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Uptake of surfactant-coated poly(methyl methacrylate)-nanoparticles by bovine brain microvessel endothelial cell monolayers

Gerrit Borchard [a,b,*], Kenneth L. Audus [a], Fenglin Shi [a], Jörg Kreuter [b,*]

[a] *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA*
[b] *Institut für Pharmazeutische Technologie, J.W. Goethe-Universität, Frankfurt, Germany*

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Abstract

The objective of this study was to determine the influence of coating of radiolabeled poly(methylmethacrylate) nanoparticles (PMMA nanoparticles) with surfactants on their uptake by bovine microvessel endothelial cell cultures (BMEC cultures). For this purpose, BMEC cultures were grown in 24-well culture plates, where they formed confluent monolayers 10–12 days after seeding. The nanoparticle suspensions were then incubated with the cell cultures at 37°C, and the radioactivity within the cell cultures and the supernatant was measured after 30 min, 2 h and 6 h. The rates of uptake of the coated nanoparticles and of a control group of uncoated nanoparticles were compared.

Key words: Blood-brain barrier; Bovine microvessel endothelial cell; Poly(methylmethacrylate) nanoparticles; Endocytosis

1. Introduction

The targeting of drugs to certain organs and tissues and the corresponding benefits, i.e., lower drug dose and fewer drug side effects, have become one of the major objectives in recent drug delivery research. In order to achieve drug delivery to specific organs, special drug carriers are required, especially if the targeting of drugs to the brain and their transport through the blood-brain barrier (BBB) into the central nervous system is intended.

The use of drug carriers such as liposomes (Zhou and Huang, 1992), erythrocyte ghosts (Zimmermann et al., 1978), antibody conjugates (Edwards, 1983; Frankel et al., 1986), monoclonal antibody conjugates (Illum et al., 1983; Brodsky, 1988), and nanoparticles (Kreuter, 1983a; Tröster et al., 1990) for targeted drug delivery has been examined. One of the major problems in targeted drug delivery is the rapid opsonization and uptake of injected carriers by the reticuloendothelial system (RES), especially by macrophages in the liver and spleen. This obstacle may be partially overcome in the case of liposomes by the incorporation of so-called 'stealth' lipids, such as

* Corresponding author.

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phosphatidylinositol (Wassef et al., 1984), monosialganglioside, or sulfogalactosylceramide (Wassef et al., 1991). In the case of nanoparticles, it was shown in an in vivo study that the body distribution of intravenously administered radiolabeled poly(methyl-methacrylate) (PMMA) nanoparticles as a model carrier in rats could be altered by coating with nonionic surfactants such as poloxamers, poloxamines, and polysorbates (Illum et al., 1986, 1987; Tröster et al., 1990). The modification of the surface properties of the particles, especially an increase in hydrophilicity, led to a prolonged circulation time in the blood stream. This, in turn, led to lower particle concentrations in organs and tissues belonging to the RES, such as liver, spleen, lungs, bone marrow and lymph nodes, and significantly higher levels in the blood and non-RES organs, i.e., heart, brain, intestine, kidneys, muscles, and blood.

An in vitro model of the blood-brain barrier (BBB) gave us the opportunity to elucidate differences in the interactions of nanoparticles coated with various surfactants with bovine brain microvessel endothelial cells (BMEC). This model has been extensively characterized morphologically, biochemically, and immunohistochemically in previous studies (Audus and Borchardt, 1987; Audus et al., 1990), and used in several transport and metabolism investigations (Van Bree et al., 1988; Guillot et al., 1990; Raub and Audus, 1990). The characteristics of the model's functions were found to be consistent with the BBB in vivo.

The surfactant coatings showing the greatest enhancing effects on brain uptake of radiolabeled PMMA nanoparticles in the in vivo studies (polysorbates 20 and 80, poloxamers 188, 338, 407, and 184, polyoxyethylene 23-lauryl ether and poloxamine 908) were tested, and compared to a control group of uncoated nanoparticles.

