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# CONTROLLED RELEASE SYSTEMS: FABRICATION TECHNOLOGY

## Volume II

Dean S. T. Hsieh

CRC PRESS

# Controlled Release Systems: Fabrication Technology

## Volume II

Editor

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CRC Press, Inc.
Boca Raton, Florida

**Library of Congress Cataloging-in-Publication Data**

Controlled release systems.

Bibliography: p.
Includes indexes.
1. Drugs--Controlled release. 2. Controlled release preparations. I. Hsieh, Dean, 1948-
RS201.C64C665 1988 615'.19 87-21785
ISBN 0-8493-6013-7 (v. 1)
ISBN 0-8493-6014-5 (v. 2)



Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.



International Standard Book Number 0-8493-6013-7 (volume I)
International Standard Book Number 0-8493-6014-5 (volume II)
Library of Congress Card Number 87-21785
Printed in the United States

# FOREWORD

Controlled release technology is a rapidly emerging field. Two decades ago, formulations based on this technology were scarcely in existence. Today, the number of controlled release products is large and growing rapidly. With the advent of biotechnological advances and genetic engineering, the development of new and more complex drugs is imminent. These developments have necessitated more than ever before the creation of effective delivery systems which can protect these precious molecules from destruction, yet continuously deliver them to the body safely. Central to the successful development of any controlled release system is the fabrication of formulation procedure — it must be safe, reproducible, not damaging to the drug, and amenable to scale-up. The choices as to the types of controlled release systems one might use and the different ways these systems can be fabricated are significant. Such choices can only be made once an understanding of the principles underlying these systems and fabrication procedures is in hand.

While many books have been written on controlled release, this book stands alone in its effort to bring together an understanding of the ways in which controlled release systems can be fabricated. Dr. Hsieh is certainly well qualified to edit and put together such a book, as he is a skilled formulator of controlled release systems and has studied them extensively both as a postdoctoral scientist in our laboratory at MIT, as a Professor at Rutgers, and at his company, Conrex Pharmaceutical Corporation. This book brings something new to readers in the exciting field of controlled release technology/fabrication technology, an area that should be of practical laboratory value in the design of these important systems.

**Robert Langer**

# PREFACE

Controlled release technology has well deserved its recent growth in popularity and widespread acclaim. Its advantages have been recognized and utilized not only by the pharmaceutical industry, but by several other industries as well. Advances in controlled release research enable innovative refinements in many currently manufactured household products. Employment of these refinements helps the sponsors of such research to achieve a competitive edge. However, before controlled release products can be manufactured, further research and development must take place. Fabrication technology refers to the methods by which controlled release products are manufactured. Throughout the development of controlled release devices, fabrication technologies have played a key role in the process of innovation. The purpose of these volumes is to compile and generalize principles of fabrication methods which have been previously published. These volumes thus provide a framework for the study of fabrication technology. It is the editor's hope that they will form the basis for future innovation.

The first volume is concerned with fabrication procedures for currently marketed products or mature technologies. The second volume is concerned with fabrication procedures in various stages of development. Some of the technologies described in the second volume may be mature, yet may belong to a class of products wherein the majority are still under development. Volume I, Chapter 1 is concerned with this classification and explores it in detail. Briefly, there are three stages in the development of controlled release technology:

1. Encapsulation technologies are covered in Chapters 2 through 7. These fabrication procedures have matured, now comprising, for instance, coacervation, film coating, and mechanical blending.
2. Transdermals and other advanced drug delivery systems are covered in Chapter 8 and Volume II, Chapters 1 through 7. These include multiple lamination for transdermal patches, injection molding, extrusion, gelation, multiple emulsion, and other methods for fabricating bioerodible and hydrogel drug delivery systems.
3. Selective drug targeting systems are surveyed in Chapter 8. These systems represent the latest and most promising stage in controlled release technologies. They include monoclonal antibodies, liposome delivery systems, dextran and magnetic microspheres, and polymeric delivery systems.

