Cells and Materials

Volume 3 | Number 1 Article 5

1993

Evaluation of Drug Delivery Systems by Electron Microscopy Techniques

Y. V. Pathak
South Dakota State University

V. D. Labhashetwar
University of Michigan

Cells and Materials, Vol. 3, No. 1, 1993 (Pages 51-66) 1051-6794/93$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

EVALUATION OF DRUG DELIVERY SYSTEMS BY ELECTRON MICROSCOPY TECHNIQUES

Y.V. Pathak* and V.D. Labhashetwar[1]

College of Pharmacy, South Dakota State University, Brookings, SD
[1]Department of Pediatric Cardiology, University of Michigan, Ann Arbor, MI

(Received for publication December 26, 1992, and in revised form February 27, 1993)

Abstract

This review covers the applications of electron microscopic techniques to the characterization and evaluation of drug delivery systems. Various electron microscopic (EM) related techniques such as transmission electron microscopy, scanning electron microscopy (SEM), SEM with energy dispersive X-ray microanalysis, and freeze-fracture electron microscopy are extensively used for this purpose. Microcapsules, microspheres, nanocapsules, liposomes, polymeric carriers and other drug delivery systems are characterized using EM related techniques for their surface topography, size and shape analysis, biodegradation, *in vitro - in vivo* evaluation and drug excipient interactions and characterization. Electron microscopy methods are very useful in understanding the mechanism of drug carrier formation, drug release in *in vitro - in vivo* situations, and drug carrier - body fluid interactions. EM techniques have also been used to study ultra-structural disposition of drug carriers in body compartments.

Key Words: Electron microscopy, drug delivery systems, drug carriers.

*Address for correspondence:
Y.V. Pathak
College of Pharmacy
Box 2202C
South Dakota State University
Brookings, South Dakota 57007-0099
Telephone number: 605-688-5314
FAX number: 605-688-6232

Introduction

Drug use must be as old as the human race. The administration of drugs to the human body has been a constant challenge for the scientists working in health related fields. The two major approaches for the administration of drugs are: **a**). Conventional dosage forms, for examples: tablets, capsules, emulsions, solutions, etc.; and **b**). Controlled drug delivery systems, for examples: microcapsules, nanocapsules, polymeric carriers, liposomes, etc.

Both approaches have two common ingredients: the active drug and the excipients being used to formulate the dosage. The broad differences between these two are the mechanism and site of release. These factors have played an important role in the advantageous applications of drug delivery systems over conventional dosage forms.

In recent years, considerable attention has been targeted on the development of new drug delivery systems. A few of the important reasons for this extensive research on drug delivery systems by the pharmaceutical industry, academia, and related health scientists are:

a) The high cost of new drug development has initiated research to find suitable, efficient ways of delivering existing drugs with a higher therapeutic efficiency.

b) The repatentability of the drug delivery systems for the existing drugs.

c) New systems of delivery are needed for the transport of novel genetically engineered pharmaceuticals, i.e., peptides and proteins, to their sites of action without incurring significant adverse immunogenical response or biological inactivation.

d) The targeting of very potent drugs, especially in cancer therapy, to avoid excessive side effects.

e) The therapeutic efficacy and safety of the drugs can be improved by administering them as controlled release or polymeric delivery systems for transdermal administration.

f) Finally drug delivery systems can utilize electronic and computer technology to deliver the drug at a particular target in the body; for example, pulsed and/or self regulated drug delivery systems.

Table 1. EM related techniques used in characterizing and evaluating drug delivery systems (DDS).

EM Technique	Drug Delivery System	References
Scanning Electron Microscopy	Microspheres	6, 8, 10, 12, 16, 17, 21, 27, 36, 38, 49, 50, 55, 57, 66, 73, 90, 95, 110, 113, 115, 116, 137, 138, 141, 149, 156, 157, 161, 162, 165, 166, 172, 173.
	Microcapsules	8, 15, 20, 23, 48, 63, 76, 77, 81, 82, 83, 93, 96, 102, 105, 107, 109, 114, 119, 126, 134, 135, 139, 146, 147, 150, 151, 152, 160, 170, 179, 181.
	Matrix Tablets	28, 29, 68, 92, 98, 103, 112, 121, 180.
	Liposomes	45, 94, 118, 124, 132, 155, 176, 177.
	Nanocapsules	37, 46, 47, 91, 183.
	Polymeric Films	9, 11, 24, 26, 61, 69, 75, 86, 100, 120, 125, 130, 131, 133, 140, 159, 167, 182.
	Miscellaneous	19, 25, 34, 41, 52, 67, 78, 79, 80, 99.
Transmission Electron Microscopy	Liposomes	2, 4, 132.
	Nanocapsules	3, 5, 13, 14, 111.
	Polymeric	11, 140.
	Microcapsules	42, 77, 136, 142, 148, 174.
	Implants	72, 178.
SEM with X-Ray Microanalysis	Polymeric	61, 75, 101, 130.
	Matrix Carrier	56, 145.
	Microspheres	137.
Freeze-fracture EM	Liposomes	33, 44, 117, 122, 144, 155.

Electron microscopic (EM) techniques have been used extensively in characterizing and/or evaluating various drug delivery systems. The purpose of the present paper is to review various EM techniques used for these purposes as well as to bring together different areas related to drug delivery systems in which EM techniques have been used to characterize and/or evaluate the systems (Table 1). EM techniques have been used to characterize polymeric implantable delivery systems and some excipients used in the formulation of drug delivery systems in our laboratories. Application of scanning electron microscopy related techniques in drug delivery system formulation, development and evaluation can be classified in three phases: **a**) preformulation (Table 2); **b**) formulation and *in vitro* characterization (Table 3); **c**) *in vivo* evaluation and effectiveness (Table 4).

Various EM related techniques used for this purpose are given in Table 1, which includes scanning electron microscopy (SEM), transmission electron microscopy (TEM), SEM energy dispersive X-ray microanalysis, and freeze-fracture electron microscopy.

Applications of EM in preformulation stage

Shape, surface, and particle size distribution of drug and excipient particles

The shape, size, and surface characteristics of the drugs as well as the excipients significantly affect the therapeutic efficacy of the drug delivery systems [135]. The drug incorporation, distribution, and release from the drug delivery systems, such as, controlled release matrix tablets [28, 29, 71], microcapsules [6, 102, 105], microspheres [12, 115, 166], polymeric carriers [9, 24, 159], are affected by the size and shape of drug-excipient particles. EM techniques have been used to study the surface properties of drugs particles [35, 37] and excipients [129, 140, 163] and to relate them to their usefulness in delivery systems.

In our laboratories, maltodextrins (D-glucose saccharide polymers) for use as excipients in drug delivery formulations, have been characterized. Maltodextrins are non-sweet, nutritive saccharide polymers that consist of D-glucose units linked primarily by alpha 1-4 bonds and have a dextrose equivalent (DE) value less than 20. We evaluated maltodextrins (normal, agglomerated, and superagglomerated with different DE values), as excipients in matrix based delivery systems [123]. The bulk density of various maltodextrins M-040 (DE = 4), M-100 (DE = 10), M-150 (DE = 15), M-700 (Superagglomerated M-100) was measured by tapping 10 grams of the powder more than 100 times or until there was no change in volume. Flow properties were determined by the cone and funnel method and the angle of repose calculated (Table 5). Surface properties of maltodextrin derivatives were studied using SEM (Fig. 1 for M-040 and Fig. 2 for M-150). It was observed

Table 2. SEM applications in preformulation stage of drug delivery systems.

Parameter	References
Shape, size and surface characterization of drug and excipients	35, 38, 55, 62, 73, 84, 88, 89, 94, 97, 98, 102, 107, 108, 114-116, 128, 131, 134, 139, 143, 156, 163, 172, 181.
Particle size distribution	23, 36, 49, 53, 90, 93, 102, 105, 151.
Effect of Processing and formulation variables on drug delivery system characterization	10, 12, 14, 16, 17, 27, 30, 31, 32, 46, 48, 53, 59, 61, 63, 81, 84, 97, 103, 116, 124, 143, 156, 179.
Mechanism of compression and deformation of particles	40, 51, 54, 129.
Crystal structure and formation studies	51, 54, 129.
Granulation processing effect on particle surface	35, 38, 39, 54.
Polymeric film studies	24, 56, 69, 103, 121, 140, 145, 174.
Drug - Excipient Interaction	39, 40.
Empty microcapsule/nanocapsule studies	13, 17, 53, 58, 115.
Mechanism oriented studies	26, 29, 63.

Table 3. SEM applications in formulation and *in vitro* evaluation of drug delivery systems.

Parameter	Reference
Effect of drug loading on surface morphology of microspheres/nanocapsules	16, 20, 25, 53, 63, 81, 84, 90, 93, 97, 104, 105, 114, 115, 116, 122, 136, 139, 166, 173.
Drug distribution, morphology, permeability, *in vitro* release in polymeric carriers	56, 59, 61, 81, 84, 98, 100, 103, 112, 115, 117, 119, 130, 132, 169, 172.
Matrix tablet surface morphology and changes during *in vitro* release	28, 29, 68, 121, 180, 182.
Physical structure of dry absorbed emulsions	19, 79, 80.
Liposomes: collagen gel matrix surface characterization	99, 176.
Calcification of carriers/implant studies	72, 75, 99, 101, 158, 164, 175.
Mechanism of drug carrier formation, degradation and drug release mechanisms	15, 19, 28, 43, 53, 85, 97, 112, 113, 115, 127, 130, 140, 167, 171.

Table 4. SEM applications in characterizing and evaluating drug delivery systems *in vivo*.

Parameter	References
Effect of *in vivo* conditions on size, shape and surface of microcapsules and other drug delivery systems	42, 60, 65, 70, 71, 86, 87, 100, 133, 144, 157, 161, 162, 168.
Ultra structural disposition/distribution	42, 44, 47, 66, 74, 86, 155.
Pathological effects of drugs released	7, 45, 66, 71, 86, 142, 178.
Blood-plasma compatibility of drug carriers	1, 3, 11, 125, 168, 178.
Mechanism of drug penetration: corneal uptake, lymphatic uptake, etc.	64, 178, 183.
Bioerosion of drug delivery systems in body	22, 43, 115, 157, 161, 170.
Mechanism of drug release	65, 86.

