Drug Development and Industrial Pharmacy, 1:43–57, 2005
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ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1081/DDC-200043985

Reduction in the Initial-Burst Release by Surface Crosslinking of PLGA Microparticles Containing Hydrophilic or Hydrophobic Drugs

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ABSTRACT Sustained-release approaches are emerging for the delivery of drugs from polymer encapsulation. However, the most persistent problem that remains is the initial burst release of the drug, which can exceed the toxic limits. Dexamethasone, a hydrophobic drug, was encapsulated in poly(lactide-co-glycolide) (PLGA) microparticles using the solvent evaporation method. The drug release profile of these microparticles was studied and the initial burst was reduced by crosslinking of the microparticle surface using ethylene glycol dimethacrylate and tri(ethylene glycol) dimethacrylate. Due to surface crosslinking, an additional diffusional resistance was created, which prevented easy dissolution of the drug into the release medium and brought about a substantial reduction in the initial burst release. Moreover, the time required for reaching a stationary-state release was also observed to be delayed, prolonging the sustained drug delivery. This concept was further tested with a hydrophilic drug, the sodium salt of dexamethasone phosphate, encapsulated in PLGA polymer microparticles and was observed to reduce the burst release as well. For synthesizing the polymer microparticles containing dexamethasone, an o/w microemulsion and solvent evaporation technique was used; whereas, for those containing dexamethasone phosphate, w/o/o/o phase separation/coacervation technique was used. The surface crosslinking was performed by ultraviolet radiation.

KEYWORDS Dexamethasone, Dexamethasone phosphate, PLGA, Controlled drug release, Sustained release formulations, EGDMA, Tri(EG)DMA, UV surface crosslinking, HPLC

INTRODUCTION

In sustained-drug release applications, the drug is usually given at one time in a bulk quantity to a specific target in the body and is expected to be delivered over a period of typically several months. The drug is encapsulated

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in a biodegradable polymer and is initially released by diffusion through the pores in the microparticles and later on by the erosion of the polymer within the particle. However, a serious problem is the initial-burst effect. Burst effect in sustained-release formulations is termed as a large initial quantity of drug released from the encapsulated drug capsule before the release rate reaches a stable profile (Huang & Brazel, 2001). This phenomenon could be useful in some cases like cancer treatment, wound treatment, etc (Setterstrom et al., 1984). However, the burst release is not desirable in most of the sustained-drug delivery applications, because the dosage of the drug encapsulated is large to be released over a long period of time. Even a small percentage of the drug released would increase its local concentration to an extent near or above that at which the drug becomes a toxin in vivo (Shively et al., 1995). As a result, the defense mechanism of the body would try to resist the increase in the toxicity level by forming additional layers of tissues on the polymer surface, thereby disrupting the entire release profile and mechanism (Basic Pathology, 1992; Biomaterials Science: An Introduction to Materials in Medicine, 1996). Any drug released during the burst stage may not be metabolized and excreted without even being effectively utilized. Even if the drug is not toxic, this amount of drug released is essentially wasted, which is undesirable inside the body as well as for the cost of the pharmaceuticals (Jeong et al., 2000). Hence, there is a need to reduce the burst effect.

Several researchers have observed burst release without giving advanced explanations. Others have tried to explore the mechanisms of burst and prevent it by several methods such as surface coating of the polymer (Hsieh et al., 1983; Peracchia, et al., 1997; Redhead et al., 2001), changing the surfactant (Park et al., 1992), changing the molecular weight of the polymer (Song et al., 1997), and including the burst effect in models to simulate the release process (Cascone et al., 2002; Narasimham & Langer, 1997; Patil et al., 1996).

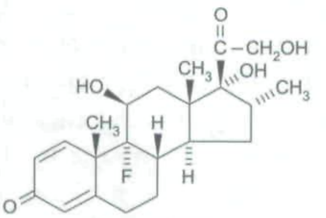
Burst release has been attributed to various physical, chemical, and processing parameters, but for the most part, no substantial studies have been carried out to examine the underlying mechanisms of burst release in monolithic polymeric systems (Huang & Brazel, 2001). There are several possible causes for burst release. One is the desorption or release of the drug that has been adsorbed or trapped on the surface of the device (Chung et al., 2001; Coombes et al., 1998; Rafati et al., 1997). Another is nonuniform drug loading. Migration or redistribution of drug, facilitated by convection during evaporation, can lead to higher surface concentrations as the polymer is dried (Huang et al., 1999; Mallapragada et al., 1997). Lastly, the porous structure of some polymer networks can also lead to an initial burst as drug diffusion is less hindered in water-filled macropores formed during solvent evaporation (van de Weert et al., 2000). Pores are usually formed on the polymer particle surface by the use of salts and volatile compounds that evaporate during the formation and solidification of the particles.

Reduction in burst release of drugs is vital in the pharmaceutical industry because it is unpredictable and hence undesirable in the majority of the cases. Even if it is desirable in some cases, the amount of burst release of drugs cannot be well controlled (Mandal et al., 2002). Earlier attempts to reduce burst release include surface extraction (Huang et al., 2002) to reduce the burst release of proxyphylline drug from PVA hydrogels, surface coating (Chiou et al., 2001) of lidocaine encapsulated in PLLA (poly-l-lactide), and use of chitosan of varying molecular weights and viscosity to form double-walled polymer microspheres. Chitosan, being easily biodegradable and relatively nontoxic to the body and skin, is a potential drug-delivery agent (Gupta & Ravi Kumar, 2000; Ravi Kumar, 2000). Azidated chitosan was photo-cross-linked to azidated epichlorohydrin using UV light and was used as a drug carrier for a model drug, such as theophylline. The process of UV crosslinking was analyzed by ATR/FTIR to observe functional group changes upon crosslinking, and the drug carrier was demonstrated to show controlled release of the drug with a reduction in the initial burst release (Jameela et al., 2002).