All three stages in controlled release technology must utilize various sterilization procedures according to individual fabrication processes. Chapter 9 provides an overview of sterilization procedures for controlled release products.

Much of the knowledge contained within these volumes will prove valuable to the scientific community. For this reason, it has been difficult for contributors to disclose proprietary information. The organizations and individuals who have offered the results of costly and painstaking research deserve greater rewards than I can offer. Nevertheless, I take this opportunity to thank all the contributors for the time and effort they have devoted to this project. I also thank those friends whose guidance directed me to these outstanding individuals. These volumes are truly the product of a concerted effort by a talented and enthusiastic group of colleagues. Those with expertise and a willingness to share it have made this book possible to complete. Even those authors whose companies vetoed their contributing chapters added stimulus to the project with their initial enthusiasm.

In addition to the major contributors to these volumes, there have been several individuals whose continuous support has sustained me through the project. I thank my wife, Mrs. Phyllis Hsieh, for her unqualified patience, endurance, and encouragement. I also thank J. C. Lorber for the capable and prudent input as well as the cooperative effort necessary

to make constant and steady progress. I would also like to acknowledge the assistance of my colleagues at the Rutgers College of Pharmacy: Drs. Y. Chien, K. Tojo, and C. Liu, among others. Their criticism concerning the pursuit of this project, both positive and negative, has been appreciated. This appreciation extends to several graduate students at Rutgers, including P. Mason, C. C. Chiang, E. Tan, and R. Bogner, who helped to proofread the communications involved in the project. Furthermore, I must thank the editorial staff at CRC Press, Inc., for the experienced coordination of communication which led to final agreements with the contributing authors. Finally, my thanks to CRC Press for giving me this opportunity to pursue this most significant and greatly rewarding project.

**Dean Hsieh**

## EDITOR

**Dr. Dean Hsieh** is the founder and president of Conrex Pharmaceutical Corporation, Brandamore, Pennsylvania since 1985. He obtained his Ph.D. ('78) and M.S. ('74) degrees from M.I.T., followed by postdoctoral training with Professor Langer at the Boston Children's Hospital, associated with the Harvard Medical School. In January, 1981, he was promoted to Instructor at Harvard Medical School. In October 1982, he became an Assistant Professor at the College of Pharmacy, Rutgers — The State University of New Jersey. He has authored and co-authored more than 60 papers and abstracts in the area of drug delivery systems and controlled release technologies. He is also the holder of several patents and pending patents. Recently, his efforts have focused on the research and development of proprietary permeation enhancers, leading to the commercialization of this technology.

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Chapter 1

# NANOPARTICLES: SOLID SUBMICRON DRUG DELIVERY SYSTEMS

**Richard C. Oppenheim**

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# I. INTRODUCTION

When a drug delivery system is fabricated with controlled release, it is normal that the mechanism and rate of drug release from the system have been identified and built into the product. However, there is usually a poor definition of or control over the site of release in relationship to the actual or perceived site of action.

Most controlled release products are developed to bypass some inconvenient physiological situation (e.g., those created by enteric coated products) or to have some form of sustained or extended release profile. In neither case is there any real attempt to ensure that the released drug encounters an appropriate site of action. Provided that the circulating concentration is sufficient to ensure the correct response at the desired site of action and that any responses at undesired sites of action are not life threatening, then such a controlled release system is adequate. It does, however, represent poor application of modern pharmaceutics.

With the current regulatory difficulties in getting new but not revolutionary drugs marketed, pharmaceutical companies are developing new delivery systems for approved drugs. In general terms, the site of action is known. A reasonable estimate of the concentration of the drug in the immediate neighborhood of that site, if not at the site itself, can be developed knowing the pharmacokinetic and pharmacodynamic properties of the drug. Hence, there is often sufficient information available to address questions in the second area of controlled release.