Table 5. Micromeritic properties of maltodextrins

Maltodextrin derivative	DE value	Bulk density* g/cc	Angle of repose*
M-040	4	0.33	63°
M-100	10	0.42	39°
M-150	15	0.48	38°
M-700	10	0.15	36°

*Average of three readings.

---

that M-150 with DE value of 15 had many more elongated particles than M-040 with DE value 4. These exhibited different micromeritic and compressional properties when used as a directly compressible excipient in tablets [123]. A detailed report of the morphological characterization of maltodextrins using SEM and the correlation with micromeritic properties is being published in this issue of Cells and Materials [153].

Effect of processing techniques on drug or excipient particles

SEM has been used widely in studying particle properties and changes in surface characteristics during various processes like spheronization [88, 89], partial dissolution, swelling due to aqueous contacts, spray-drying [90], granulation, and agglomeration [35, 38, 39, 54]. SEM is a very effective tool in studying surfaces. In our studies of maltodextrins (Figs. 3 and 4), the agglomeration process significantly increased the particle size, and thus affected the bulk densities of these products (Table 5). The low bulk density of M-700 can be attributed to the highly fluffy nature of these super agglomerated products. Figs. 3 and 4 show the micrographs of M-700 powders. It is observed that the agglomeration process has led to the formation of bigger particles with large void volumes in the particles, thereby decreasing the bulk density. The spherical particles are seen to attach to each other at one point. Every particle has an empty hollow space. The agglomeration process has increased the surface area enormously thus increasing the area of contact with other components such as air, moisture, and body fluids (when incorporated in drug delivery systems).

The mechanism of compression and deformation of powders

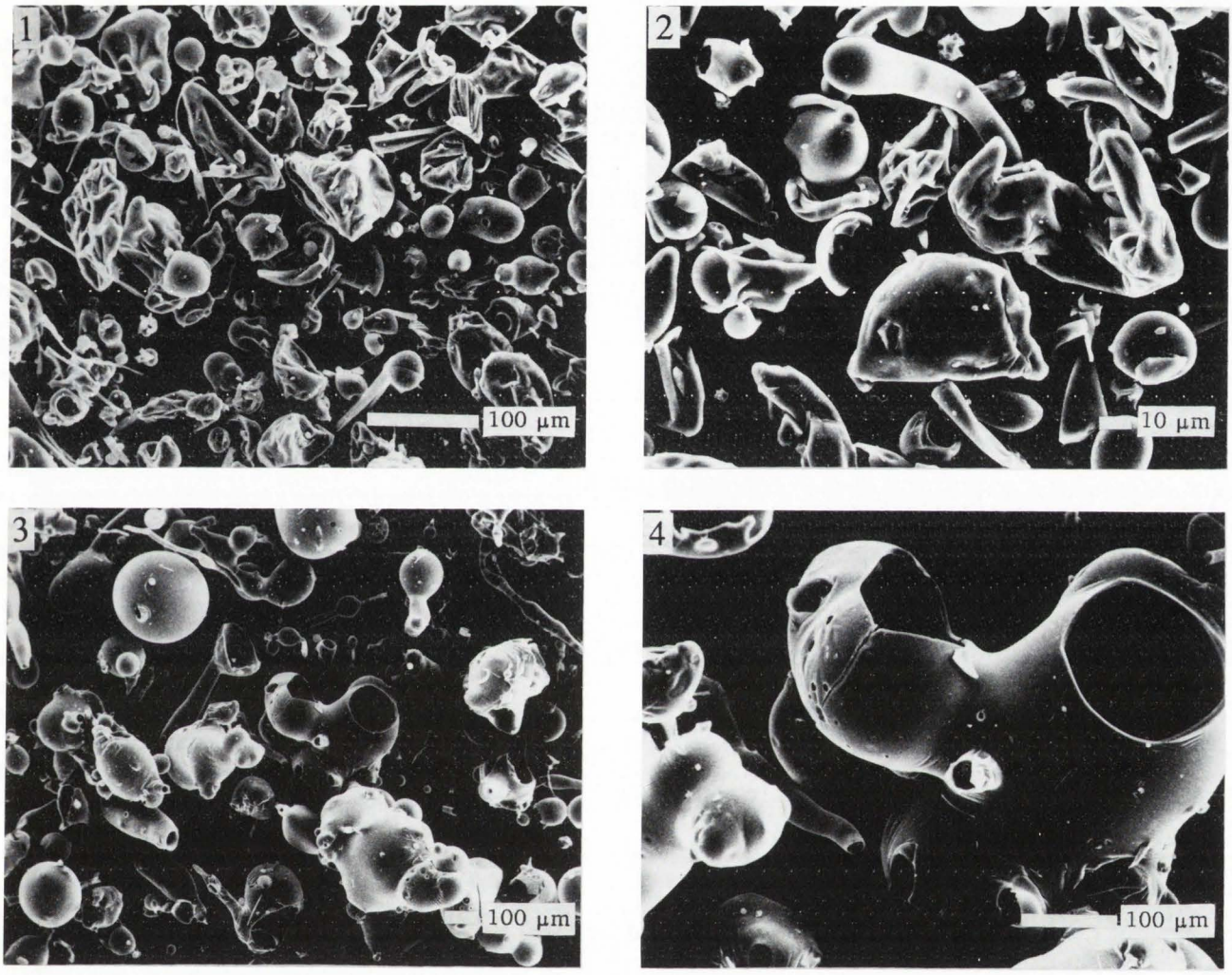
SEM techniques have been employed to study the mechanical properties of drug/excipient compacts [128], as well as the effect of compression on drug/excipient surface properties. The deformation of powders during compression and breaking has helped in understanding the mechanism of compression as well as the constitutive properties of matrix tablets which are one of the most widely used delivery systems for controlled release of drugs [39, 51, 54]. A study by Padmadisastra and Gonda [129] showed that significant changes occurred in morphological properties of bagasse based microcrystalline cellulose (MCC) during compression. It was observed that significant differences in the particle morphology of MCC from different sources affected the mean hardness, friability loss, and disintegration time of tablets prepared from these excipients.

Drug crystal formation and structure

Application of SEM in the preformulation stage of drug delivery systems is reported by Otsuka and Kaneniwa [128]. They studied the polymorphic transformation of chloramphenicol palmitate during the grinding process and have used SEM to characterize and identify the polymorphs and transformations in particle surface topography during processing. Another similar study has also reported the effect of compression on crystal structure [51]. A model has been developed to predict the uniaxial compaction behavior of compacts using the hardness and elastic moduli of brittle crystals determined by the Vickers microindentation test. SEM has been used to characterize the crystals and compacts during and after compression of acetaminophen, sucrose, and adipic acids. Electron micrographs of a fractured sucrose compact showed substantial crushing at the asperities. The SEM observations supported the assumption that the fragments arising from crushing bear no load at lower relative densities [51].

Polymeric film casting and film studies

SEM and TEM have been used to characterize polymeric films and castings of polymeric strips as drug carriers [18]. SEM has been used to give direct evidence of the morphology differences among ethylcellulose cast films with and without a drug [9]. Depending on the formulation and casting procedures, the film surfaces have been observed to be either smooth or rough with engulfed air pockets. TEM has been used in this study to reveal the influence of glass on the texture of samples [11]. It is reported that randomly distributed crystalline zones formed at the air side of the films which might account for the extreme heterogeneity of the films. Micrographs of the cross-section of films clearly indicated the heterogenous internal structure of the polymer. Hsiue *et al.* [72] in their studies on the preparation and properties of poly(2-hydroxyethyl methacrylate) (PHEMA) grafted styrene-butadiene-styrene triblock copolymer (SBS) used SEM to characterize the surface morphology of copolymers with different degrees of grafting. They observed that the morphology of the surface of the graft copolymer changed with the degree of grafting [72]. The vertical sections of the membrane showed a lower SBS layer and an upper PHEMA layer [11]. Bakker *et al.* [11] studied the biocompatibility of two silicone rubber polymers and a polyurethane polymer *in vitro*. Using SEM/TEM and quantitative analysis the epithelial morphology showed that polyurethanes and polyether-polyester copolymer have better biocompatibility than that of silastic rubber polymers [72].



Figures 1-4. Scanning electron micrographs of maltodextrin M-040 (**Fig. 1**); M-150 (**Fig. 2**); and M-700 (**Fig. 3 and 4**) powders. In **Figure 4**, one of the agglomerated particle is viewed showing spherical hollow particles agglomerated together. Bars = 100 μm (Figs. 1, 3 and 4) and 10 μm (Fig. 2).

Drug excipient interaction and characterization

The interaction between drugs and excipients which are either inert additives, sustained release polymeric matrices, or films have been extensively studied by SEM [38, 39]. Our investigation of maltodextrins has shown that the agglomeration affected the excipient properties. The micrographs for M-040 (Fig. 1), M-150 (Fig. 2), and M-700 (Fig 4) show the effects of agglomeration and superagglomeration on the particle shape and surface of maltodextrin. The M-700 powder had a low bulk density (Table 5) which correlated with hollow structures containing air pockets in the maltodextrin (see Fig. 3). A study of the drug loading mechanism in cross-linked polymers has been reported by Carli and Garbassi [34] using X-ray photoelectron spectroscopy. SEM also has been used to examine the surface characteristics of drug excipient mixtures [39]. Figure 5 shows a micrograph of griseofulvin-crospovidone matrix system used as a drug carrier [34].

Empty microcapsule and nanocapsule studies

SEM has been employed in studying the development of empty microcapsule and nanocapsule formulations using several polymers [13, 17]. Benita *et al.* [17] reported the preparation of carnauba wax microspheres with and without a drug (5-Fluorouracil) to show how various process parameters affect the preparation and properties of carnauba wax: 5-fluorouracil microspheres [17]. A similar study has been reported for nanocapsules [13]. The surface characterization of empty carriers has been performed SEM and TEM in both studies [13, 17]. These studies have been aimed at exploring the effects of various formulation and processing variables on surface, shape and size of microcapsules [53, 58] and microspheres [115]. TEM was found to be more useful for characterizing nanocapsule formulations [3, 5, 13, 15, 111].