2. Materials and methods

2.1. Phosphate-buffered saline (PBS)

The buffer used for the surfactant solutions as well as for the incubation of the particles consisted of 7.6 g Na_2HPO_4, 1.5 g KH_2PO_4 and 4.8 g NaCl in 1000 ml distilled water, with the resulting physiological pH of 7.4.

2.2. Poly(methylmethacrylate) nanoparticles

The particles were prepared by polymerisation out of a methyl[2-^{14}C]methacrylate solution in PBS by γ-radiation (^{60}Co source, 500 krad with a rate of 2.2 krad/min) (Kreuter, 1983b) at the Eidgenössische Forschungsanstalt Wädenswil, Switzerland. The resulting freeze-dried powder contained 43.7% PMMA nanoparticles, and showed an activity of 4 mCi/g (37 MBq/g).

2.3. Surfactants

For the coating of the nanoparticles prior to incubation, the following surfactants were used as received: Poloxamers 184, 188, 338, 407 (POE-POP block copolymers, obtained from C.H. Erbsloeh, Düsseldorf, Germany); poloxamine 908 (ethylenediamine POE-POP block copolymer, C.H. Erbsloeh); polysorbates 20 and 80 (Atlas Chemie, Essen, Germany); and polyoxyethylene 23-lauryl ether (Fluka, Buchs, Switzerland).

2.4. Cell isolation and culture

The BMEC were isolated from the gray matter of the cerebral cortex of bovine brain and stored in dimethylsulfoxide at −70° C prior to use (Audus and Borchardt, 1987). Thawed and rinsed BMEC were suspended in culture medium (45% (v/v) mimimum essential medium, 45% (v/v) F-12 nutrient mixture (Ham), 10% (v/v) equine serum, 100 μg/ml heparin, 50 μg/ml gentamycin, and 2.5 μg/ml amphotericin B), and were seeded at 50000 cells/cm^3 into 24-well culture plates that had been precoated with rat tail collagen and 25 μg/ml human fibronectin.

The plates were then incubated at 37°C with 5% (v/v) CO_2. The formation of confluent monolayers was established after 10–12 days.

2.5. Coating of particles

0.5 ml of a nanoparticle stock suspension (1% (w/v) in PBS) was mixed with 0.1 ml 0.5% (w/v)

surfactant solution, and incubated overnight at room temperature.

2.6. Incubation of particle suspension

To each well, 0.6 ml particle/surfactant suspension and 0.9 ml culture medium were added, with a resulting surfactant concentration of 0.03% (w/v). The plates were incubated at 37°C with 5% (v/v) CO_2 for 30 min, 2 h, and 6 h. 0.5 ml of uncoated nanoparticle suspension was used as a control. The experiments were run in triplicate for each surfactant solution and the control.

2.7. Measurement of radioactivity

After incubation, the supernatant of each well was pipetted into scintillation vials and the wells were washed three times with ice-cold PBS. The cell monolayers were then solubilized with 1 ml 0.05% (w/v) Triton® X-100 solution and also pipetted into scintillation vials.

The radioactivity present in the monolayers was considered to be due to nanoparticles taken up by or firmly attached to the cell membranes of BMEC. 10 ml of scintillation cocktail was added to each vial, and the radioactivity was measured in a Beckman scintillation counter.

3. Results

BMEC layers treated with the control suspension of uncoated nanoparticles showed only a small increase in radioactivity, which remained stable for 6 h. Coating of the nanoparticles with poloxamer 338 and poloxamine 908 led to no significant increase in uptake, compared to the control group (Fig. 1 and 2).