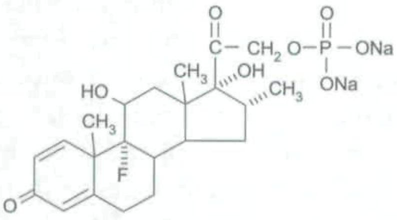
A novel microencapsulation technique was developed (Mandal et al., 2002) with high efficiency of encapsulation and low burst effect by microencapsulating pentamidine/polyvinyl alcohol (PVA) hydrogel for crosslinking in poly(lactide-co-glycolide) (PLGA) using a solvent evaporation technique. They observed reduction in the burst effect and drug loss, and controlled in vitro release of the drug pentamidine for 80 days. A similar study was conducted (Cascon et al., 2002) by encapsulating dexamethasone in PLGA nanoparticles based on a single oil-in-water (o/w) emulsion and further crosslinking these nanoparticles in PVA hydrogels. The in vitro drug release tests showed that the crosslinking of PVA hydrogel, which acts as a diffusional barrier for the drug release, is the controlling parameter in the release of dexamethasone. Another attempt (Patil et al., 1996) was to synthesize macroporous poly(sucrose acrylate) hydrogel containing high-density spherical microgels embedded inside low-density macrogel phase for the controlled release of macromolecules through the hydrogel matrix. The release of protein from the low-density macrogel resulted in the initial burst, while the release from the high-density microgels gave rise to the sustained release of protein.

Highly crosslinked hydrogel spheres were fabricated (Mellott et al., 2001) using UV photopolymerization of poly(ethylene glycol) diacrylate (PEG-DA) and pentaerythritol triacrylate (PETA) with 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) as the photoinitiator. A comonomer that can create noncovalent interactions between the hydrogel and the encapsulated drug was added (Graham & McNeill, 1984) which can significantly affect the drug release rates. Using ATR/FTIR, a reduction in the swelling rates of the hydrogels and controlled drug release rates with a reduction in initial burst for increasing concentrations of comonomer additions was observed.

Dexamethasone (Fig. 1) is a synthetic adrenocortical steroid possessing basic glucocorticoid activity. It is very sparingly soluble in water (0.1 mg/mL) (Merck Index, 1996). It is among the most active members of its class, being about 25–30 times as potent as hydrocortisone. Moreover, dexamethasone is readily absorbed from the gastrointestinal tract. It can be used as a glucocorticosteroid in various applications including apoptosis (Distelhorst, 2002), anti-inflammatory (Brahmapurikar et al., 1980), protective agent against the neurotoxic actions of cisplatin (Scott et al., 1995), for the treatment of chronic eustachian tube dysfunction (Silverstein et al., 2003), for the prevention of post operative nausea and vomiting (Wang et al., 2000). Dexamethasone 21-phosphate disodium salt (Fig. 1) is a crystalline, water-soluble salt of the same drug. It is highly soluble in water.



Dexamethasone



Dexamethasone phosphate

FIGURE 1 **Chemical Structure of Dexamethasone and Dexamethasone Phosphate.**

In order to encapsulate hydrophobic drugs, an oil-in-water (o/w) solvent evaporation method has been utilized to synthesize polymer microparticles of biodegradable polymers such as PLGA or PLA with the drug encapsulated in them (Bodmeier & McGinity, 1987a). However, this method is not very successful for water-soluble drugs because the drug gets partitioned out into the external aqueous phase, leading to decreased drug loading (Bodmeier & McGinity, 1987b). As a result, a water-oil-water (w/o/w) double emulsion technique has been used to encapsulate water-soluble drugs (Heya et al., 1991; Yamakawa et al., 1992). However, there are concerns with the emulsion stabilities and the drug getting carried away from the inner aqueous phase to the external aqueous phase during the formation of the double emulsion (Ficheux et al., 1998). Better encapsulation of water-soluble drugs has been achieved by using oil-in-oil (o/o) (Jalil & Nixon, 1989), solid-in-oil-in-oil (s/o/o) (Iwata & McGinity, 1993), and s/o/o/o (Iwata et al., 1998; Iwata et al., 1999) emulsion techniques.

In this work, an o/w solvent evaporation technique (Iwata et al., 1999; Rickey et al., 2003) was employed for the encapsulation of the hydrophobic dexamethasone and w/o/o/o phase coacervation technique for the encapsulation of the hydrophilic dexamethasone phosphate. Also, the effect of the presence of NaCl salt in the aqueous phase of o/w emulsion on the extent of drug encapsulation was studied. NaCl is known to make the aqueous phase more polar, thereby reducing the solubility of hydrophobic compounds in the aqueous phase. This should result in the reduction of drug loss during the microparticles formulation process due to drug dissolution in the aqueous phase and result in increased encapsulation efficiency.

Recently, dexamethasone was encapsulated in PLGA microparticles and burst release was observed (Hickey et al., 2002). Gaining a basic understanding of the process, the objective of this work is to reduce the burst release of dexamethasone by surface preferential crosslinking polymerization of another crosslinker on the PLGA microparticles, thereby providing an additional diffusional resistance for the release of surface drug (Mellott et al., 2001). This technique was chosen because it facilitates post process on the already existing polymer microparticles without having to change the formulation procedure and still achieving a reduction in the initial burst release. Polymerized ethylene glycol dimethacrylate (EGDMA) or Tri(EG)DMA is not toxic to the human body and hence can be used as crosslinkers for parenteral depot delivery. The final product is not very different from the original microparticles and hence can be used in a similar manner for the drug-delivery process.

Also, the effects of multiple layers of crosslinked polymer on the reduction in the burst release and control of release profile were also studied. This work was further extended to the reduction of burst release of highly hydrophilic salt of the same drug, to demonstrate that this technique is also applicable for highly water-soluble drugs. In all the further discussions and explanations, the crosslinking polymerization of an additional crosslinker over the PLGA microparticle surface will be referred to as surface crosslinking of microparticle surface or just surface crosslinking.