SEM Applications in Formulation and *In Vitro* Characterization of Drug Delivery Systems

Effect of drug loading

The objectives of the formulator in designing drug delivery systems is to explore the possibility of administering the desired amount of drug to the body. The desired amount varied from drug to drug. Hence, incorporation of different amounts of drugs (drug loading) in drug carriers has been extensively studied in drug delivery systems. SEM and TEM have been employed in studying drug loading effects on microcapsules [106, 107], microspheres [16, 110], nanocapsules [16, 46, 93], and polymeric film carriers [130, 131]. It was observed that the surface characteristics were significantly changed by the degree of drug loading. In one of our studies [12, 16] we have incorporated nifedipine in polymethacrylate microspheres with drug loadings from 5 to 31% and found that the smooth surface present at 5% drug loading was gradually shifted to a very rough surface at 31% incorporation which affected the stability and *in vitro* drug release from these microspheres. In a study by Kawashima *et al.* [90] controlled release microspheres have been prepared by a quasi emulsion solvent diffusion method for Ibuprofen. SEM has been used to study the surface topography of various microsphere formulations depicting the differences in surface topography with different polymers [90].

Drug distribution, morphology, permeability, and *in vitro* release in polymeric film carriers (matrices)

Polymeric carriers are widely used, especially in the field of cardiovascular and contraceptive drug delivery systems. SEM is used for the study of drug distribution in the polymeric matrix, morphological characterization of matrices [61], permeability studies [11, 26, 133], drug loading effects [100, 101], and surface characterization during *in vitro* drug release studies [130, 131]. We have used SEM in investigating drug distribution and surface morphology during release studies for verapamil polymeric carriers (154). Figures 6 and 7 show the surface of films during pre- and post-release studies. It was observed that the drug had been uniformly distributed in the polymeric matrix. During the *in vitro* release process the particles swell and gradually leave the matrix forming holes and channels in the polymeric matrix surface (Fig. 7). A similar mechanism of release has also been observed in our other studies in which Fe^{3+}, Al^{3+}, or levamisole have been incorporated in polymeric carriers (polyurethane or silastic rubber polymers) for the prevention of heart valve calcification [130, 131].

Sustained release matrix tablets

SEM has been used for characterizing surface properties of sustained release matrix tablets as well as studying the changes which occur in the tablet surface during release [71, 98]. Surface properties and uniformity of film coating in coated tablets used for sustaining the drug release have been also characterized [28, 29].

Emulsions

Another interesting application of SEM has been in characterizing emulsions. Oral sustained release of drugs has been reported to be obtained by adsorbing the emulsion on silica. SEM was used to locate the drug crystals engulfed in the oil droplet adsorbed on silica particles [19]. SEM was also used in characterizing emulsions carrying microcapsules [80]. Microspheres containing multiple emulsions and coated emulsion particles with polysaccharides have been characterized using SEM. In a study of the coating of surfaces of oil in water (o/w) emulsions using polysaccharides bearing a cholesterol moiety, Iwamoto *et al.* [79] successfully used SEM to determine the shape, size, and surface properties of the emulsion systems (Fig. 8).

Formulation, surface characterization of liposomes

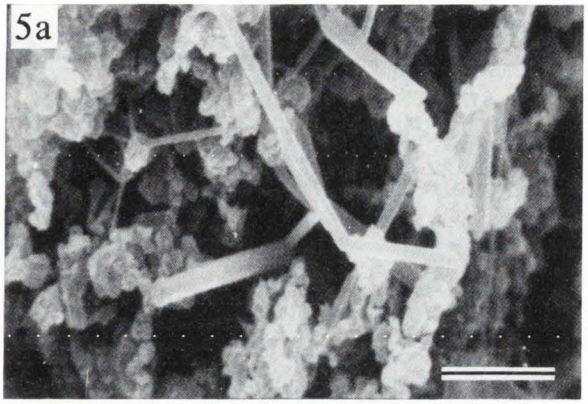
TEM [2, 5, 132] and freeze fracture electron microscopy [33, 44, 117] have been used extensively to characterize surface morphology of liposome-drug delivery systems [122]. TEM was used to study the bilayer structure of polymerized liposomes. TEM helped in revealing that the polymerization reaction (between phosphatidyl choline vesicles stabilized by polycholesteryl methacrylate and carboxy methyl chitosan) increased the vesicle membrane stability [2]. Freeze fracture electron microscopy was employed in investigations of surface properties as well as drug incorporation studies [145, 155]. Metha *et al.* [118] evaluated liposomes containing amphotericin B for toxic and antifungal activities. Figure 9 shows a micrograph of liposomes.

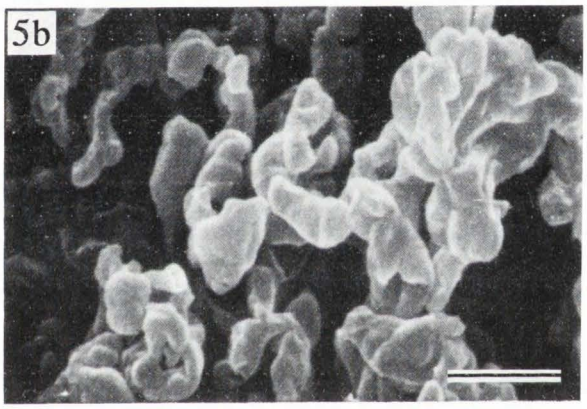
Calcification of drug carriers

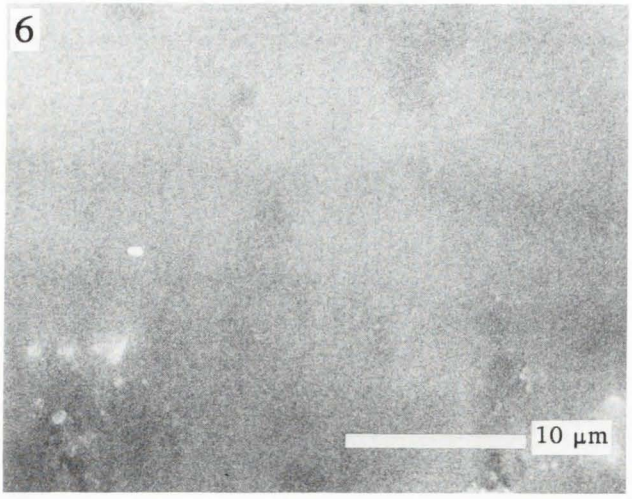
Calcification is a normal physiological phenomenon. Several drug carriers, such as, polyurethane and hydrogels [75, 158] undergo calcification. Furthermore, bioprostheses based on either natural tissues or synthetic polymers also undergo calcification [69, 99, 101]. SEM has been used to characterize the surface changes in the heart valve bioprosthesis due to the calcification processes when implanted in rats [146, 175].

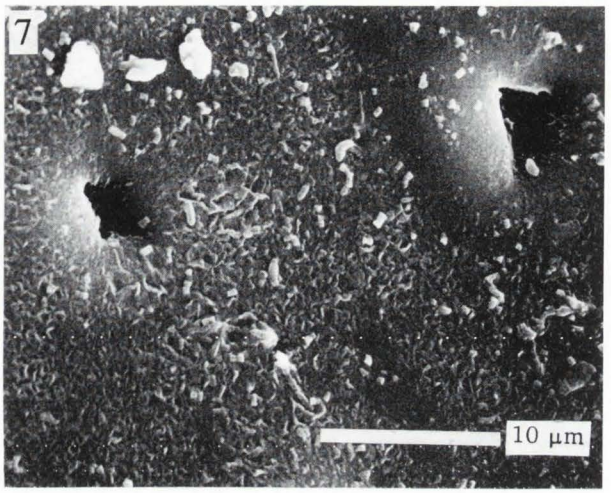
Mechanism of drug carrier formation, degradation and drug release mechanism from biodegradable polymeric carriers

SEM has been used in characterizing the formation of microcapsules and microspheres by such techniques as polycondensation [8], coacervation [23, 48, 107], or solvent evaporation [15, 106, 157]. The effect of processing and formulation variables on surface topography was studied by SEM [20, 81, 82]. Microcapsule interactions with water or body fluids *in vitro*, and changes in their properties have been critically observed with the SEM [63, 76]. In the case of biodegradable polymeric carriers, the biodegradation during contact with body fluids was observed by SEM [53]. This helped in understanding the kinetics and mechanisms of biodegradation as well as of drug release from these carriers [85, 112, 113, 116]. TEM is reported to be useful in studying ultrastructural characterization of









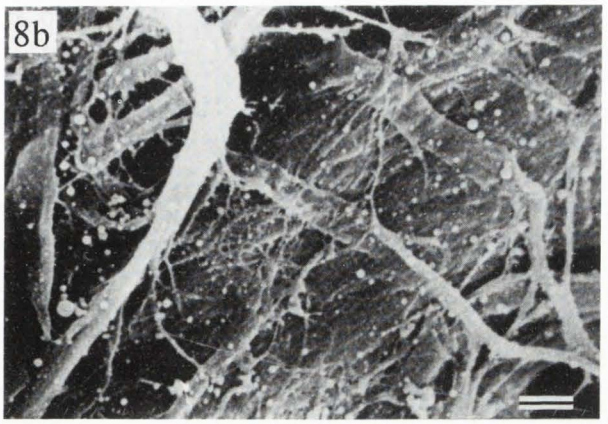


Figure 5. Scanning electron micrograph of griseofulvin-crospovidone carrier system. (**a**) 1:5 w/w, bar = 10 μm; (**b**) 1:10 w/w, bar = 4 μm (from reference 34 with permission from the Amer. Pharmaceutical Association).

Figure 6. Scanning electron micrograph of sustained release polymeric carriers (films) of verapamil, showing uniform distribution of drug in polymer film. Bar = 10 μm.

Figure 7. Scanning electron micrograph of sustained release polymeric carrier (films) of verapamil during *in vitro* release studies after 3 weeks showing particle swelling and hole formation due to loss of drug particles during *in vitro* release studies. Bar = 10 μm.

Figure 8. Scanning electron micrographs of (**a**) conventional and (**b**) CHE:200 = 1:8 coated oil droplets in o/w emulsions on a filter paper. The CHM:200 coated droplets were prepared from 1 ml of conventional o/w lecithin emulsions and 7.2 mg CHM:200 = 1:8; bars = 2 μm (from ref. 79 with permission from the Amer. Pharmaceutical Association).

microcapsules and nanocapsules. The semipermeable membranes in microcapsules have been characterized by TEM [14, 41, 77, 174]. Another study reported the application of SEM in studying the internal structure of polyamide microcapsules and the effect of irradiation on microcapsule surface properties [114]. Figures 10 and 11 show the typical nanocapsule formulations viewed by TEM [4].