The concentrations of nanoparticles coated with polyoxyethylene 23-lauryl ether and polysorbate 20 (Fig. 3 and 4) in the BMEC increased only slowly with time, but were significantly higher than for the control group after 6 h. A different rate of uptake was achieved by coating with the poloxamers 184, 188, and 407 (Fig. 5–7): here, the initially low concentrations remained stable for 2 h, but increased after 6 h. The highest

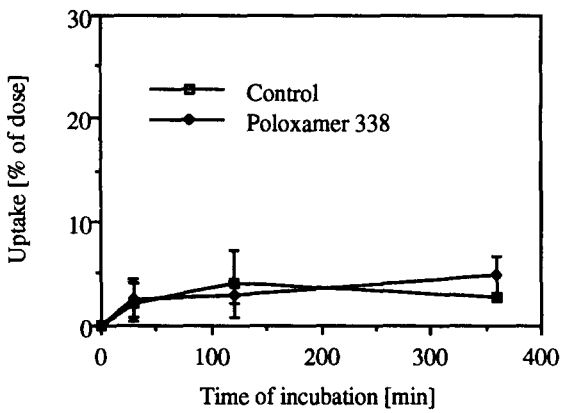

Fig. 1. Uptake of poloxamer 338-coated nanoparticles, compared to control.

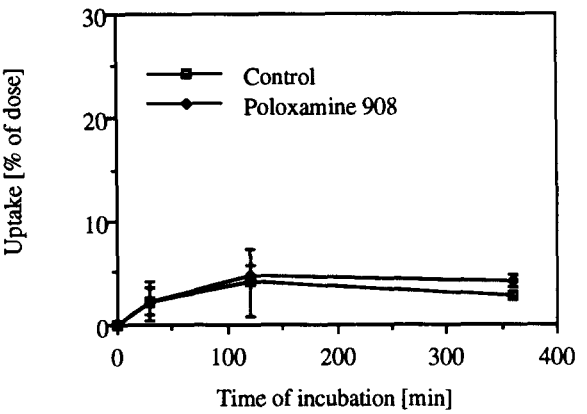

Fig. 2. Uptake of poloxamine 908-coated nanoparticles, compared to control.

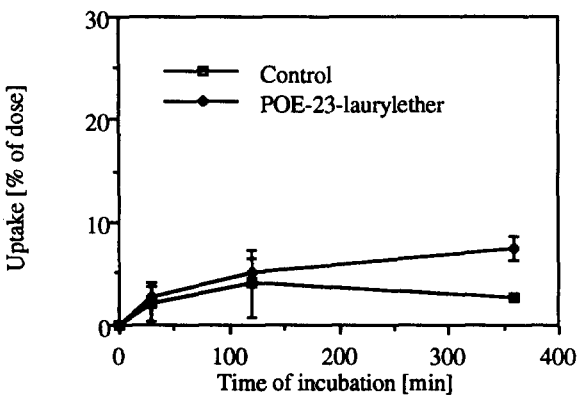

Fig. 3. Uptake of polyoxyethylene 23-lauryl ether-coated nanoparticles, compared to control.

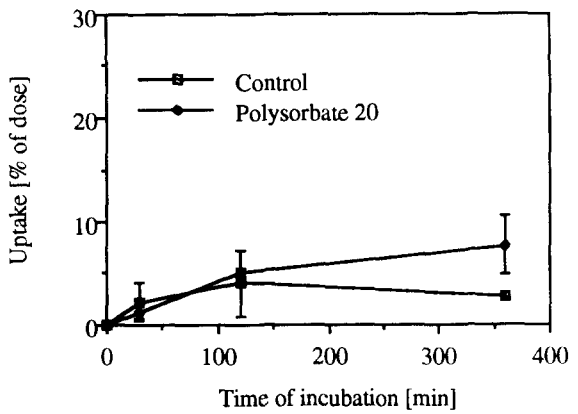

Fig. 4. Uptake of polysorbate 20-coated nanoparticles, compared to control.