MATERIALS

Poly(lactide-co-glycolide) (PLGA 50:50; inherent viscosity = 0.39 dL/g in HFIP @ 30°C) was obtained from Birmingham Polymers. Polyvinyl alcohol (PVA; avg. mol. wt. = 30,000–70,000), dexamethasone, sodium salt of dexamethasone-21-phosphate, and rhodamine-B were obtained from Sigma Chemicals (St. Louis, MO, USA). Dichloromethane (99.9% ACS grade), silicon oil (dimethicone, 350 cSt), ethylene glycol dimethacrylate (EGDMA, 98%), Tri(ethylene glycol) dimethacrylate, (Tri(EG)DMA, 95%), 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 99%) were purchased from Sigma Aldrich, and methanol (HPLC grade), acetonitrile (HPLC grade), phosphate buffer saline (PBS, Labchem Inc., pH = 7.4), hexane (HPLC grade), and distilled deionized water (DIUF) were obtained from Fisher Scientific (Atlanta, GA, USA). All the materials were used as received.

APPARATUS AND PROCEDURE

Microsphere Preparation

The PLGA microspheres loaded with dexamethasone were prepared by oil-in-water (o/w) emulsion technique (Hickey et al., 2002). The organic (oil) phase consisted of 20 mg dexamethasone and 100 mg PLGA dissolved in the mixture of 4.5 mL dichloromethane and 0.5 mL methanol. The aqueous phase was constituted of 1 wt. % PVA in 100 mL distilled water. To study the effect of addition of salt to the aqueous phase, NaCl salt was added to the aqueous phase to form a 1 N NaCl solution. The PVA acts as a water based surfactant and NaCl makes the aqueous phase more polar, thereby reducing the solubility of hydrophobic drug in the aqueous phase. Separate experiments were conducted in the presence and absence of NaCl salt in the aqueous medium to study the effect of the salt on the encapsulation efficiency. A microemulsion of organic phase containing the drug and the polymer was formed in the aqueous phase by means of stirring using a Corning® magnetic stirrer at 1000 rpm. On mechanical stirring, the organic phase is broken down into smaller droplets in the aqueous phase and stabilized by the surfactant. The organic solvent from the droplets starts evaporating, causing the drug and the polymer to precipitate in the form of microspheres. The drug gets encapsulated in the polymer microspheres during the hardening process. The resulting emulsion was stirred overnight on the magnetic stirrer at a relatively low rpm to allow for complete evaporation of dichloromethane and hardening of polymer microspheres. After sufficient evaporation, the resulting solution was then centrifuged at 3400 rpm (Fisher Scientific) to sediment the particles down. The microspheres were then washed twice with DIUF water to remove excess surfactant and salt (in the case of experiments where salt was used in the aqueous phase), and to wash off any drug adhered to the polymer surface. The concentrated suspension was then lyophilized using Labconco® lyophilizer for complete removal of solvent. For this purpose, the microparticles were initially frozen and then subjected to high vacuum of 100 mTorr at −60°C. As a result, water undergoes sublimation, leaving behind dry microparticles. The particles obtained were then stored in a desiccator for further processing.

The PLGA microspheres loaded with dexamethasone phosphate were prepared by w/o/o/o phase separation/coacervation technique (Iwata et al., 1998). Fifty mg of dexamethasone phosphate was dissolved in 0.25 mL of water and added to 5 mL of dichloromethane to form a water-in-oil suspension by using ultrasonic vibration at 120 W for 1 min. Five mL of 350 cSt silicon oil was then added to this metastable suspension, resulting in the phase coacervation of the organic phase with continuous mixing using a Corning magnetic stirrer at around 1000 rpm. This caused PLGA to precipitate in the form of microspheres. This solution was then added to 75 mL of hexane, pre-cooled to 0−5°C to extract the dichloromethane and silicon oil. Successive washings with pure hexane and then drying under heavy vacuum using the Labconco lyophilizer resulted in the formation of PLGA microparticles with dexamethasone phosphate encapsulated in them.

Surface Crosslinking

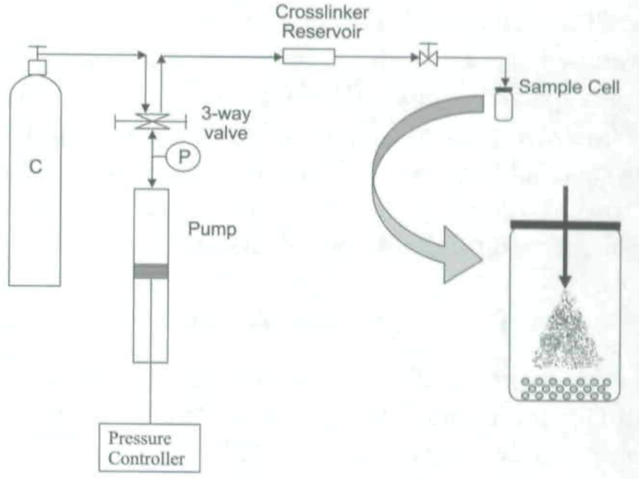


FIGURE 2 **Apparatus for Crosslinker and Photoinitiator Spraying on PLGA Microparticles.**