SEM Applications in Characterizing and Evaluating Drug Delivery Systems *In Vivo*

Effect of *in vivo* conditions on size, shape, and surface of drug delivery systems

In vivo interaction of various drug delivery systems, such as microcapsules [41, 144], microspheres [65, 157, 162], liposomes [44, 45], and polymeric carriers, have been studied extensively using EM and other related techniques [70, 71, 86, 100, 133]. Biodegradation [46, 157], phagocytosis [161], uptake by body compartments such as liver, Kupffer cells, etc. has been observed [45, 74]. Clayton *et al.* [41] studied the effect of capsule composition on the biocompatibility of alginate-poly-l-lysine capsules. Empty capsules from high mannuronic acid alginate have been prepared and coated with poly-l-lysine alone, poly-l-lysine and guluronic acid and poly-l-lysine and high mannuronic acid alginate. The capsules have been placed in renal subcapsular space or the peritoneal cavity and retrieved after 3 weeks for histological and electron microscopical study to evaluate interaction of body cells and drug carriers as well as the fate of drug carriers in *in vivo* conditions. The infiltrating cells in the capsule surface have been characterized to be mostly fibroblasts and macrophages. Similar studies have also been reported [157, 170]. It is also reported by Krause *et al.* [92] that liposomes formed aggregates (when administered intravenously) during circulation thus affecting the drug release.

Ultrastructural disposition and distribution of carrier particles

The uptake of the carrier particles in various body compartments [8, 45, 66], their distribution throughout the body [44, 46, 74], the accumulation [75], and ultrastructural disposition has been studied using SEM. The microcapsules and nanocapsules administered orally [74] or by a parenteral route [157] have been monitored to study their distribution and accumulation in the body. SEM was found to be helpful in localizing these carriers in various tissues in the body [161]. Illum *et al.* [74] used SEM to study the distribution of model carrier particles (polystyrene microspheres) in the reticuloendothelial system. Figures 12 and 13 show the accumulation of these microspheres in Kupffer cells [74].

Pathological effects of drugs

Several research papers have reported the application of EM techniques to the study of pathological effects of drugs released through drug delivery systems at the target site [8, 45, 66]. This has been an especially useful in delivery system where accumulation of the drug carrier in cancer or body tissues releases the drug over sustained periods of time. The tissues have been characterized using SEM for the pathological effects due to constantly released drug over longer periods of time [68, 86]. The effects of drugs from a delivery system on cell structure have also been studied [144, 178], as part of *in vivo* evaluation of the carriers.

Blood-plasma compatibility of drug carrier

Delivery systems such as nanocapsules and liposomes can be intravenously injected and can come in contact with blood-plasma components immediately after the injection. Their compatibility with circulating blood and its contents becomes of prime importance. Absolom *et al.* [1] studied the role that substrate surface properties play in influencing the extent of endothelialization of polymer surfaces during *in vivo* implantation and the interaction of body components with polymer carriers. Published micrographs show different degrees of endothelialization for different polymer surfaces when exposed to blood *in vitro*. Other similar reports have shown applications of EM techniques in studying the biocompatibility between drug carriers and blood, interactions between them, and changes occurring during the contact time [11, 126, 168, 178].

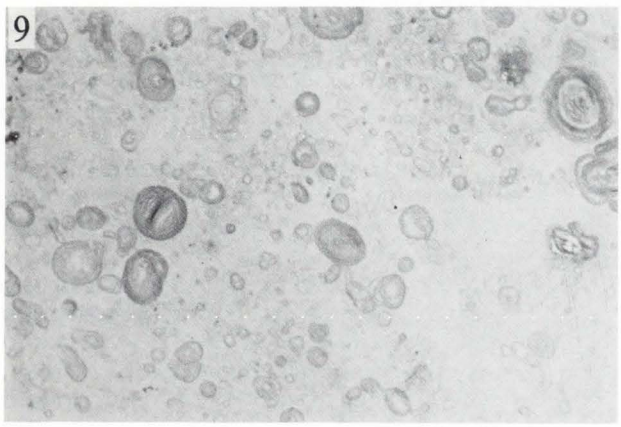
Mechanism of drug penetration from drug delivery system

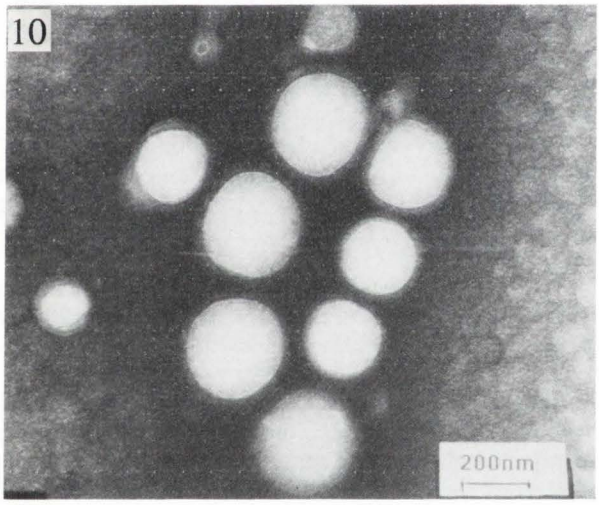
Application of SEM is reported in understanding the mechanism of drug penetration in the cornea through ocular drug delivery systems [64]. Zimmer *et al.* [180], during their studies on the transport of PBCA nanoparticles in ocular tissues, showed that the PBCA nanoparticles penetration in eye tissue is limited to the precorneal area only. Penetration through tight junctions or through whole corneal tissue did not occur. They also studied the corneal sections by laser scanning confocal microscopy and suggested an uptake mechanism by phagocytosis [183].

SEM is also used to understand the mechanism of drug uptake through lymphatic systems. Williams *et al.* [178] have reviewed the electron microscopy of endothelial cell-biopolymeric interaction.

Bioerosion of drug delivery systems *in vivo*

The drug delivery systems based on biodegradable polymers such as polyanhydrides [113, 115, 116] or polycaprolactone [14] have been studied using SEM and the bioerosion of the carriers has been monitored [167]. SEM has been useful in understanding the mechanism of surface erosion of microspheres [42, 116]. Brem *et al.* [22] studied the brain biocompatibility of a biodegradable controlled release polymer consisting of an anhydride copolymer of fatty acid dimer and sebacic acid. The biodegradable carriers were implanted in the brain. The localized reaction evoked due to polymer implantation and bioerosion of drug carrier was monitored using EM techniques. The study showed an important application of these polymers in delivering a water soluble drug by bypassing the blood-brain barrier.





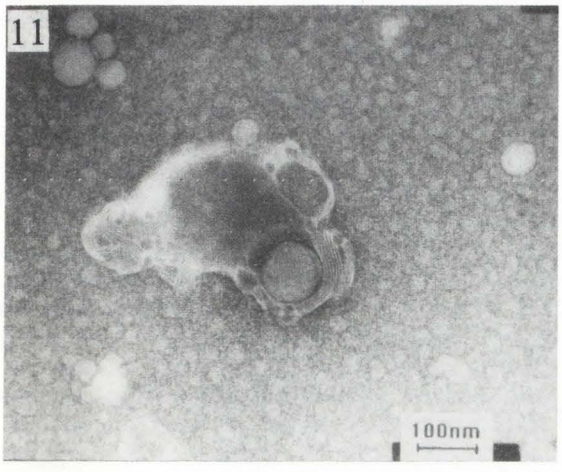
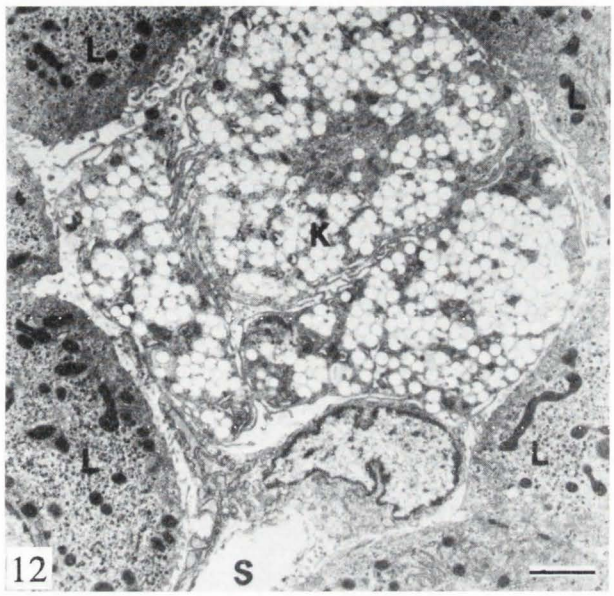


Figure 9. Electron microscopic profiles of amphotericin B containing DPL vesicles. The size distribution of polymerized DPL vesicles is quite heterogenous as is typical for multilamellar vesicles. Large multilayered vesicles in the 2-4 μm size range are visible along with an abundant background of smaller vesicles. From reference 118 with permission from the American Pharmaceutical Association.



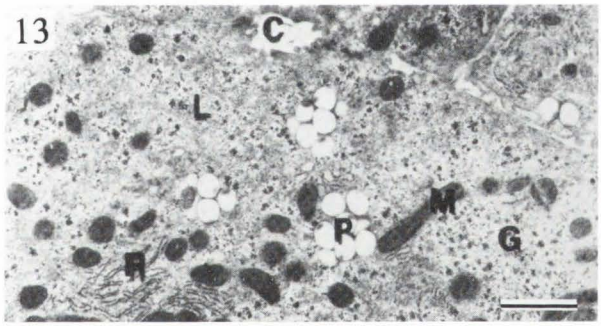


Figure 10. Transmission electron micrograph of PLA nanocapsules prepared according to standard conditions by interfacial deposition of poly (D,L-lactide) polymer. From reference 5 with permission from the American Pharmaceutical Association.

Figure 11. Transmission electron micrograph of PLA nanocapsules showing the presence of large multilamellar phospholipid vesicles. From reference 5 with permission from the American Pharmaceutical Association.