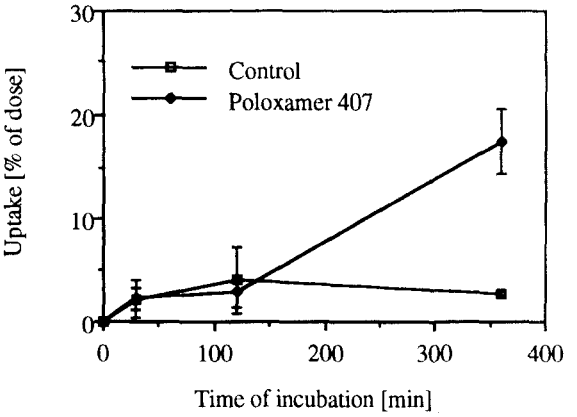

Fig. 7. Uptake of poloxamer 407-coated nanoparticles, compared to control.

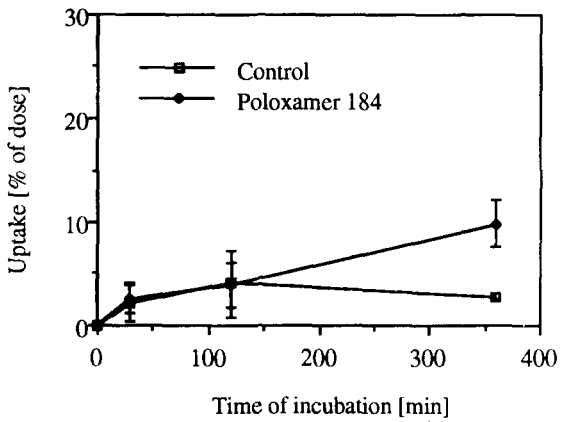

Fig. 5. Uptake of poloxamer 184-coated nanoparticles, compared to control.

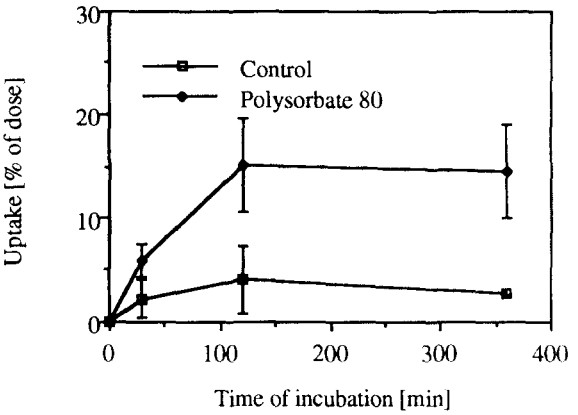

Fig. 8. Uptake of polysorbate 80-coated nanoparticles, compared to control.

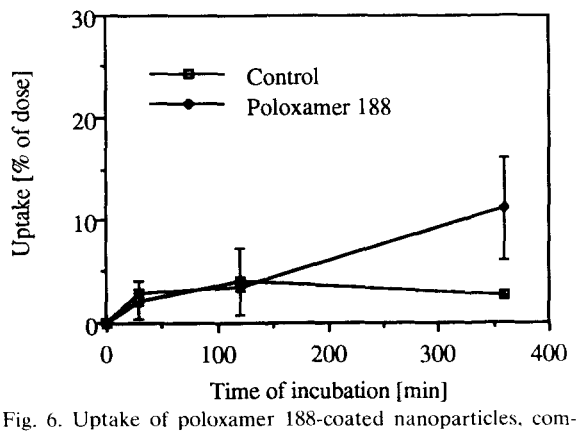

Fig. 6. Uptake of poloxamer 188-coated nanoparticles, compared to control.

concentration after 6 h was found for nanoparticles coated with poloxamer 407 (17.47 ± 3.09% of the total activity).

Coating with polysorbate 80, the most efficient substance for brain targeting in the in vivo studies (Tröster et al., 1990), led to an uptake of 15.07 ± 4.48% of the total amount of radioactivity after 2 h, which remained stable for up to 6 h (14.64 ± 4.43%) (Fig. 8).