A portion of these microparticles obtained from the above processes were exposed to a solution of UV crosslinker and photoinitiator for UV polymerization of the crosslinker over the surface of the microparticles. The crosslinking process was carried out using two different crosslinkers, EGDMA and Tri(EG)DMA in different experiments. Both the crosslinkers were allowed to equilibriate overnight with inhibitor remover beads to remove hydroquinone inhibitors before use. The photoinitiator used was 2,2-dimethoxy-2-phenyl-acetophenone (DMPA). Being completely soluble in both EGDMA and Tri(EG)DMA, DMPA was dissolved up to 10 wt. % and used as a homogenous solution. The apparatus for applying crosslinker and photoinitiator onto the microparticle surface is shown in Fig. 2. CO_2 was taken in a syringe pump and pressurized to 1000 psi and 5°C. This liquid CO_2 was passed through a crosslinker reservoir in which 0.1 mL of EGDMA containing 10 wt. % DMPA was taken. CO_2 carried the EGDMA-DMPA solution in liquid form and was sprayed using a 75 μ nozzle onto microparticles in the form of a smokelike mist. As a result, this mist formed a very thin coating of the crosslinker and photoinitiator over the microparticle surface. The flow of CO_2 kept the particles fluidized and the sample cell containing the particles was continuously rotated and shaken to ensure that all the particles are well mixed and well coated. After making sure that the particles are well coated, they were exposed to UV light at 254 nm (UV-A) at 5 mW/cm^2 using a Spectrolinker XL-1500 UV crosslinker (Spectronics Corporation, Westbury, NY, USA) for 1 hour in 3 intervals of 20 minutes each. This process was carried out in intervals to prevent overheating of the microparticles due to prolonged UV exposure. The system was allowed to cool down for about 5 minutes between the 20-minute intervals. This time of exposure and time intervals were previously deduced by trial runs to ensure complete polymerization of EGDMA and Tri(EG)DMA so that no uncrosslinked crosslinkers were left behind. A stream of nitrogen was allowed to flow through the microparticles to keep the particles well mixed and prevent them from getting heated at the same time. Nitrogen also knocks off atmospheric oxygen, thereby preventing the free radical scavanging.

To study the effect of multiple layers of surface crosslinking, increasing layers of EGDMA as well as Tri(EG)DMA were sprayed and polymerized on PLGA microparticles in individual experiments. For this purpose, two sets of 200 mg of microparticles were each sprayed with EGDMA and Tri(EG)DMA and exposed to UV for 1 hour in three intervals of 20 minutes each. Fifty mg of these microparticles were separated for release rate study and the rest was again sprayed with another layer of EGDMA and Tri-(EG)DMA, respectively. This procedure was repeated twice to obtain 50 mg of microparticles sprayed and crosslinked with 1 layer, 2 layers, and 3 layers of polymerized EGDMA and Tri(EG)DMA, respectively.

Particle Size Analysis

The microparticles were analyzed for their sizes and surface morphology using a scanning electron microscope (SEM: Zeiss, model DSM 940). The microparticles were initially spread on a carbon tape glued to an aluminum stub and coated with Au/Pd using a Sputter Coater® (Pelco, model Sc-7 and Electron Microscopy Systems, model EMS 550) under a vacuum of 0.2 mbar. The sputter-coating process involves applying a vacuum of 0.1 mbar to the microparticles in a closed chamber. The particles are then coated with a Au/Pd vapor in presence of a small purge of Argon gas at 0.2 mbar. The Au/Pd layer is coated to make the microparticle surface conductive to electrons in the SEM. The microparticles were coated four times for a 30 sec interval instead of a single coat of 2 minutes to avoid melting of the polymer because of excessive heating. The sputter coater adds Au/Pd layer of about 10–15 nm thick in 2 minutes. Hence, the amount of Au/Pd coated in 2 minutes is insubstantial as compared to the size of the microparticles (about 50–100 μm so as not to alter the microparticles' morphology or surface texture on a micron level. The microparticles were then observed under SEM and micrographs were recorded. Multiple images of different areas of the SEM stub were recorded for microparticle size analysis. In order to gain an idea of the condition of the microparticle surface during the release study, a small portion of original and surface crosslinked microparticles, after about 75 days of the particles being in the release medium, were lyophilized, and observed using SEM for particle size, morphology, and pores.

Confocal Laser Scanning Fluorescence Microscopy

In order to observe the presence of coating over the microparticle surface, a solution of rhodamine-B dye was prepared in the mixture of EGDMA and DMPA and sprayed over the PLGA microparticles as described above. These microparticles, after surface crosslinking using UV light at 254 nm, were observed under confocal laser scanning microscope (CLSM: BioRad® MRC 1024 using LaserSharp 2000 software). Ar/Kr laser was used to excite rhodamine-B dye. Rhodamine-B is a fluorescent dye that can be excited by laser in all the Red (647 nm), Green (568 nm), and Blue (488 nm) regions. Hence, triple labeling images were used to represent an overlapping combination of all of them. Z-series optical cross-sections of the microparticles were obtained to observe the distribution of the dye around the microparticle surface at different cross-sections of the microparticle.

Extent of Encapsulation Measurement

In order to determine the content of dexamethasone actually encapsulated in the polymer matrix, a known quantity of the microparticles was mixed in acetonitrile. The resulting solution was analyzed for the dexamethasone content using HPLC (Model 600, Waters Corp.), which was equipped with a 7725i Rheodyne® six-port injection valve, model 600 HPLC pump, model 2410 refractometer, and model 2487 Dual λ UV spectrometer with the wavelengths set to 242 nm and 211 nm. A Spectronic Genesys2® UV spectrophotometer was used to initially detect the wavelength at which dexamethasone absorbs the maximum of UV light and was found to be 242 nm. The mobile phase used for HPLC analysis of dexamethasone was 0.05 M PBS buffer and acetonitrile in the ratio of 60:40 flowing at 1 mL/min through a 3.9 mm × 150 mm Novapak C-18 column (Lamiable et al., 1986).