Figure 12. Electron micrograph showing Kupffer cells situated between a sinusoid(s) and liver cells (L) following administration of 10^{13} polystyrene microspheres of 359 nm diameter. The Kupffer cells are filled with the microspheres. From reference 74 with permission from the American Pharmaceutical Association. Bar = 1 μm.

Figure 13. Electron micrograph showing groups of particles (P) within a liver cells (L) 8 days following administration of 10^{13} polystyrene microspheres of 359 nm diameter. c: bile canaliculus; m: mitochondrion; g: glycogen; r: rough endoplasmic reticulum. From reference 74 with permission from the American Pharmaceutical Association. Bar = 1 μm.

Mechanism of drug release, absorption, phagocytosis and elimination in drug delivery systems

SEM techniques have been used to study the *in vitro - in vivo* interaction of drug delivery systems such as microspheres [16, 65], microcapsules [20, 41], or nanocapsules [3, 4] undergoing phagocytosis by macrophages [13]. The surface morphology of polymeric films *in vivo* after implantation has been studied by SEM and it is reported that the drug particles underwent swelling and were released from the surface forming holes and in time, channels [53]. Release mechanisms of biodegradable [81, 84] or non-biodegradable microspheres [12, 14] have been reported from a study of the surface topography during *in vivo* observations. Povey *et al.* [135] studied the delivery of chemical carcinogens with magnetic polyethyleneimine microcapsules by *in vivo* trapping of electrophiles from N-methyl-N-nitrosourea and recovery from feces. The mean diameter of administered and recovered microcapsules has been reported to be different. The micrographs of these recovered microcapsules showed the adsorption of particles of an unknown character as well as surface changes following the *in vivo* exposure [135].

Conclusions

Electron Microscopy techniques have been used extensively in characterizing and evaluating drug delivery systems. Due to the complex nature and microstructure of most of these delivery systems, EM is very useful in understanding surface topography. It is used to study the effects of formulation and processing variables on the properties of drug delivery systems. It is also a very useful tool in the understanding of the mechanisms of a drug delivery system formation release, ultrastructural disposition and distribution, *in vivo* uptake and bioerosion. In the last few years, EM has achieved an indispensable status in drug delivery systems research, development, characterization and evaluation.

Acknowledgements

We are thankful to Ms. Deborah Robison for her help in EM techniques. We acknowledge the help of Ms. Kathi Loban for typing this manuscript.

References

1. Absolom DR, Hawthorn LA, Chang G (1988) Endothelialization of polymer surfaces. J. Biomed. Mater. Res. **22**: 271-285.

2. Alamelu S, Panduranga Rao K (1990) Effect of surfactants on the stability of modified egg-yolk phosphatidyl choline liposomes. J. Microencapsulation **7**: 541-551.

3. Ammoury N, Fessi H, Devissaguet JP, Puisieux F, Benita S (1989) Physicochemical characterization of polymeric nanocapsules and *in vitro* release evaluation of indomethacin as a drug model. S. T. P. Pharma. **5**: 647-651.

4. Ammoury N, Fessi H, Devissaguet JP, Puisieux F, Benita S (1990) *In vitro* release kinetic pattern of indomethacin from poly(D,L-lactide) nanocapsules. J. Pharm. Sci. **79**: 763-767.

5. Amselem S, Gabizon A, Barenholz Y (1990) Optimization and upscaling of doxorubicin-containing liposomes for clinical use. J. Pharm. Sci. **79**: 1045-1052.

6. Arshady R (1989) Preparation of microspheres and microcapsules by interfacial polycondensation techniques. J. Microencapsulation **6**: 13-28.

7. Artursson P, Edman P, Laakso T, Sjöholm I (1984) Characterization of polyacryl starch microparticles as carriers for proteins and drugs. J. Pharm. Sci. **73**: 1507-1513.

8. Arturson P, Arro E, Edman P, Ericsson JLE, Sjöholm I (1987) Biodegradable microspheres V: Stimulation of macrophages with microparticles made of various polysaccharides. J. Pharm. Sci. **76**: 127-133.

9. Azoury R, Elkayam R, Friedman M (1988) Nuclear magnetic resonance study of an ethyl cellulose sustained-release delivery system I: Effect of casting solvent on hydration properties. J. Pharm. Sci. **77**: 425-427.

10. Babay D, Hoffman A, Benita S (1988) Design and release kinetic pattern evaluation of indomethacin microspheres intended for oral administration. Biomaterials **9**: 482-488.

11. Bakker D, Van Blitterswijk CA, Daems WT, Grote JJ (1988) Biocompatibility of six elastomers in vitro. J. Biomed. Mater. Res. **22**: 423-439.

12. Barkai A, Pathak YV, Benita S (1990) Polyacrylate (Eudragit retard) microspheres for oral controlled release of Nifedipine I: Formulation design and process optimization. Drug Dev. Ind. Pharm. **16**: 2057-2075.

13. Barratt GM, Yu WP, Fessi H, Devissaguet JP, Petit JF, Tenu JP, Israel L, Mòrère JF, Puisieux F (1989) Delivery of MDP-L-alanyl-cholesterol to macrophages: comparison of liposomes and nanocapsules. Cancer Journal **2**: 439-443.

14. Barratt G, Pathak YV, Fessi H, Devissaguet JP (1992) Levamisole-PLA biodegradable nanocapsules. Proceed. Intern. Symp. Controlled Release Bioactive Materials. Controlled Release Society Inc., Deerfield, IL. 19 (abstract).

15. Benita S, Donbrow M (1982) Dissolution rate control of the release kinetics of water-soluble compounds from ethyl cellulose film-type microcapsules. Int. J. Pharm. **12**: 251-264.

16. Benita S, Barkai A, Pathak YV (1990) Effect of drug loading extent on the *in vitro* release kinetic behaviour of nifedipine from polyacrylate microspheres. J. Control. Rel. **12**: 213-222.

17. Benita S, Zouai O, Benoit JP (1986) 5-Fluorouracil: Carnauba wax microspheres for chemoembolization: An *in vitro* evaluation. J. Pharm. Sci. **75**: 847-851.

18. Benita S, Dor P, Aronhime M, Marom G

(1986) Permeability and mechanical properties of a new polymer: cellulose hydrogen phthalate. Int. J. Pharm. **33**: 71-80.

19. Berthod A, Rollet M, Farah N (1988) Dry adsorbed emulsions: an oral sustained drug delivery system. J. Pharm. Sci. **77**: 216-221.

20. Beyger JW, Nairn JG (1986) Some factors affecting the microencapsulation of pharmaceuticals with cellulose acetate phthalate. J. Pharm. Sci. **75**: 573-578.

21. Bindschaedler C, Leong K, Mathiowitz E, Langer R (1988) Polyanhydride microsphere formulation by solvent extraction. J. Pharm. Sci. **77**: 696-698.

22. Brem H, Domb A, Lenartz D, Dureza C, Olivi A, Epstein JL (1992) Brain biocompatibility of biodegradable controlled release polymer consisting of anhydride copolymer of fatty acid dimer and sebacic acid. J. Control. Rel. **19**: 325-330.

23. Berg J, Sundberg D, Kronberg B (1989) Microencapsulation of emulsified oil droplets by *in-situ* vinyl polymerization. J. Microencapsulation **6**: 327-337.

24. Blumenthal NC, Posner AS, Cosma V (1988) The effect of glass-ceramic bone implant materials on the *in vitro* formation of hydroxyapatite. J. Biomed. Mater. Res. **22**: 1033-1041.

25. Boag AH, Sefton MV (1987) Microencapsulation of human fibroblasts in a water-insoluble polyacrylate. Biotech. Bioeng. **30**: 954-962.

26. Bodmeier R, Paeratakul O (1990) Drug Release from Laminated Polymeric Films Prepared from Aqueous Latexes. J. Pharm. Sci. **79**: 32-36.

27. Bodmeier R, Paeratakul O (1989) Spherical agglomerates of water-insoluble drugs. J. Pharm. Sci. **78**: 964-967.

28. Bodmeier R, Paeratakul O (1990) Theophylline tablets coated with aqueous latexes containing dispersed pore formers. J. Pharm. Sci. **79**: 925-928.

29. Bodmeier R, Paeratakul O (1991) Constant potassium chloride release from microporous membrane-coated tablets prepared with aqueous colloidal polymer dispersions. Pharm. Res. **8**: 355-359.

30. Bodmeier R, Wang J, Bhagwatwar H (1992) Process and formulation variables in the preparation of wax microparticles by a melt dispersion technique. I. Oil-in-water technique for water-insoluble drugs. J. Microencapsulation **9**: 89-98.

31. Bodmeier R, Wang J, Bhagwatwar H (1992) Process and formulation variables in the preparation of wax microparticles by a melt dispersion technique. II. W/O/W multiple emulsion technique for water-soluble drugs. J. Microencapsulation **9**: 99-107.

32. Bogataj M, Mrhar A, Kristl A, Kozjek F (1991) Eudragit E microspheres containing bacampicillin: preparation by solvent removal methods. J. Microencapsulation **8**: 401-406.

33. Bonanomi MH, Velvart M, Weder HG (1987) Fate of different kinds of liposomes containing dexamethasone palmitate after intra-articular injection into rabbit joints. J. Microencapsulation **4**: 189-200.

34. Carli F, Garbassi F (1985) Characterization of drug loading in crospovidone by X-ray photoelectron spectroscopy. J. Pharm. Sci. **74**: 963-967.

35. Chan HK, Gonda I (1989) Respirable form of crystals of cromoglycic acid. J. Pharm. Sci. **78**: 176-180.

36. Chasin M, Lewis D, Langer R (1988) Polyanhydrides for Controlled Drug Delivery. Biopharm. Manuf. February, 10-16.

37. Chitambara B, Jayakrishnan A (1989) Radioopaque hydrogel microspheres. J. Microencapsulation **6**: 233-244.

38. Chowhan ZT, Chi LH (1986) Drug-excipient interactions resulting from powder mixing IV: Role of lubricants and their effect on *in vitro* dissolution. J. Pharm. Sci. **75**: 542-545.

39. Chowhan ZT, Chi LH (1986) Drug-excipient interactions resulting from powder mixing III: Solid state properties and their effect on drug dissolution. J. Pharm. Sci. **75**: 534-541.

40. Chu CC, Browning A (1988) The study of thermal and gross morphologic properties of polyglycolic acid upon annealing and degradation treatments. J. Biomed. Mater. Res. **22**: 699-712.

