown of the particle. SEM examinations, however, revealed intact particles after finishing drug release experiments (data not shown), an observation which is consistant with the slow hydrolytic degradation of the polymer (Brady et al., 1973).

Although a lot of work has been done to examine the biodegradation and histocompatibility of polylactic acid (Gurny et al., 1981; Brady et al., 1973; Ratcliffe et al., 1984; Anderson et al., 1976), nothing is known concerning the biodistribution of PLA particles after intravenous injection. Colloidal particles were usually enriched in organs of the reticuloendothelial system (Davis et al., 1982), but this pattern can be altered by changing the surface properties of the injected material (Davis, 1983). Table 2 shows that the PLA nanoparticles were mainly accumulated in liver, kidney and bone marrow. Some activity was found in the lungs, probably caused by particles mechanically trapped in the capillaries.

Thus, PLA nanoparticles prepared by a solvent evaporation procedure may be a valuable carrier system for targeting to the liver, particularly in the therapy of parasitic diseases.

References

Anderson, L.C., Wise, D.L. and Howes, J.F., An injectable sustained release fertility control system. Contraception, 13 (1976) 375–384.

Beck, L.R., Flowers, Ch.F. and Cowsar, D.R., Microparticles for treating the interior of female sexual organs. German patent, No. 2940146 (1980a).

Beck, L.R., Cowsar, D.R. and Lewis, D.H., Systemic and local delivery of contraceptive steroids using biodegradable microcapsules. In Hafez, E.S.E. (Ed.), Biodegradables and Delivery systems for Contraception, Vol. 1, MTP Press, 1980b, pp. 63–81.

Beck, L.R., Cowsar, D.R. and Pope, V.Z., Injectable particulate contraceptive systems. In Zatuchini (Ed.), Research frontiers in fertility regulation, 1980c, pp. 213–229.

Brady, J.M., Cutright, D.E., Miller, R.A. and Battistone, G.C., Resorption rate, route of elimination, and ultrastructure of the implant site of polylactic acid in the abdominal wall of the rat. J. Biomed. Mater. Res., 7 (1973) 155–166.

Brasseur, F., Couvreur, P., Kante, B., Deckers-Passau, L., Roland, M., Deckers, C. and Speiser, P., Actinomycin D adsorbed on polymethylcyanoacrylate nanoparticles: increased efficiency against an experimental tumor. Eur. J. Cancer, 16 (1980) 1441–1445.

Couvreur, P., Kante, B., Roland, M. and Speiser, P., Adsorption of antineoplastic drugs to polyalkylcyanoacrylate nanoparticles and their release in calf serum. J. Pharm. Sci., 68 (1979a) 1521–1524.

Couvreur, P., Kante, B., Roland, M., Guiot, P., Bauduin, P. and Speiser, P., Polyalkylcyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. J. Pharm. Pharmacol., 31 (1979b) 331–332.

Davis, S.S. and Hansrani, P.K., The evaluation of parenterally administered emulsion formulations. In Wilson, C. (Ed.), Radionuclide Imaging and Drug Research, Croom Helm, London, 1982, pp. 217–241.

Davis, S.S., Emulsion systems for the delivery of drugs by the parenteral route. In Bundgaard, H. (Ed.), Optimization of Drug Delivery, Munksgaard, Copenhagen, 1983, pp. 333–347.

Florey, K., Analytical Profiles of Drug Substances, Academic Press, New York-London, 1972, pp. 397–421.

Gardner, D.L. and Fink, D.J., Biodegradable microcapsules and microparticles for contraception. In Hafez, E.S.E. (Ed.), Biodegradables and Delivery Systems for Contraception, Vol. 1, MTP Press, 1980, pp. 47–61.

Gurny, R., Peppas, N.A., Harrington, D.D. and Banker, G.S., Development of biodegradable and injectable latices for controlled release of potent drugs. Drug Develop. Ind. Pharm., 7 (1981) 1–25.