4. Discussion

The application of an in vitro model of the blood brain barrier gave us the opportunity to

examine the interaction of coated nanoparticles with specific tissues. However, this simplification – like any model – implies the limitation to fewer parameters than actually existing in vivo. In this model, the influence of serum components, blood cells and enzymes, or even of the blood flow itself, are neglected. In order to achieve specific targeting, the coating of the particles should lead to the following effects: prolongation of circulation in the blood stream, specific attachment to certain tissues, and enhancement of uptake by the cells of the targeted tissue. In the present study, the second and third effects are examined by comparing quantitatively the influence of several coatings on the uptake of PMMA nanoparticles by BMEC to a control group of uncoated nanoparticles.

Depending on their influence on the rate of uptake of the nanoparticles by BMEC monolayers, the several coating materials can be divided into four groups.

4.1. Control, poloxamer 338 and poloxamine 908

Incubation of the control suspension resulted, as expected, in low concentrations in the cell layer over the whole experimental time period. Coating with ploxamer 338 and poloxamine 908 also yielded low concentrations after 6 h (Fig. 1 and 2), in contrast to the in vivo data (Tröster et al., 1990), where both substances significantly increased nanoparticle uptake into the brain (up to 12-fold after 30 min).

4.2. Polyoxyethylene 23-lauryl ether and polysorbate 20

Compared to the control group, coating with these two substances led to slowly increasing concentrations (Fig. 3 and 4). These results correlate to the in vivo study (Tröster et al., 1990), where these two substances also showed only a slight enhancing effect on the particle uptake by brain cells.

4.3. Poloxamers 184, 188 and 407

Different rates of uptake were observed for the particles coated with poloxamers 184, 188, and 407 (Fig. 5–7). During the initial 2 h, their concentrations within the endothelial cells were at the same level as those of the control group. Thereafter, they increased considerably, those of the poloxamer 407 group (Fig. 7) after 6 h being the highest concentrations observed in this study.

This effect might be explained by alterations of the cell membranes, even though surfactant concentrations were held far below the concentration inducing hemolysis of human red blood cells (Geyer et al., 1955), and the BMEC did not show any signs of lysis under the microscope.

However, the values for the 30 min and 2 h time points in the present in vitro study did not correlate to the in vivo data (Tröster et al., 1990), where coating of the nanoparticles with poloxamers 184 and 407 resulted in an increase in concentrations of up to 7-fold after 30 min.

4.4. Polysorbate 80

Coating with polysorbate 80 enhanced nanoparticle uptake by a factor of 5, compared to the control group of uncoated nanoparticles (Fig. 8). This result strongly supports the findings in the in vivo studies (Tröster et al., 1990), where coating with polysorbate 80 resulted in an increase in uptake by a factor of up to 20 after 6 h. In former studies (Azmin et al., 1985; Sakane et al., 1989), the potency of polysorbate 80 to increase the uptake of drugs like D-kyotorphin and methotrexate had been shown. Our in vivo and in vitro studies now seem to reveal the ability of polysorbate 80 to increase the uptake of particulate carriers like nanoparticles by the cells of the blood brain barrier.

A comparison of the in vivo uptake (as a percentage of the dose initially administered), and the uptake enhancement factor of the in vitro experiments (BMEC concentration of coated nanoparticles/BMEC concentration of uncoated nanoparticles) of the surfactant coatings used in this study after 30 min and 2 h are depicted in Fig. 9 and 10. However, the correlation coefficients between the in vivo and in vitro data for most of the surfactant coatings examined are rather low. Nevertheless, polysorbate 80-coated nanoparticles in both cases, in vivo and in vitro,