41. Clayton HA, London NJM, Colloby PS, Bell PRF, James RFL (1991) The effect of capsule composition on the biocompatibility of alginate-poly-1-lysine capsules. J. Microencapsulation **8**: 221-233.

42. Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R (1991) Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm. Res. **8**: 713-720.

43. Couvreur P, Vauthier C (1991) Polyalkylcyanoacrylate nanoparticles as drug carries: present state and perspectives. J. Control. Rel. **18**: 187-198.

44. Cudd A, Nicolau C (1985) Intracellular fate of liposome encapsulated DNA in mouse liver: Analysis using electron microscope autoradiography and subcellular fractionation. Biochem. Biophys. Acta **845**: 477-491.

45. Cudd A, Nicolau C (1986) On the use of Percoll as a marker for liposomes in electron microscopy of mouse spleen tissue. J. Microencapsulation **3**: 275-282.

46. Damgé C, Aprahamian M, Balboni G, Hoeltzel A, Andrieu V, Devissaguet JP (1987) Polyalkylcyanoacrylate nanocapsules increase the intestinal absorption of a lipophilic drug. Int. J. Pharm. **36**: 121-125.

47. DeKeyser JL, Poupaert JH, Dumont P (1991) Poly(diethyl methylidenemalonate) nanoparticles as a potential drug carrier: Preparation, distribution and elimination after intravenous and peroral administration to mice. J. Pharm. Sci. **80**: 67-70.

48. Donbrow M, Benita S, Hoffman A (1984) Microencapsulation of dichromate and paracetamol with eudragit retard polymers using phase separation by non-solvent addition. App. Biochem. Biotech. **10**: 245-249.

49. Dubernet C, Benoit JP, Peppas NA, Puisieux F (1990) Ibuprofen-loaded ethylcellulose microspheres: Release studies and analysis of the matrix structure through the Higuchi model. J. Microencapsulation **7**:

555-565.

50. Dubernet C, Rouland JC, Benoit JP (1991) Ibuprofen-loaded ethylcellulose microspheres: Analysis of the matrix structure by thermal analysis. J. Pharm. Sci. **80**: 1029-1033.

51. Duncan-Hewitt WC, Weatherly GC (1990) Modeling the uniaxial compaction of pharmaceutical powders using the mechanical properties of single crystals. II: Brittle materials. J. Pharm. Sci. **79**: 273-278.

52. Eklheshen S, Zia H, Needham TE, Badawy A, Luzzi LA (1992) Coating charcoal with polyacrylate-polymethacrylate copolymer for haemoperfusion. I: Fabrication and evaluation. J. Microencapsulation **9**: 41-51.

53. Embleton JK, Tighe BJ (1992) Polymers for biodegradable medical devices. IX: Microencapsulation studies: effects of polymer composition and process parameters on poly-hydroxybutyrate-hydroxyvalerate microcapsule morphology. J. Microencapsulation **9**: 73-87.

54. Fassihi AR, Parker MS (1987) Inimical effects of compaction speed on microorganisms in powder systems with dissimilar compaction mechanisms. J. Pharm. Sci. **76**: 466-470.

55. Fassihi AR, Parker MS (1988) Influence of gamma radiation on the gel rigidity index and binding capability of gelatin. J. Pharm. Sci. **77**: 876-879.

56. Forni F, Coppi G, Iannuccelli V, Vandelli MA, Bernabei MT (1989) Distribution of drugs in polymers loaded by swelling. J. Pharm. Sci. **78**: 25-27.

57. Forni F, Vandelli MA, Cameroni R (1992) Influence of drug loading level on drug release and dynamic swelling of crosslinked gelatin microspheres. J. Microencapsulation **9**: 29-39.

58. Ganguly S, Ramana Rao KV, Krishna M (1989) Structure of hollow microspheres: an SEM study. J. Microencapsulation **6**: 193-198.

59. Gangrade N, Price JC (1991) Poly(hydroxybutyrate-hydroxyvalerate) microspheres containing progesterone: preparation, morphology and release properties. J. Microencapsulation **8**: 185-202.

60. Germine N, Parsons JR (1988) Deconvoluted X-ray diffraction analysis of bone and mixtures of bone and particulate hydroxyapatite. J. Biomed. Mater. Res. **22**(A1): 55-67.

61. Golomb G, Dixon M, Smith MS, Schoen FJ, Levy RJ (1987) Controlled-release drug delivery of diphosphonates to inhibit bioprosthetic heart valve calcification: Release rate modulation with silicone matrices via drug solubility and membrane coating. J. Pharm. Sci. **76**: 271-276.

62. Goosen MFA, O'Shea GM, Gharapetian HM, Chou S, Sun AM (1985) Optimization of microencapsulation parameters: Semipermeable microcapsules as a bioartifical pancreas. Biotech. Bioeng. **28**: 146-150.

63. Goto S, Kawata M, Nakamura M, Nagatsuma Y, Fujinaga K, Aoyama T (1988) Evaluation of the sustained release properties of Eudragit RS, RL and S (acrylic resins) microcapsules containing ketoprofen in beagle dogs. J. Microencapsulation **5**: 343-360.

64. Grass GM, Robinson JR (1988) Mechanisms of corneal penetration II: Ultrastructural analysis of potential pathways for drug movement. J. Pharm. Sci. **77**: 15-23.

65. Gupta PK, Hung CT (1989) Albumin microspheres II: applications in drug delivery. J. Microencapsulation **6**: 463-472.

66 Gupta PK, Hung CT, Rao NS (1989) Ultrastructural disposition of adriamycin-associated magnetic albumin microspheres in rats. J. Pharm. Sci. **78**: 290-294.

67. Häkkinen L, Yli-Urpo A, Heino J, Larjava H (1988) Attachment and spreading of human gingival fibroblasts on potentially bioactive glasses *in vitro*. J. Biomed. Mater. Res. **22**: 1043-1059.

68. Hartman LC, Bessette RW, Baier RE, Meyer AE, Wirth J (1988) Silicone rubber temporomandibular joint (TMJ) meniscal replacements: Postimplant histopathologic and material evaluation. J. Biomed. Mater. Res. **22**: 475-484.

69. Heughebaert M, LeGeros RZ, Gineste M, Guilhem A, Bonel G (1988) Physicochemical characterization of deposits associated with HA ceramics implanted in nonosseous sites. J. Biomed. Mater. Res. **22**(A3): 257-268.

70. Horbett TA, Waldburger JJ, Ratner BD, Hoffman AS (1988) Cell adhesion to a series of hydrophilic-hydrophobic copolymers studied with a spinning disc apparatus. J. Biomed. Mater. Res. **22**: 383-404.

71. Horiuchi Y, Fumitoshi H, Uekama K (1990) Slow-release characteristics of diltiazem from ethylated β-cyclodextrin complexes. J. Pharm. Sci. **79**: 128-132.

72. Hsiue GH, Yang JM, Wu RL (1988) Preparation and properties of a biomaterial: HEMA grafted SDS by γ-ray irradiation. J. Biomed. Mater. Res. **22**(A3): 405-415.

73. Huang HP, Ghebre-Sellassie I (1989) Preparation of microspheres of water-soluble pharmaceuticals. J. Microencapsulation **6**: 219-225.

74. Illum L, Thomas NW, Davis SS (1986) Effect of a selected suppression of the reticuloendothelial system on the distribution of model carrier particles. J. Pharm. Sci. **75**: 16-22.

75. Imai Y, Masuhara E (1982) Long-term *in vivo* studies of poly(2-hydroxyethyl methacrylate). J. Biomed. Mater. Res. **16**: 609-617.

76. Iso M, Shirahase T, Hanamura S, Urushiyama S, Omi S (1989) Immobilization of enzyme by microencapsulation and application of the encapsulated enzyme in the catalysis. J. Microencapsulation **6**: 165-176.

77. Iso M, Masumoto H, Urushiyama S, Omi S (1990) Application of encapsulated enzyme dispersed in a continuous stirred tank reactor. J. Microencapsulation **7**: 167-177.

78. Itoh T, Xia J, Magavi R, Nishihata T, Rytting JH (1990) Use of shed snake skin as a model membrane for *in vitro* percutaneous penetration studies: Comparison with human skin. Pharm. Res. **7**: 1042-1047.

79. Iwamoto K, Kato T, Kawahara M, Koyama N, Watanabe S, Miyake Y, Sunamoto J (1991) Polysaccha-

ride-coated oil droplets in oil-in-water emulsions as targetable carriers for lipophilic drugs. J. Pharm. Sci. **80**: 219-224.

80. Iwata M, McGinity JW (1992) Preparation of multi-phase microspheres of poly(D,L-lactic acid) and poly(D,L-lactic-co-glycolic acid) containing a W/O emulsion by a multiple emulsion solvent evaporation technique. J. Microencapsulation **9**: 201-214.

81. Jalil R, Nixon JR (1990) Microencapsulation using poly(D,L-lactic acid) I: Effect of preparative variables on the microcapsule characteristics and release kinetics. J. Microencapsulation **7**: 229-244.

82. Jalil R, Nixon JR (1990) Microencapsulation using poly(D,L-lactic acid) III: Effect of polymer molecular weight on the release kinetics. J. Microencapsulation **7**: 357-374.

83. Jalil R, Nixon JR (1990) Microencapsulation using poly(D,L-lactic acid) IV: Effect of storage on the microcapsule characteristics. J. Microencapsulation **7**: 375-383.

84. Jalil R, Nixon JR (1989) Microencapsulation using poly(L-lactic acid) I: Microcapsule properties affected by the preparative technique. J. Microencapsulation **6**: 473-484.

85. Jalil R, Nixon JR (1990) Microencapsulation using poly(L-lactic acid) III: Effect of polymer molecular weight on the microcapsule properties. J. Microencapsulation **7**: 41-52.

86. John T (1991) Interactions of bacteria and amoebae with ocular biomaterials. Cells and Materials **1**: 129-139.

87. Kang IK, Ito Y, Sisiso M, Imanishi Y (1988) Serotonin and β-thromboglobulin release reaction from platelet as triggered by interaction with polypeptide derivatives. J. Biomed. Mater. Res. **22**: 595-611.