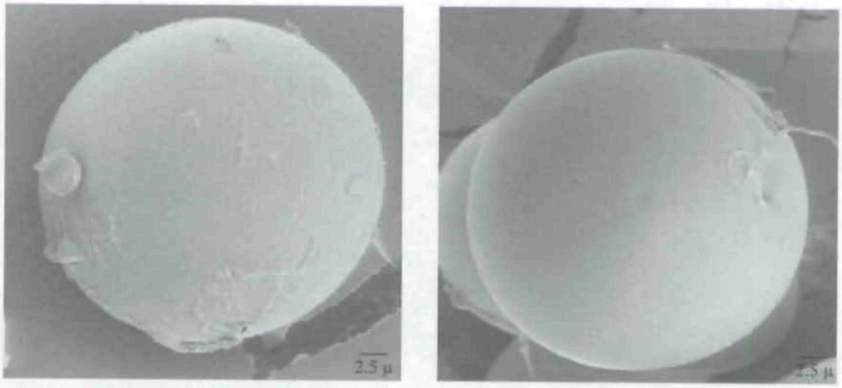
To determine the same for dexamethasone phosphate, a different technique was utilized. The polymer is soluble in dichloromethane, whereas the drug is soluble in water. Using this concept, 11 mg of PLGA microparticles containing dexamethasone phosphate were dissolved in 1 mL of dichloromethane and this was then equilibriated with 10 mL of distilled water. This aqueous phase, assuming that the drug was totally partitioned in the aqueous phase, was then tested for dexamethasone phosphate content using HPLC analysis method for dexamethasone phosphate. This method of analysis was validated by mixing known amounts of polymer and drug in known quantities of distilled water and dichloromethane. After complete mixing, the two phases were allowed to separate. The resulting aqueous phase was analyzed for drug content using HPLC and was observed to retain almost 98% of the total drug taken. In the case of dexamethasone phosphate, the mobile phase had to be changed to an acidic medium to maintain the sodium salt of dexamethasone phosphate in undissociated form. Hence, the mobile phase was modified to 0.05 M PBS:ACN:75% acetic acid (AA) in the ratio of 70:28:2. Dexamethasone phosphate also was analyzed by UV spectrophotometer and was observed to show maximum absorption at 244 nm. Hence, the UV wavelength for dexamethasone phosphate in UV spectrometer of HPLC was set to 244 nm and 211 nm.

In Vitro Release Rate Study

The original and the surface crosslinked polymer particles were then used to study the release rate of drugs from the polymer. For this purpose, about 30–35 mg of microparticles were suspended in 100 mL of 0.05 M phosphate buffer saline (PBS, pH=7.4) at 37°C in an incubator, and stirred at 50 rpm. For in vitro release study, PBS at pH=7.4 at 37°C resembles the environment of the drug when it is in contact with blood inside human body. Fifty rpm is USP standard to simulate the hydrodynamic behavior inside body (Bempong et al., 1999). Samples, after being filtered using a 5 μm filter, were taken approximately once or twice a day and analyzed for UV absorbance of dexamethasone in the PBS buffer using Waters HPLC as described above. Only the original set of experiments were conducted in triplicate (n=3) for testing for the consistency of the results as described in Fig. 8. Once the consistency was established, all the remaining experiments were conducted only once (n=1). The dual wavelength spectrometer was set to 242 nm for dexamethasone and 244 nm for dexamethasone phosphate. The second wavelength was set at 211 nm, although EGDMA shows UV absorption both at 242 and 211 nm. So, a verification of no additional peak at 211 nm would verify the fact that EGDMA was totally polymerized during the crosslinking process. In order to make sure that the crosslinkers did not mask the HPLC analysis of the drugs, separate samples containing the drug and the crosslinkers were analyzed using HPLC for their retention times of the HPLC column.

RESULTS AND DISCUSSION

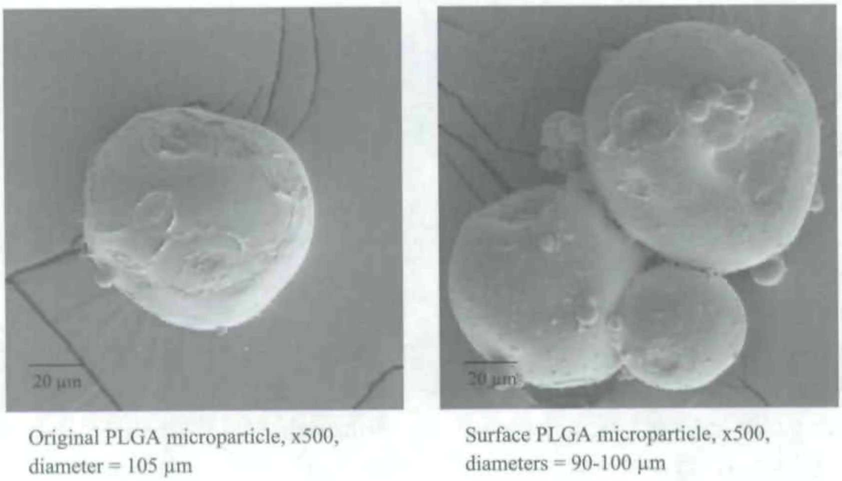
During the particle formation process, when the organic phase is added to the aqueous media, methanol is the first to diffuse into the aqueous phase and carries dichloromethane along with it. However, dichloromethane, having a limited solubility in water, starts to evaporate out. The PLGA and dexamethasone (solubility in water: 0.1 mg/mL) (Merck Index, 1996) being poorly soluble in water, precipitate and form microspheres. They are held apart and not allowed to



Original PLGA microparticle, x2000, diameter = 40 μm

Surface Crosslinked PLGA particle, x2000, diameter = 40 μm

FIGURE 3 SEM Micrographs of Dexamethasone Encapsulated PLGA Original and Surface Crosslinked Polymer Particles Made From Solvent Evaporation Technique. Crosslinker Used: EGDMA.