This chapter will focus on those submicron particulate delivery systems in which a deliberate atttempt is made to respond to the problems in both of these areas. Hence, systems which cause uncontrolled or serendipitous therapeutic responses will not be discussed. Examples of such excluded particulate systems are inhaled cigarette smoke, metal or asbestos dusts, viruses or pollen, contaminating silica particles leached off blood defoaming bags used in open heart surgery, or particulate contamination derived from the administration of large- or small-volume parenterals. Similarly, simple diagnostic and therapeutic colloidal systems such as technetium-labeled colloidal sulfur or colloidal gold as $^{198}Au$ have been discussed previously.[1]

Widder et al.[2] clearly identified the three levels of targeting at which modern parenteral drug delivery systems should be aimed: (1) a local perfusion of an organ by drug released from the delivery system, (2) interaction of the released drug only with part of an organ or even with the external membrane of the target cells, and (3) no release of drug until the delivery system is inside the cell containing the site of action. Conceptually the fourth and ultimate level is delivery to and release at the site of action. As this targeting approach to drug delivery is more widely accepted and achieved, there will be a much reduced need for biopharmaceutics as it is now practiced.

Tomlinson[3] has modified this approach by dividing targeting into two types, passive and active. In passive targeting, the size of the particulate delivery system is used to define the type of automatic response by the body to that delivery system. Capillary blockage is possible for particles greater than approximately 4 μm in diameter. If the particle diameter is between 30 nm and 7 μm, there can be reticuloendothelial system (RES) uptake and general lysosomotropism, whereas if the particles are less than 100 to 200 nm in diameter, they can pass through basement membranes and escape from the circulatory systems.

In active targeting, which seems to be useful for particles with a size of 0.05 to 4 μm, the formulation and/or the user guides the particle in some way as it interacts with the body. This guiding procedure could be antibody attachment to the particle so that specific cell antigens can be identified. Alternatively, magnetite could be incorporated into the particle and an external magnet focused on the target part of the circulatory system, so that as the particles arrive at that part, they are trapped there.

There are a number of consequences[1] of controlling the release of a drug by targeting it

in some way. The circulatory levels of free and protein-bound drugs are reduced drastically. The total amount of drug used is reduced. The cost of the delivery increases due to the technology of making the delivery system. Provided that this increased cost is not as great as the savings achieved in reduced drug dosage, reduced hospital residence time, and reduced repair of adverse and toxic reactions to the drug, the targeted controlled release system could be economically viable.

A variety of terms have been used to describe the solid particulate systems used to achieve controlled parenteral release of a drug. Tomlinson[3] describes all particles with a size of between 20 nm and 300 μm as microspheres. While this has the advantage of simplicity, it is clear that capillary blockage cannot be achieved by submicron- or nanometer-sized particles. Consequently Oppenheim[1] and Kreuter[4] have argued that a functional definition based on size of the particle is appropriate. Hence nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm where the active ingredient is dissolved, entrapped, encapsulated, adsorbed, or attached. It follows that nanoparticles have a direct interaction with individual target cells, i.e., either the second or third level of targeting.[2] Microspheres are similar particles, but with a size greater than about 4 μm, and as such can be embolized in capillaries. The released drug can then interact with the target cells. This corresponds to the first level of targeting.[2] It is suggested that particles in the overlap size range of 1 to 7 μm be classified on the basis of their embolism (microspheres) or alternatively their RES interaction or active targeting approach (nanoparticles).

It is vital to differentiate these two fundamentally different approaches to drug delivery because each requires a different approach to controlling the release of the drug. Microsphere delivery involves the payload of drug being released by leaching from pores, diffusing through an external coat, or becoming available as the microsphere slowly degrades. A small release of drug prior to embolization is not desirable, but is not disastrous from a formulation point of view. On the other hand, nanoparticle delivery can only be at the surface of the target cell or, preferably within the target cell. Hence there should be no leakage prior to this target dumping. This stringent control of release is difficult to achieve. It also leads to a dilemma for the system designer. The desired intracellular release is controlled only by the amount and character of the intracellular, often lysosomal, enzymes of the target cell over which the designer has no control. Therefore, an increase in targeting efficiency can lead to a loss of control over release rate.