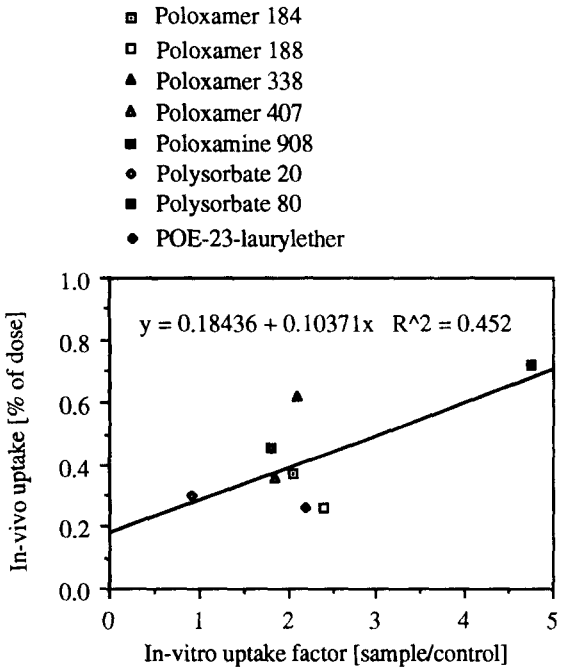

Fig. 9. Correlation between in vivo uptake and in vitro uptake factor after 30 min.

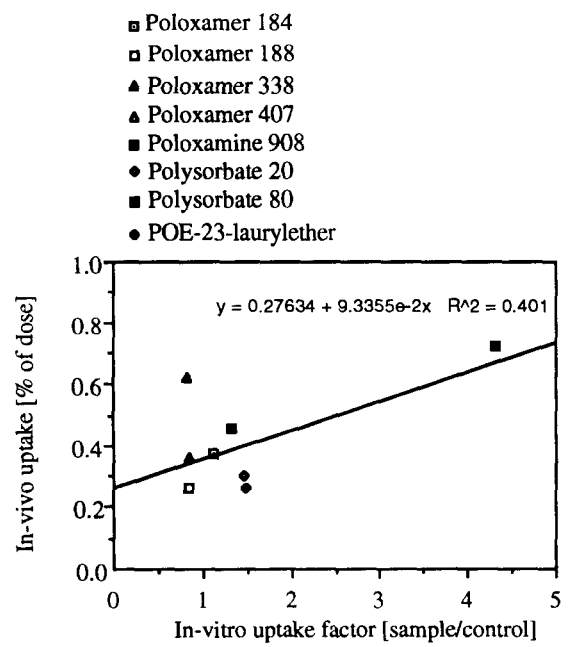

Fig. 10. Correlation between in vivo uptake and in vitro uptake factor after 2 h.

yielded increased accumulation or uptake in the brain tissues compared to the other surfactant coatings used in this study.

5. Conclusions

Cultured BMEC monolayers can serve as a model to study the influence of coating on the uptake of nanoparticles by the blood-brain barrier. Previous in vivo results were confirmed for one 'lead substance' for brain targeting, polysorbate 80.

As pointed out above, in this study it was not possible to differentiate between firm attachment of the particles to the cell membranes and uptake into the cells. However, even the enhanced attachment of polysorbate 80-coated drug carriers to the endothelial cells of the BBB would increase local drug concentrations and uptake into the target organ, the brain. Transport studies with side-by-side diffusion cells may show a possible uptake and transport process by means of persorption and/or transcytosis of the particles. In order to determine more about the mechanism of uptake, the temperature dependence of the process should be elucidated.

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References

Audus, K.L. and Borchardt, R.T., Bovine brain microvessel endothelial cell monolayers as a model for the blood-brain barrier. *Ann. N.Y. Acad. Sci.*, 507 (1987) 9–18.

Audus, K.L., Bartel, R.L., Hidalgo, I.J. and Borchardt, R.T., The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. *Pharm. Res.*, 7 (1990) 435–451.

Azmin, M.N., Stuart, A.F.B. and Florence, A.T., The distribution and elimination of methotrexate in mouse blood and brain after concurrent administration of polysorbate 80. *Cancer Chemother. Pharmacol.*, 14 (1985) 238–242.