Kleine, J. and Kleine, H.H., Über hochmolekulare, insbesondere optisch aktive Polyester der Milchsäure, ein Beitrag zur Stereochemie makromolekularer Verbindungen. Makromol. Chem., 30 (1959) 23–38.

Kramer, P.A., Albumin microspheres as vehicles for achieving specificity in drug delivery. J. Pharm. Sci., 63 (1974) 1646–1647.

Krause, H.-J., Schwarz, A. and Rohdewald, P., Interfacial polymerization, a useful method for the preparation of polymethylcyanoacrylate nanoparticles. Drug. Dev. Ind. Pharm., submitted for publication.

Kreuter, J., Täuber, U. and Illi, V., Distribution and elimination of poly(methyl-2-[^{14}C]-methacrylate) nanoparticle radioactivity after injection in rats and mice. J. Pharm. Sci., 68 (1979) 1443–1447.

Kreuter, J., Nefzger, M., Liehl, E., Czok, R. and Voges, R., Distribution and elimination of poly(methyl methacrylate) nanoparticles after subcutaneous administration to rats. J. Pharm. Sci., 72 (1983) 1146–1149.

Kulkarni, R.K., Moore, E.G., Hegyeli, A.F. and Leonard, F., Biodegradable poly(lactic acid) polymers. J. Biomed. Mater. Res., 5 (1971) 169–181.

Luft, J.H., Improvements in epoxy resin embedding methods. J. Biophys Biochem. Cytol., 9 (1961) 409–414.

Mauger, J.W., Howard, St. A. and Amin, K., Dissolution profiles for finely divided drug suspensions. J. Pharm. Sci., 72 (1983) 190–193.

Oppenheim, R.C., Marty, J.J. and Steward, N.F., The labelling of gelatin nanoparticles with ^{99m}technetium and their in vivo distribution after intravenous injection. Aust. J. Pharm. Sci., 7 (1978) 113–117.

Oppenheim, R.C. and Stewart, N.F., The manufacture and tumour cell uptake of nanoparticles labelled with fluorescein isothiocyanate. Drug Dev. Ind. Pharm., 5 (1979) 563–571.

Rak, J., Ford, J.L., Rostron, C. and Walters, V., Preparation and characterization of poly(D,L-lactic acid) (PLA) for use as a biodegradable drug delivery system. J. Pharm. Pharmacol., 35 Suppl. (1983) 107 P.

Ratcliffe, J.H., Hunneyball, I.M., Smiht, A., Wilson, C.G. and Davis, S.S., Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs. J. Pharm. Pharmacol., 36 (1984) 431–436.

Reynolds, E.S., The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., 7 (1963) 208–212.

Roland, M. and Couvreur, P., Les Vecteurs Nanoparticulaires De Medicaments. Bull. Men. Acad. Med. Belg., 136 (1981) 107–124.

Souder, J.C. and Ellenbogen, W.C., Laboratory control of dextro-amphetamine sulfate sustained release capsules. Drug Standards, 26 (1958) 77–83.

Wakiyama, N., Juni, K. and Nakano, M., Preparation and evaluation in vitro of polylactic acid microspheres containing local anesthetics. Chem. Pharm. Bull., 29 (1981) 3363–3368.

Watson, M.L., Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol., 4 (1958) 475–479.

Weissler, A., Sonochemistry: the production of chemical changes with sound waves. J. Acoust. Soc. Am., 25 (1953) 651–657.

Widder, K.J., Morris, R.M., Poore, G. and Howard, D.P., Tumor remission in Yoshida sarcoma-bearing rats by selective targeting of magnetic albumin microspheres containing doxorubicin. Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 579–581.

Widder, K.J., Senyei, A. and Ranney, D.F., In vitro release of biologically active adriamycin by magnetically responsive albumin microspheres. Cancer Res., 40 (1980) 3512–3517.

Yoshioka, T., Hashida, M., Muranishi, S. and Sezaki, H., Specific delivery of mitomycin C to the liver, spleen and lung: nano- and microspherical carriers of gelatin. Int. J. Pharm., 81 (1981) 131–141.