Many more microsphere systems than nanoparticle systems have been developed. In general terms, this is because they are simpler to make and use. A more complete detailed compilation of the properties and behavior of the more important microsphere delivery systems is available in recent books edited by Illum and Davis[5] and Davis et al.[6] In this review of nanoparticles, the relevant key parts of the information available about the manufacture, storage, and use of microspheres will be included. A rational approach will be developed for fabricating and fomulating nanoparticle delivery systems which will be able to achieve upper-level targeting.

## II. SYSTEM LIMITATIONS

Any drug delivery system is a combination of the active drug and the base carrier molecules. Physical, chemical, and physiological properties of each part of the system limit the choice and the combination of choices for each part. As nanoparticles will deliver the drug to the membrane or to the intracellular fluid of the target cell, the system designer must have information about the interaction mechanism and strength between the drug and the external membrane; the partition coefficient of the drug between the intracellular fluid, the cell membrane, and the surrounding fluid; and the drug concentration required either in the membrane or in the intracellular fluid for effective therapy; as well as the metabolic pattern of the drug associated with that target cell.

For parenteral administration of particles, the individual as well as the associated, aggregated, or polymerized carrier molecules must be nontoxic, nonimmunogenic, and biodegradable. Acceptance of these criteria severely limits the choices available to the system designer. Many systems have been developed and tested in which a compromise has been reached with these criteria.

The combination of the drug and the carrier molecules used to make the drug delivery system must meet a number of criteria. The system must be pharmaceutically acceptable with regard to shelf-life. Hence most of the vast literature on liposomes does not help formulate better solid submicron drug delivery systems.[7] The system must be sterile before administration: filtration cannot be used since the nanoparticles are similar in size to the contamination particles. Additionally the system should be easy to administer with standard equipment without causing any localized reaction. The system should not cause any hapten production or other immunological reaction; as the carrier molecules are not immunogenic, any such problem would be the result of the drug and the carrier molecules interacting. The delivery system must have an acceptably large payload of drug. The system must be designed so that any opsonization that takes place after administration either facilitates or at least does not hinder the targeting. The system must release its payload of drug only when the system can identify the site of action of the drug. Release must then occur at a predetermined controlled rate by the release mechanism built into the system. Much work has been undertaken on release mechanisms and rates without due regard to all the other system criteria. Finally, if there are parallel shifts in the $LD_{50}$ and the minimum effective dose, then the therapeutic index of the drug in the particulate and free form is the same. Similarly, the particulate delivery system could increase the concentration of the drug in the target cell and in important nontarget cells equally.[8] In neither case does the controlled release product offer any advantage.

These system limitations are severe and have not been totally overcome. Consequently nanoparticle manufacture is generally limited to small-scale, noncommercial batches. Scale-up problems will be encountered, but as considerable experience has been accumulated with microspheres, scale-up is not expected to be a major difficulty with nanoparticles. When the first types of nanoparticles (those based on the acrylates) are discussed, the approach adopted to try to solve the extra- and intrabody questions posed at the beginning of this chapter will be highlighted. As each of the other types of nanoparticles is discussed, mention will be made of these approaches, particularly as they influence manufacturing, storage, and administration procedures. Detailed discussion of the use to which these controlled release nanoparticles are being put, experimentally and clinically, is not given here, but is available in the references cited.

## III. ACRYLATE SYSTEMS

There are a number of base molecules which may be used and their choice is determined by how the nanoparticle is to be used. Kreuter[9] recommends the almost nonbiodegradable poly(methylmethacrylate) nanoparticles for vaccination purposes because the antigen is retained for a long time, leading to prolonged immunostimulation. Similarly, enzymes[10] and proteins[11] have been immobilized in polyacrylamide microbeads and nanoparticles to ensure long-term activity in industrial processes. On the other hand, polycyanoacrylate nanoparticles are more suitable for use as parenterally administered drug carriers because they have a controllable, quicker biodegradation rate.[9]

All polyacrylic particles are made by techniques based on emulsion, suspension, or bead polymerization. Emulsion polymerization, either with or without added surfactant, generally leads to submicron particles. Suspension polymerization yields microspheres while the beads formed by the third technique usually have millimeter-sized diameters.