Brodsky, F.M., Monoclonal antibodies as magic bullets. *Pharm. Res.*, 5 (1988) 1–9.

Edwards, D.C., Targeting potential of antibody conjugates. *Pharm. Ther.* 23 (1983) 238–242.

Frankel, A.E., Houston, L.L., Issel, B.F. and Fatham, G., Prospects for immunotoxin therapy in cancer. *Annu. Rev. Med.*, 39 (1986) 125–142.

Geyer, R.P., Olsen, F.R., Andrus, S.B., Waddell, W.R. and Stare, F.J., Preparation of fat emulsion for intravenous alimentation. *J. Am. Oil Chem. Soc.*, 32 (1955) 365; cited in product information, C.H. Erbslöh, Düsseldorf.

Guillot, F.L., Audus, K.L. and. Raub, T.J., Fluid-phase endocytosis by primary cultures of bovine brain microvessel endothelial cell monolayers. *Microvasc. Res.*, 39 (1990) 1–14.

Illum, L., Davis, S.S., Müller, R.H., Mak, E. and West, P., The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a blockcopolymer – poloxamine 908. *Life Sci.*, 40 (1987) 367–374.

Illum, L., Hunneyball, I.M. and Davis, S.S., The effect of hydrophilic coatings on the uptake of colloidal particles by the liver and by peritoneal macrophages. *Int. J. Pharm.*, 29 (1986) 53–65.

Illum, L., Jones, P.D.E., Kreuter, J., Baldwin, R.W. and Davis, S.S., Adsorption of monoclonal antibodies to polyhexylcyanoacrylate nanoparticles and subsequent immunospecific binding to tumour cells in vitro. *Int. J. Pharm.*, 17 (1983) 65–76.

Kreuter, J., Evaluation of nanoparticles as drug-delivery systems: III. Materials, stability, toxicity, possibilities of targeting and use. *Pharm. Acta Helv.*, 58 (1983a) 242–250.

Kreuter, J., Physicochemical characterization of polyacrylic nanoparticles. *Int. J. Pharm.*, 14 (1983b) 43–58.

Raub, T.J. and Audus, K.L., Adsorptive endocytosis and membrane recycling by cultured primary bovine brain microvessel endothelial cell monolayers. *J. Cell Sci.*, 97 (1990) 127–138.

Sakane, T., Tanaka, C., Yamamoto, A., Hashida, M., Sezaki, H., Ueda, H. and Takagi, H., The effect of polysorbate 80 on brain uptake and analgesic effect of D-kyotorphin. *Int. J. Pharm.*, 57 (1989) 77–83.

Tröster, S., Müller, U. and Kreuter, J., Modification of the body distribution of poly(methylmethacrylate) nanoparticles in rats by coating with surfactants. *Int. J. Pharm.*, 61 (1990) 85–100.

Van Bree, J.B.M.M, Audus, K.L. and Borchardt, R.T., Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharm. Res.*, 5 (1988) 369–371.

Wassef, N.M., Matyas, G.R. and Alving, C.R., Complement-dependent phagocytosis of liposomes by macrophages: suppressive effects of 'stealth' lipids. *Biochem. Biophys. Res. Commun.*, 176 (1991) 866–874.

Wassef, N.M., Roerdink, F., Richardson, E.C. and Alving, C.R., Suppression of phagocytic function and phospholipid metabolism in macrophages by phosphatidylinositol liposomes. *Proc. Natl. Acad. Sci. USA*, 81 (1984) 2655–2659.

Zhou, X. and Huang, L., Targeted delivery of DNA by liposomes and polymers. *J. Controlled Release*, 19 (1992) 269–274.

Zimmermann, U., Pilwat, G. and Esser, B., The effect of encapsulation in red blood cells on the distribution of methotrexate in mice. *J. Clin. Chem. Clin. Biochem.*, 16 (1978) 135–144.