88. Kawashima Y, Lin SY, Ogawa M, Handa T, Takenaka H (1985) Preparations of agglomerated crystals of polymorphic mixtures and a new complex of indomethacin-epirizole by the spherical crystallization technique. J. Pharm. Sci. **74**: 1152-1156.

89. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y, Furuyama S (1991) Characterization of polymorphs of tranilast anhydrate and tranilast monohydrate when crystallized by two solvent change spherical crystallization techniques. J. Pharm. Sci. **80**: 472-478.

90. Kawashima Y, Niwa T, Handa T, Takeuchi H, Iwamoto T, Itoh K (1989) Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method. J. Pharm. Sci. **78**: 68-72.

91. Kost J, Langer R (1986) Magnetically modulated drug delivery systems. Pharm. Intnl. **7**(3): 60-63.

92. Krause JH, Juliano RL, Regen S (1987) *In vivo* behavior of polymerized lipid vesicles. J. Pharm. Sci. **76**: 1-5.

93. Kreuter J (1988) Possibilities of using nanoparticles as carriers for drugs and vaccines. J. Microencapsulation **5**: 115-127.

94. Kwok KK, Groves MJ, Burges DJ (1991) Production of 5-15 μm diameter alginate-polyolsine microcapsules by an air-atomization technique. Pharm. Res. **8**: 341-344.

95. Laakso T, Sjöholm I (1987) Biodegradable microspheres X: Some properties of polyacryl starch microparticles prepared from acrylic acid-esterified starch. J. Pharm. Sci. **76**: 935-939.

96. Laakso T, Artursson P, Sjöholm I (1986) Biodegradable microspheres IV: Factors affecting the distribution and degradation of polyacryl starch microparticles. J. Pharm. Sci. **75**: 962-967.

97. Lalla JK, Ahuja PL (1991) Drug targeting using non-magnetic and magnetic albumin-globulin mix microspheres of mefenamic acid. J. Microencapsulation **8**: 37-52.

98. Leong KW, Brott BC, Langer R (1985) Bioerodible polyanhydrides as drug-carrier matrices. I: Characterization, degradation, and release characteristics. J. Biomed. Mater. Res. **19**: 941-955.

99. Levy RJ, Schoen FJ, Sherman FS, Nichols J, Hawley MA, Lund SA (1986) Calcification of subcutaneously implanted type I collagen sponges. Am. J. Pathol. **122**: 71-82.

100. Levy RJ, Wolfrum J, Shcoen FJ, Hawley MA, Lund SA, Langer R (1985) Inhibition of calcification of bioprosthetic heart valves by local controlled-release diphosphonate. Science **228**: 190-192.

101. Levy RJ, Schoen FJ, Levy JT, Nelson AC, Howard SL, Oshry LJ (1983) Biologic determinants of dystrophic calcification and osteocalcin deposition in glutaraldehyde-preserved porcine aortic valve leaflets implanted subcutaneously in rats. Am. J. Pathol. **113**: 143-155.

102. Levy MC, Andry MC (1991) Mixed-walled microcapsules made of cross-linked proteins and polysaccharides: preparation and properties. J. Microencapsulation **8**: 335-347.

103. Li LC, Peck GE (1989) Water based silicone elastomer controlled release tablet film coating II - Formulation considerations and coating evaluation. Drug Dev. and Ind. Pharm. **15**: 499-531.

104. Lin SY, Tzans YL, Weng CN, Lee CJ (1991) Preparation of enteric-coated microspheres of *Mycoplasma hyopneumoniae* vaccine with cellulose acetate phthalate: (II). Effect of temperature and pH on the stability and release behaviour of microspheres. J. Microencapsulation **8**: 537-545.

105. Lin SY, Ho LT, Chiou HL (1985-86) Microencapsulation and controlled release of insulin from polylactic acid microcapsules. Biomat. Med. Dev. Art. Org. 13(**3&4**): 187-201.

106. Lin SY, Tzan YL, Lee CJ, Weng CN (1991) Preparation of enteric-coated microspheres of *Mycoplasma hyopneumoniae* vaccine with cellulose acetate phthalate: I. Formation condition and micromeritic properties. J. Microencapsulation **8**: 317-325.

107. Lin SY, Yand JC, Jiang SS (1985) Studies on microencapsulation (I) Micromeritic properties of theophylline microcapsules prepared by coacervation-

phase separation method. J. Taiwan Pharm. Assoc. **37**(1): 1-10.

108. Lowes MMJ, Caira MR, Lotter AP, VanDer-Watt JG (1987) Physicochemical properties and X-ray structural studies of the trigonal polymorph of carbaazepine. J. Pharm. Sci. **76**: 744-752.

109. Mac A, Negi D, Friend D (1989) Preparation and characterization of poly(styrene) microcapsules containing corrosion inhibitors. J. Microencapsulation **6**: 361-357.

110. Markus A, Pelah Z (1989) Encapsulation of vitamin A. J. Microencapsulation **6**: 389-394.

111. Malaiya A, Vyas SP (1988) Preparation and characterization of indomethacin magnetic particles. J. Microencapsulation **5**: 243-253.

112. Marcotte N, Polk A, Gossen MFA (1990) Kinetics of protein diffusion from a poly(D,L-lactide) reservoir system. J. Pharm. Sci. **79**: 407-410.

113. Mathiowitz E, Langer R (1987) Polyanhydride microspheres as drug carriers I. Hot-melt microencapsulation. J. Cont. Rel. **5**: 13-22.

114. Mathiowitz E, Cohen MD, Langer R (1987) Novel microcapsules for delivery systems. Reactive Poly. **6**: 275-283.

115. Mathiowitz E, Amato C, Dor P, Langer R (1990) Polyanhydride microspheres: 3. Morphology and characterization of systems made by solvent removal. Polymer **31**: 547-555.

116. Mathiowitz E, Kline D, Langer R (1990) Morphology of polyanhydride microsphere delivery systems. Scanning Microsc. **4**: 329-340.

117. Mayer LD, Bally MB, Cullis PR (1990) Strategies for optimizing liposomal doxorubicin. J. Liposome Res. **1**: 463-480.

118. Mehta R, Hsu MJ, Juliano RL, Krause HJ, Regen SL (1986) Polymerized phospholipid vesicles containing Amphotericin B: Evaluation of toxic and antifungal activities *in vitro*. J. Pharm. Sci. **75**: 579-581.

119. Moldenhauer MG, Nairn JG (1990) Formulation parameters affecting the preparation and properties of microencapsulated ion-exchange resins containing theophylline. J. Pharm. Sci. **79**: 659-666.

120. Morita M, Sasada T, Hayashi H, Tsukamoto Y (1988) The corrosion fatigue properties of surgical implants in a living body. J. Biomed. Mater. Res. **22**: 529-540.

121. Munday DL, Fassihi AR (1989) Controlled release delivery: effect of coating composition on release characteristics of mini-tablets. Int. J. Pharm. **52**: 109-114.

122. Nastruzzi C, Gambari R, Menegatti E, Walde P, Luisi PL (1990) Tumor cell growth inhibition by liposome-encapsulated aromatic polyamidines. J. Pharm. Sci. **79**: 672-677.

123. Nath S, Pathak YV (1993) Evaluation of maltodextrins as tablet excipients I. micromeritic and compressional characterization. Powder Technology **75**: 97-101.

124. Nixon JR, Yeung VW (1989) Preparation of microencapsulated liposomes, II. Systems containing nylon-gelatin and nylon-gelatin-acacia walling material. J. Microencapsulation **6**: 43-52.

125. Okada J, Asano S, Kondo T (1991) A new concept for interpretation of first-order release from albumin microspheres. J. Microencapsulation **8**: 483-496.

126. Olson ME, Ruseska I, Costerton JW (1988) Colonization of n-butyl-2-cyanoacrylate tissue adhesive by *Staphylococcus epidermidis*. J. Biomed. Mater. Res. **22**: 485-495.

127. Omi S, Umeki N, Mohri H, Iso M (1991) Microencapsulation of pheromone-analogue and measurement of the sustained release. J. Microencapsulation **8**: 465-478.

128. Otsuka M, Kaneniwa N (1986) Effect of seed crystals on solid-state transformation of polymorphs of chloramphenicol palmitate during grinding. J. Pharm. Sci. **75**: 506-511.

129. Padmadisastra Y, Gonda I (1989) Preliminary studies of the development of a direct compression cellulose excipient from bagasse. J. Pharm. Sci. **78**: 508-514.

130. Pathak, YV, Boyd J, Johnston TP, Levy JT, Golomb G, Schoen FJ, Levy RJ (1991) Scanning electron microscopy studies of the prevention of bioprosthetic heart valve calcification with controlled release polymeric matrices. Cells and Materials **1**: 65-72.

131. Pathak YV, Boyd J, Levy RJ, Schoen FJ (1990) Prevention of calcification of glutaraldehyde pretreated bovine pericardium through controlled release polymeric implants: studies of Fe^{3+}, Al^{3+}, protamine sulphate and levamisole. Biomaterials **11**: 718-723.

132. Payne NI, Timmins P, Ambrose CV, Ward MD, Ridgway F. (1986) Proliposomes: A novel solution to an old problem. J. Pharm. Sci. **75**: 325-329.

133. Paynter RW, Askill IN, Glick SH, Guidoin R (1988) The hydrolytic stability of Mitrathane (a polyurethane urea) - An X-ray photoelectrol spectroscopy study. J. Biomed. Mater. Res. **22**: 687-698.

134. Pitt CG (1990) The controlled parenteral delivery of polypeptides and proteins. Int. J. Pharm. **59**: 173-196.

135. Povey AC, Brouet I, Nixon JR, O'Neill IK (1987) Trapping of chemical carcinogens with magnetic polyethyleneimine microcapsules: III. *In vivo* trapping of electrophiles from N-methyl-N-nitrosoureal and recovery from feces. J. Pharm. Sci. **76**: 201-207.

136. Povey AC, Nixon JR, O'Neill IK (1987) Membrane formation and characterization of semi-permeable magnetic polyhexamethyleneterephthalamide microcapsules containing polyethyleneimene (PEI) for trapping carcinogens. J. Microenacapsulation **4**: 299-314.

137. Quigley KJ, Deasy PB (1992) Use of deacetylated gellan gum (Gelrite) for the production of sulphamethizole containing beads. J. Microencapsulation **9**: 1-7.