Despite the fact that these techniques are used on a large scale by a variety of industries, polyacrylic particles are still only produced in small batches for experimental therapeutic use. However, there seem to be no significant scale-up problems apparent, once a drug-containing product has been shown to release its payload at a controlled rate by a known mechanism at the desired physiological site. Many potential scale-up problems have been encountered and resolved in the enzyme immobilization industry. Whether the scale-up would lead to an economically viable product depends on the balance of manufacturing costs and health care savings. The particles can be made in either organic or aqueous phase using polymerization procedures described in the following sections.

### A. Manufacture in Organic Phase

Initially[12] Sjöholm's group in Sweden used 200 mℓ of a 4:1 toluene:chloroform organic phase into which 5 mℓ of a buffered aqueous solution of acrylamide, drug or active macromolecule, and ammonium peroxodisulfate was emulsified using a high-speed homogenizer and 0.25% Pluronic F68 as emulgent. The composition of the organic phase was chosen to give a density of 0.98 g/mℓ in order to facilitate a stable emulsion. *N,N*$^1$-Methylenebisacrylamide can be added to the aqueous phase when a reduced biodegradation rate and a more porous particle is required. After adding *N,N,N*$^1$,*N*$^1$-tetramethylethylenediamine as the initiator, radical polymerization proceeded with gentle stirring at room temperature to produce 1000-nm nanoparticles. Oxygen had to oe excluded to ensure successful polymerization. Ekman et al.[13] had found earlier that high-speed homogenization during the polymerization led to 1- to 10-μm particles, presumably due to collisional coalescence of the polymerizing particles.

Edman et al.[14] used much the same manufacturing technique to make nanoparticles based on molecules which had dextran or other polysaccharides incorporated into the acrylic monomer. Large molecular weight materials such as albumins or enzymes can be immobilized in each type of system partly by entrapment in the polymeric network and partly by physical fixation within the polymeric bundles. The polysaccharide particles immobilize a greater payload of protein than the polyacrylamide particles because as the pore size is reduced in the polysaccharide system, the extent of entrapment increases. Albumin payloads of 35 to 40% can be achieved[15] in the polysaccharide particles. However, although the salicylate binding capacity of albumin polyacrylamide nanoparticles is not impaired, it is reduced by 50% when used in maltodextrin particles. These polysaccharide particles also leach their payload more rapidly than the polyacrylamide particles, presumably due to a much faster degradation of the starch component of the particles in both in vitro and in vivo situations.[16] Hence, a balance of the starch component and the hydrocarbon chain is essential to ensure an optimal release profile in vivo.

Low molecular weight drugs cannot be effectively entrapped in either type of acrylamide-based nanoparticle because they readily leach from the pores. El-Samaligy and Rohdewald[17] found similar results for 100 to 600-μm polyacrylamide microbeads. However, it may be possible to conjugate such drugs to the glucose residues, make the nanoparticles, and then use lysosomal enzymes to cleave the drug-glucose link and release the drug. Assuming that such a conjugation does not enhance the degradation of the nanoparticle before it encounters its target cell and that this type of nanoparticle can be taken up by that cell, the release rate is determined only by the amount and character of the lysosomal enzymes in the target cells and not by any feature built into the delivery system by its designer. The pore size of these nanoparticles is too great to be of use to the designer in controlling the drug release. Considerable work remains to be done to control the pore size of these and all other porous nanoparticles. If this can be achieved, then pore diffusion could become a rate-limiting step in controlled drug delivery from nanoparticles.