Original PLGA microparticle, x500, diameter = 105 μm

Surface PLGA microparticle, x500, diameters = 90-100 μm

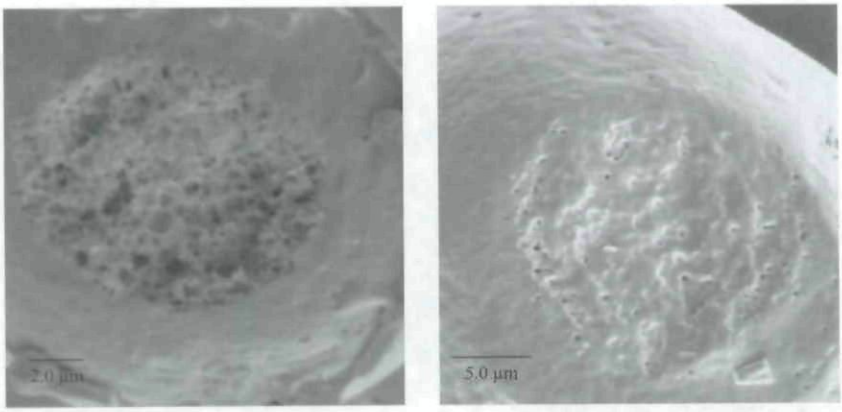
FIGURE 4 SEM Micrographs of Dexamethasone Phosphate Encapsulated PLGA Original and Surface Crosslinked Microparticles Made from Phase Separation/Coacervation Technique. Crosslinker Used: EGDMA.

agglomerate due to continuous mixing of the bulk phase and PVA present in the aqueous phase.

In the case of dexamethasone phosphate, the drug has a higher affinity towards the aqueous phase and prefers to be in the aqueous phase. On sonication, the aqueous phase is broken down into small droplets forming a metastable water-in-oil suspension in the dichloromethane phase. On addition of silicon oil, with sufficient mixing, phase separation takes place, leading to precipitation of the PLGA polymer in the form of microspheres. This gives a w/o/o suspension of the aqueous phase entrapped in dichloromethane, which is further entrapped in the silicon oil. This silicon oil is extracted by the hexane phase, which on the application of vacuum, is evaporated to give dry microparticles of PLGA polymer with dexamethasone phosphate encapsulated in them.

Particle Size and Morphology

Microparticles loaded with dexamethasone were observed under the scanning electron microscope (SEM) for particle size analysis. These microparticles were observed to be spherical with sizes ranging from 30–40 μm for those loaded with dexamethasone using the solvent evaporation method (Fig. 3), and about 100–120 μm for those loaded with dexamethasone phosphate using the phase coacervation method (Fig. 4).



Original PLGA microparticle, x5000, crater of 20 μm with pore size < 1 μm

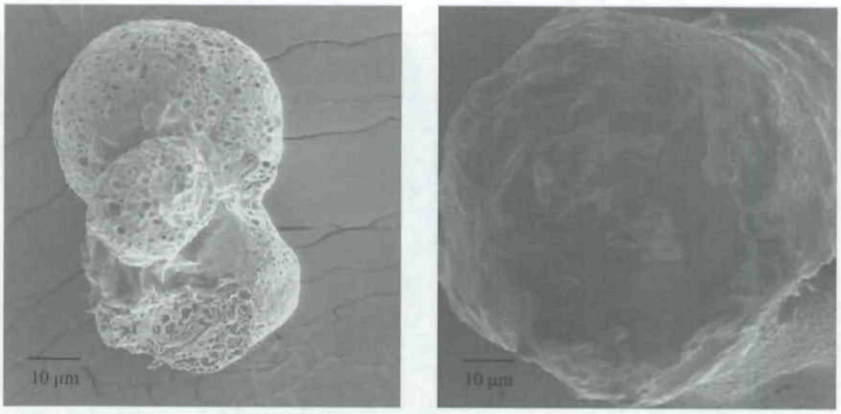
Crosslinked PLGA microparticle, x3000, less number of pores due to surface crosslinking

FIGURE 5 Magnified Images of Microparticle Surface Containing Dexamethasone Phosphate Made Using the Coacervation Method to Provide an Evidence to Reduction in Porosity on the Surface Due to Surface Crosslinking of EGDMA on PLGA Microparticles.

The surface morphology was observed to be slightly irregular for particles that were not crosslinked. Upon surface crosslinking of the particles, the surface texture did not seem to show a drastic change as can be seen from Figs. 3 and 4. However, direct evidence showing the reduction in the number of pores due to surface crosslinking of the microparticle surface can be seen in Fig. 5, which shows zoomed-in images of microparticle surface. This reduction in number of pores was from only one layer of EGDMA. Multiple layers would further reduce the number of pores. Reduction in number of pores is directly related to the reduction in burst release of the drug from the microparticles. This implies that EGDMA or Tri(EG)DMA not only formed a very thin, crosslinked layer on the particle surface, but also diffused into the mesh of the microparticle surface prior to crosslinking, got crosslinked on the microparticle surface, and closed the pores.

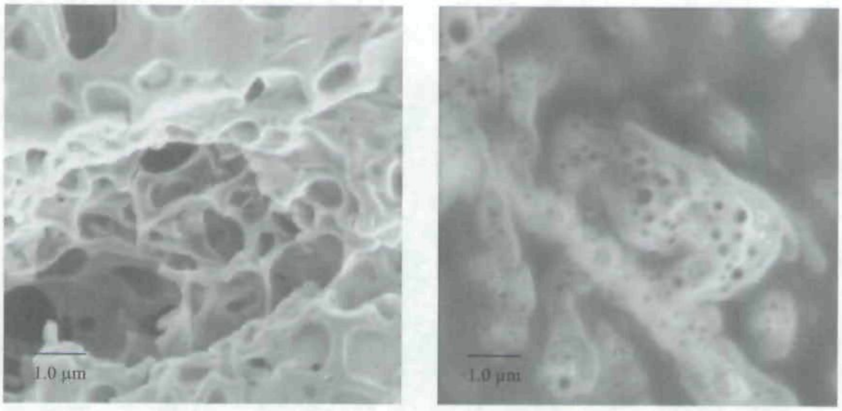
The particle diameter did not change substantially, suggesting that the surface layer was very thin. There was no substantial increase in the mass of the microparticles after surface crosslinking. This implied that CO_2 sprayed the crosslinker in the form of a very fine mist, just enough for the crosslinker to penetrate into the pores and to add a very thin layer on the microparticle surface. Moreover, percentage encapsulation studies were conducted on the original microparticles and surface crosslinked microparticles in order to account for the addition of mass, if any.