138. Ramadan MA, Tawashi R (1990) Effect of surface geometry and morphic features on the flow char-

acteristics of microsphere suspensions. J. Pharm. Sci. **79**: 929-933.

139. Rosenberg M, Talmon Y, Kopelman IJ (1988) The microstructure of spray-dried microcapsules. Food Microstructure **7**: 15-23.

140. Rosilio V, Roblot-Treupel L, Costa MdL, Baszkin A (1988) Physico-chemical characterization of ethylcellulose drug-loaded cast films. J. Control. Rel. **7**: 171-180.

141. Rosilio V, Benoit JP, Deyme M, Thies C, Madelmont G (1991) A physicochemical study of the morphology of progesterone-loaded microspheres fabricated from poly(D,L-lactide-co-glycolide). J. Biomed. Mater. Res. **25**: 667-682.

142. Sanghvi SP, Nairn JG (1992) Effect of viscosity and interfacial tension on particle size of cellulose acetate trimellitate microspheres. J. Microencapsulation **9**: 215-227.

143. Sano A, Kuriki T, Handa T, Takeuchi H, Kawashima Y (1987) Particle design of tolbutamide in the presence of soluble polymer or surfactant by the spherical crystallization technique: Improvement of dissolution rate. J. Pharm. Sci. **76**: 471-474.

144. Sano T, Muramatsu N, Kondo T (1986) Phagocytosis of microcapsules of guinea-pig polymorphonuclear leucocytes. J. Microencapsulation **3**: 265-273.

145. Schenk P, Ausborn M, Bendas F, Nuhn P, Arndt D, Meyer HW (1989) The preparation and characterization of lipid vesicles containing esters of sucrose and fatty acids. J. Microencapsulation **6**: 95-103.

146. Schoen FJ, Levy RJ, Nelson AC, Bernhard WF, Nashef A, Hawley M (1985) Onset and progression of experimental bioprosthetic heart valve calcification. Lab. Invest. **52**: 523-532.

147. Sefton MV, Broughton RL (1982) Microencapsulation of erythrocytes. Biochem. Biphys. Acta **717**: 473-477.

148. Sheorey DS, Sai MS, Dorle AK (1991) A new technique for the encapsulation of water insoluble drugs using ethyl cellulose. J. Microencapsulation **8**: 359-368.

149. Shimi SM, Newman EL, Hopwood D, Cushieri A (1991) Semi-permeable microcapsules for cell culture: ultra-structural characterization. J. Microencapsulation **8**: 307-316.

150. Sheorey DS, Dorle AK (1991) Effect of solvents on the characteristics of rosin walled microcapsules prepared by a solvent evaporation technique. J. Microencapsulation **8**: 71-78.

151. Singh J, Robinson DH (1990) Controlled release captopril microcapsules: effect of ethyl cellulose viscosity grade on the in vitro dissolution from microcapsules and tableted microcapsules. J. Microencapsulation **7**: 67-76.

152. Singh J, Robinson DH (1988) Controlled release captopril microcapsules: effect of non-ionic surfactants on release from ethyl cellulose microcapsules. J. Microencapsulation **5**: 129-137.

153. Singh KK, Robison D, Pathak YV (1993) Morphological characterization of maltodextrin derivatives using scanning electron microscopy. Cells and Materials **3**,

154. Singh KK, Pathak YV (1993) Sustained release of verapamil using implantable polymeric carriers. Drug Design and Discovery, (in press).

155. Singh M, Faulkner G, Meisner D, Mezei M (1990) Freeze substitution technique for identifying liposomes incorporated in emulsion and gel preparations. J. Microencapsulation **7**: 77-84.

156. Spenlehauer G, Veillard M, Benoit JP (1986) Formation and characterization of cisplatin loaded poly-(D,L-lactide) microspheres for chemoembolization. J. Pharm. Sci. **75**: 750-755.

157. Spenlehauer G, Vert M, Benoit JP, Boddaert A (1989) *In vitro* and *in vivo* degradation of poly(D,L-lactide/glycolide) type microspheres made by solvent evaporation method. Biomaterials **10**: 557-563.

158. Šprincl L, Novák M (1981) The initial stage of calcification in porous hydrophilic polymers. J. Biomed Mater. Res. **15**: 437-440.

159. Staubli A, Ron E, Langer R (1990) Hydrolytically degradable amino acid containing polymers. J. Amer. Chem. Soc. **112**: 4419-4424.

160. Sun AM, Cai Z, Shi Z, Ma F, O'Shea GM, Gharapetian H (1986) Microencapsulated hepatocytes as a bioartificial liver. Trans. Am. Soc. Artif. Intern. Organs **32**: 39-41.

161. Tabata Y, Ikada Y (1988) Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers. J. Biomed. Mater. Res. **22**: 837-858.

162. Tabata Y, Ikada Y (1989) Synthesis of gelatin microspheres containing interferon. Pharm. Res. **6**: 422-427.

163. Thibert R, Akbarieh M, Tawashi R (1988) Application of fractal dimension to the study of the surface ruggedness of granular solids and excipients. J. Pharm. Sci. **77**: 724-726.

164. Tomazic BB, Siew C, Brown WE (1991) A comparative study of bovine pericardium mineralization: a basic and practical approach. Cells and Materials **1**: 231-241.

165. Torrado JJ, Illum L, Cadorniga R, Davis SS (1990) Egg albumin microspheres containing paracetamol for oral administration. I. *In vitro* characterization. J. Microencapsulation **7**: 463-470.

166. Tripathi KP, Singh J (1992) Aminophylline targeting to lung: optimization of the size and drug loading of albumin microspheres. J. Microencapsulation **9**: 229-235.

167. VanMeeteren R, Pathak YV (1992) Sustained release Al^{3+} implants. Proceed. Intern. Symp. Control Release of Bioactive Materials. Controlled Release Society Inc., Deerfield, IL. 19 (abstract).

168. Verdon SL, Chaikof EL, Coleman JE, Hayes LL, Connolly RJ, Ramberg K, Merrill EW, Callow AD (1990) Scanning electron microscopy analysis of poly-

ethylene oxide hydrogels for blood contact. Scanning Microsc. **4**: 341-350.

169. Vinard E, Eloy R, Descotes J, Brudon JR, Guidicelli H, Magne JL, Patra P, Berruet R, Huc A, Chauchard J (1988) Stability of performances of vascular prostheses retrospective study of 22 cases of human implanted prostheses. J. Biomed. Mater. Res. **22**: 633-648.

170. Visscher GE, Pearson JE, Fong JW, Argentieri GJ, Robison RL, Maulding HV (1988) Effect of particle size on the *in vitro* and *in vivo* degradation rates of poly(D,L-lactide-co-glycolide) microcapsules. J. Biomed. Mater. Res. **66**: 733-746.

171. Vural I, Kas HS, Hincal AA, Cave G (1990) Cyclophosphamide loaded albumin microspheres II. Release characteristics. J. Microencapsulation **7**: 511-516.

172. Vyas SP, Gogoi PJ, Pande S, Dixit VK (1991) Enteric spherules diastase in enzyme preparations. J. Microencapsulation **8**: 447-454.

173. Wada R, Hyon SH, Ikada Y (1990) Lactic acid oligomer microspheres containing hydrophilic drugs. J. Pharm. Sci. **79**: 919-924.

174. Wan LSC, Heng PWS, Chia CGH (1992) Plasticizers and their effects on microencapsulation process by spray-drying in an aqueous system. J. Microencapsulation **9**: 53-62.

175. Webb CL, Schoen FJ, Levy RJ (1989) Covalent binding of aminopropanehydroxydiphosphonate to glutaraldehyde residues in pericardial bioprosthetic tissue: Stability and calcification inhibition studies. Exp. Mole. Path. **50**: 291-302.

176. Weiner AL, Carpenter-Green SS, Soehngen EC, Lenk RP, Popescu MC (1985) Liposome-collagen gel matrix: A novel sustained drug delivery system. J. Pharm. Sci. **74**: 922-925.

177. Wiessner JH, Mar H, Baskin DG, Hwang KJ (1986) Peptide-carrier interaction: Induction of Liposome fusion and aggregation by insulin. J. Pharm. Sci. **75**: 259-263.

178. Williams SK, Schneider T, Jarrell BE (1990) Electron microscopy of endothelial cell - biopolymer interaction. Scanning Microsc. **4**: 181-189.

179. Wright KC, Wallace S, Mosier B, Mosier D (1988) Microcapsules for arterial chemoembolization: appearance and in vitro drug release characteristics. J. Microencapsulation **5**: 13-20.

180. Yang TT, Swarbrick J (1986) Sustained-release delivery systems, II: In vitro dissolution of 4,4'-sulfonylbisbenzamine (dapsone)-N-dodecanoll-4,4'-sulfonylbisbenzamine comelts. J. Pharm. Sci. **75**: 264-270.

181. Yueming C, Xianjue T, Changxi, Zhongming L (1991) Preparation and performance of cross-linked agar encapsulated activated charcoal (CAAC-III). J. Microencapsulation **8**: 327-334.

182. Zhang G, Schwartz JB, Schnaare RL (1991) Bead coating. I. Change in release kinetics (and mechanism) due to coating levels. Pharm. Res. **8**: 331-335.

183. Zimmer A, Krueter J, Robinson JR (1991) Studies on the transport pathway of PBCA nanoparticles in ocular tissues. J. Microencapsulation **8**: 497-504.

Discussion with Reviewers

S.K. Williams: What drug delivery systems currently appear to be the most promising for the delivery of genetically engineered pharmaceuticals, particularly peptides and proteins?

Authors: Currently, several drug delivery systems are being evaluated as carriers for proteins and peptides. Liposomes, stealth and immunoliposomes, nanocapsules, submicronized emulsions and polymeric carriers show promising applications for delivering specific proteins and peptides. None of them can be used generically for delivering proteins and peptides. Each has advantages and disadvantages when considered as carriers especially for proteins and peptides.

M. Foldvari: Would you comment on the quantitative aspects of EM for pharmaceutical characterization purposes?

Authors: EM techniques can often be used to characterize particle size of pharmaceutical systems, especially when the dispersed phase has particles within nanometer range. Nanocapsules and nanospheres formulations are characterized by EM techniques. Quantitative characterization has become much easier and reproducible by laser beam analyses, which might be a technique of choice for scientists working in this area.