Intravenous and intraperitoneal injections of L-asparaginase-containing 250-nm polyacryl-

amide nanoparticles in mice produce an enhancement of the humoral antibody response compared to a soluble enzyme given by the same route.[18] Intramuscular or subcutaneous injection of the particles gave a much weaker immune response. However, the ability of the particle-entrapped enzyme to react with an antibody is decreased compared to the soluble enzyme.

Edman et al.[19] found that with 40-mg/kg body weight intravenous single doses of 400-nm polyacrylamide nanoparticles in mice, only insignificant and hardly detectable morphological reactions could be seen in the liver, spleen, and bone marrow. Doses of 160 mg/kg were needed to produce significant adverse reactions. If such a system is to be used as a drug delivery system, this then sets the upper limit on the amount of nanoparticles and hence attendant drug payload that can be administered. No chronic toxicity studies appear to have been reported.

Similar polyacrylic nanoparticles have been developed independently by Speiser's group in Zurich. Birrenbach and Speiser[20] used hexane as the outer phase. By using 15% of *bis*-(2-ethylhexyl)-sodium sulfosuccinate and 7.5% of polyoxyethylene-4-lauryl ether, up to 35 mℓ of water can be solublized in 80 mℓ of hexane. The acrylic monomers such as acrylamide or methylmethacrylate readily dissolved to form a clear solubilized system. The polymerization was preferably done by gamma irradation in a "cobalt bomb" (300 krad) or by UV irradiation. It could also be achieved by visible light irradiation provided small amounts of a catalyst such as riboflavin-5′-sodium phosphate or potassium peroxodisulfate had been added. This latter method produced fewer polydisperse nanoparticles than the gamma irradiation method.[21] Much smaller 40-nm water-swollen polyacrylamide particles stabilized by Aerosol OT and dispersed in toluene have been made and characterized by Candau et al.[22]

Kreuter[4] argues that the lower solubility of the acrylic monomers in the hexane compared to the toluene-chloroform mixture used by Sjöholm's group leads to a reduction in the extent of acrylic monomer molecule diffusion. This results in an increase in the stability of the system which means stirring is not required during the polymerization. This could be the reason for a greater batch-to-batch and within-batch uniformity of particle size in the systems using hexane. The problems with all these acrylamide-type systems are poor biodegradability, concern that all the unincorporated monomer is removed in the phase separation/centrifugation cleanup procedure, and the possible degradation or denaturation of the active payload upon irradiation.[23]

Kreuter[9] found that scanning electron microscopy (SEM) (after allowing for the thickness of the conducting gold coating), transmission electron microscopy (after freeze fracturing), and photon correlation spectrometry gave comparable estimates of size for both poly(methylmethacrylate) and polyacrylamide nanoparticles. Using nonadsorbing helium in a gas pycnometer, he found the density of polyacrylamide and poly(methylmethacrylate) nanoparticles to be 1.14 and 1.06 g/cm$^3$, respectively. This latter figure is consistent with the porous nature of the nanoparticles when compared to 10-μm beads of the same material which had a density of 1.15 g/cm$^3$. All the polyacrylic nanoparticles were X-ray amorphous. The electrophoretic mobility of aggregates of 120-nm poly(methylmethacrylate) nanoparticles was about $-1.3$ μm.cm/(sec.V) in an unknown-strength phosphate-buffered saline at pH 7.4. This mobility dropped to about $-0.25$ μm.cm/(sec.V) when the nanoparticles were stored in human serum, indicating opsonization of the particles. Polyacrylamide nanoparticles did not readily aggregate and no electrophoretic measurements were made. No zeta potential or surface charge calculations are possible on these systems which have aggregated to an unknown extent. This opsonization process is reflected in the drop in contact angle of water on compressed particles from 73 to 53° before and after storage for 12 hr in human serum.