In order to gain an idea of the condition of the polymer surface during the release study, SEM micrographs of original and surface crosslinked microparticles were obtained after about 75 days of the particles being in the release medium. From the SEM micrographs at 1000 magnification as shown in Fig. 6, it can be clearly seen that the original microparticles



Original PLGA microparticle, x1000, size = 30-50 μm, pores = 2-3 μm

Surface Crosslinked PLGA particle, x1000, size = 80 μm



Original PLGA microparticle surface, x10000, pores = 2-3 μm

Surface Crosslinked PLGA particle surface, x2000, pores = 0.2-0.3 μm

FIGURE 6 **SEM Micrographs of Dexamethasone Encapsulated PLGA Original and Surface Crosslinked Polymer Particles Made from Solvent Evaporation Technique After 75 days of Microparticles Being in Release Medium. Crosslinker Used: EGDMA.**

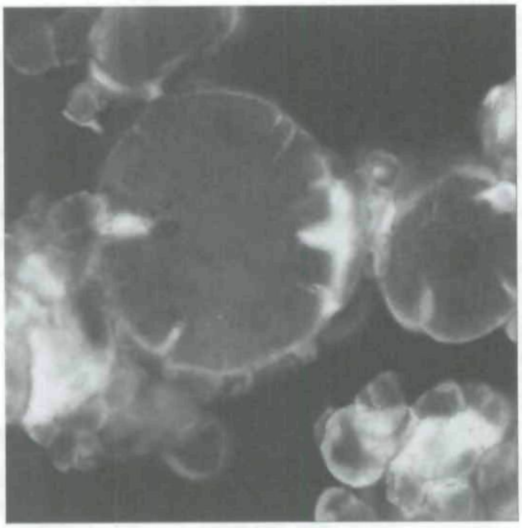


FIGURE 7 Cross-Sectional Images of PLGA Microparticles, Surface Crosslinked with EGDMA Containing Rhodamine-B, Using Confocal Laser Scanning Microscope Images. [Triple Labeling: Rhodamine-B Absorbs Laser in All the Red (647 nm), Green (568 nm) and Blue (488 nm) Regions. The Triple Labeling Image Shown Above is an Overlapping Combination of All Them.]

had degraded to a great extent, whereas the surface crosslinked microparticles were relatively less degraded. The SEM micrographs at 10,000 magnification revealed that the surface of the original microparticles show numerous pores as large as 2–3 μm on a 50 μm particle, whereas the surface crosslinked microparticles shows very fine pores of about 0.2–0.3 μm on their surface. This implies that the surface crosslinked microparticles degraded more slowly than the original microparticles and the pores developed were not as large when compared to the original microparticles. Therefore, the surface crosslinking protects the microparticle surface from easy degradation and also prevents the surface drug from being lost immediately, thereby reducing the initial burst release.

Confocal Fluorescence Microscopy

The confocal laser scanning microscopy (CLSM) (Berkland et al., 2001; Berkland et al., 2002; Berkland et al., 2003) provided direct evidence of the surface treatment. Images taken at different cross-sectional levels reveal the distribution of rhodamine-B dye dissolved in EGDMA-DMPA solution at various levels of microparticle surface. As all the particles were not present at the same level, focusing at the center of one particle might correspond to looking at the top or bottom surface of another particle, thereby representing uneven distribution of the fluorescent dye. However, the crosslinker and the dye distribution is mostly uniform except at places where they penetrate the pores present on the microparticle surface. Figure 7 shows the presence of a thin layer of the fluorescent dye only on the surface of the microparticles with occasional penetration to some extent in larger particles. Hence, the CLSM images not only provide evidence that the crosslinker penetrates the surface pores, polymerizes and closes them, but also shows that the polymerized layer is just a few microns thick such that the particle size does not change by a great extent.

Percentage Encapsulation

The microparticles were analyzed for the percentage of the dexamethasone encapsulated in the polymer matrix using o/w solvent evaporation method and were observed to have 26% of the drug encapsulated in the polymer. Dexamethasone has a solubility of 0.1 mg/mL in water (Merck Index, 1996). As a result, 10 mg out of a total of 20 mg of the drug is directly

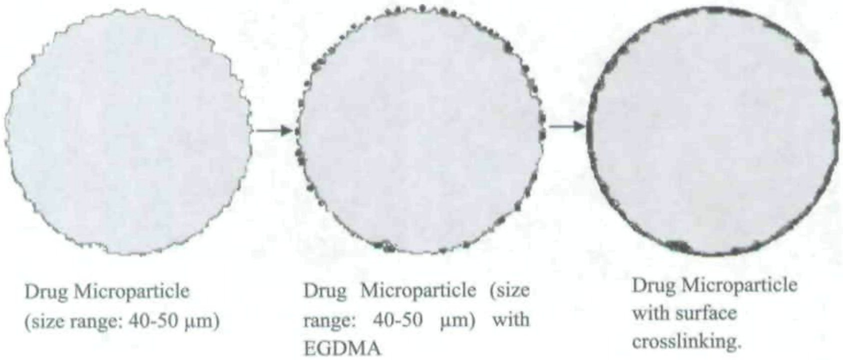


FIGURE 8 Schematic Representation of the Surface Crosslinking Process.

lost in 100 mL of water. Out of the remaining 10 mg, some of the drug stays on the polymer surface, which is washed away in the washing process, during the centrifuging and in the process of lyophilization of microparticles. As a result, only around 5 mg of drug gets encapsulated, leading to an effective encapsulation of 26%. Even though the intended drug loading for this case was 20% of the polymer (drug:polymer ratio=1:5), it was reduced down to 5% (1:20).