### B. Manufacture in Aqueous Phase

Alkylcyanoacrylates are practically insoluble in water. The alkyl groups commonly found

in the polymerized form of these acrylic materials used as biodegradable or dissolvable sutures are methyl, ethyl, propyl, and butyl. Nanoparticles can be made from these materials by solubilizing the monomer in a surfactant containing aqueous vehicle and allowing anionic polymerization to take place. Again very small batch sizes are used with, for example,[24] 0.25 mℓ of butylcyanoacrylate being added dropwise to 24.75 mℓ of a 0.2-μm filtered, stirred aqueous solution of 0.5% w/v Dextran 70 in 0.01 *M* hydrochloric acid at 20°C. A magnetic stirrer set at about 1000 rpm is used to fully disperse the monomer. After 2 hr, the polymerization is complete and the product can be cleaned up by filtration, centrifugation, or dialysis. Other surfactants which have been used include Polysorbate 20,[25] Pluronic L63,[26] and Pluronic F68[27] (both polyoxyethylene-polyoxypropylene surfactants). Illum et al.[28] used the less hydrophilic material Dextran 70 when the surface of the nanoparticle was to be the adsorption site for the Fc portion of antibodies, leaving the F(ab) portion accessible to recognize cell membrane antigens.

In a detailed study, Douglas et al.[24] found that control of particle diameter in the range of 100 to 200 nm could be achieved by varying the pH of the system between 2 and 3. At pH values below 2, the rate of the anionic polymerization is too slow and a very polydisperse system results due to the coalescence of nanoparticles swollen with unreacted monomer. A higher pH values, the increased hydroxyl ion concentration leads to a very rapid polymerization rate and an amorphous polymer mass results.

At monomer concentration below 1%, the nanoparticles were quite polydisperse. Reasonable control of the size and dispersity was achieved at monomer concentrations between 1 and 2.5%, but at higher concentrations up to the maximum of around 7% (when the resultant system was not free flowing) the particle size increased markedly and the dispersity increased slowly. Other physicochemical factors such as temperature and acidifying agent had no effect on particle size. Since the added electrolyte over the concentration range of 0.01 to 0.25 mol/dm$^3$ also had no effect on particle size, surface charge of the particle is not important with respect to particle size or maintenance of colloidal stability, inferring that the system is sterically stabilized. This is in direct contrast to the charged polystyrene latices which are highly ionic-strength dependent.[29] Douglas et al.[24] also found that stirring rate had no effect on particle size, although Kreuter[4] suggests that vigorous stirring can lead to larger butylcyanoacrylate particles. In a second report, Douglas et al.[30] found that the diameter of the nanoparticles could be controlled in the range of 20 to 770 nm by varying the molecular weight and concentration of dextrans, poloxamers, and polysorbates.

Kreuter[9] also investigated the physicochemical properties of polyalkylcyanoacrylate nanoparticles. He found that the presence of surfactants used in their manufacture led to a smooth coat over the particle surfaces, which inhibits the observation of the individual structures. Removal of the surfactants, however, often results in aggregation and leads to irreversible changes in the nanoparticles. Hence SEM is not very useful for these products, nor can it determine the fate of the surfactant when the nanoparticles are administered in vivo or whether or not the nanoparticles can remain discrete in vivo if the surfactant desorbs.

Removal of the surfactant caused nanoparticle aggregation which enabled electrophoretic mobilities to be determined. A clear chain-length effect was found in the phosphate-buffered saline at pH 7.4 in that the methyl, ethyl, and butyl derivatives had mobilities of $-1.64$, $-1.32$, and $-0.87$ μm.cm/(sec.V) respectively. This effect was completely obliterated when the measurements were done in human serum when a value of around $-0.22$ μm.cm/(sec.V) was determined. This is about the same as the mobility of the poly(methylmethacrylate) nanoparticles, indicating that the effect on the surface after opsonization was very similar for the two types of acrylic nanoparticles. The water contact angle measurement indicated that the butyl derivative was more hydrophobic than the methyl derivative. This marked hydrophobic effect was lost upon storage in human serum, and again there was little difference from the poly(methylmethacrylate) nanoparticles stored in human serum.