However, the percentage of dexamethasone encapsulated in the cases, where the aqueous phase in the o/w emulsion constituted 1 N NaCl solution, was observed to be as high as 96%. NaCl, being an ionic compound, makes the aqueous phase highly polar, thereby reducing the solubility of the drug in the aqueous phase. This helps in better encapsulation of the drug in the nonpolar polymer matrix and minimal drug loss. Hence, the drug loading in this case was 1:5.

Analysis of the extent of encapsulation of dexamethasone phosphate was carried out using the method of partitioning the drug in the aqueous phase and the polymer in the immiscible dichloromethane phase. The percentage of the dexamethasone phosphate encapsulated in the polymer matrix using w/o/o/o phase coacervation method was observed to be 41% of the drug used for encapsulation in the polymer. Part of the loss could be from the drug present on the microparticle surface and part from destabilization of the w/o emulsion. So, the drug loading in this case was dropped down from 1:5 to 2:25.

In Vitro Release Rate Study of the Drugs from PLGA Microspheres

During the formation of these microspheres, dexamethasone gets partially encapsulated within the microspheres and partially adsorbed on the microspheres. If the drug is dispersed homogenously within the microspheres, a continuous drug release would be expected. As the polymer particles start degrading, the particles become very porous and release the drug over time. Eventually, with the polymer degradation, the particle size reduces and so does the release rate due to the reduction in particle surface area. If it is encapsulated in the form of small clusters within the polymer matrix, the release rate is expected to reach a maximum in a short period of time because of channeling within the microspheres (Streubel et al., 2003).

The *in vitro* release of dexamethasone and dexamethasone phosphate was analyzed using HPLC. In order to make sure that the crosslinkers did not mask the HPLC analysis of the drugs, separate samples containing the drug and the crosslinker were analyzed using HPLC and were observed to show different retention times for the drug and the crosslinkers. Moreover, care was taken during the crosslinking process to make sure that the crosslinker was completely polymerized into solid form. Even though the crosslinking monomer might be slightly soluble in the release medium, the polymerized crosslinker is not. Hence, there would be very few chances that UV absorption by the crosslinker would mask the drug in vitro analysis.

The results of in vitro release of dexamethasone and dexamethasone phosphate are shown in Figs. 9 and 10, respectively. The release profile from the conventional microparticles was characterized by an initial burst release followed by a continuous release. However, the initial burst was reduced by two-thirds in the case of surface crosslinked particles containing dexamethasone and by half in those with dexamethasone phosphate. This can be attributed to the presence of an additional diffusional resistance to the release of the drug. The surface crosslinking polymerization of EGDMA to P(EGDMA) reduces the number of pores, thereby creating an additional barrier, to the release of the drug. The possible phenomenon is

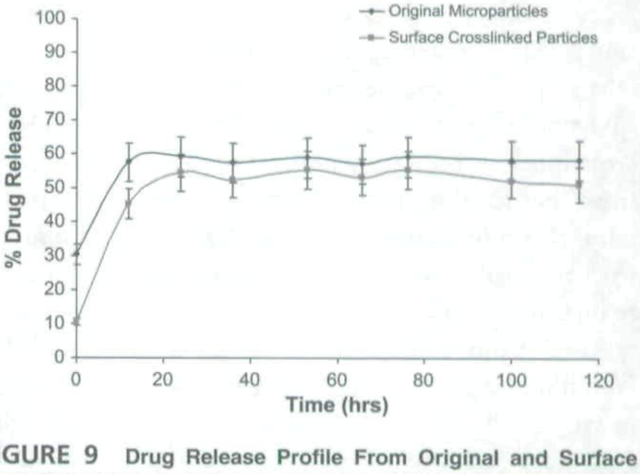


FIGURE 9 Drug Release Profile From Original and Surface Crosslinked Polymer Microparticles Loaded with Dexamethasone Formed By the Solvent Evaporation Technique. Crosslinker Used: EGDMA. Experiments Were Performed in Triplets (n=3). Plot Represents Average Release Profile. Quantity of Microparticles: 33 mg in 100 ml PBS Buffer. Actual Drug Loading: 1:20.

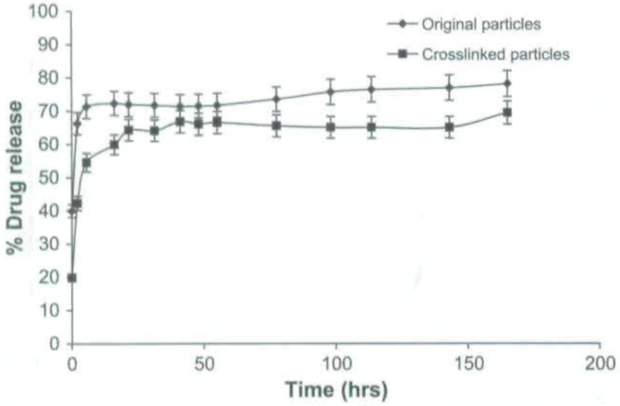


FIGURE 10 Drug Release Profile From Original and Surface Crosslinked Polymer Microparticles Loaded with Dexamethasone Phosphate Formed by the Phase Coacervation Technique. Crosslinker Used: EGDMA. Quantity of Microparticles: 30 mg in 100 ml PBS Buffer. Actual Drug Loading: 1:20.

sketched in Fig. 8. The release of dexamethasone is of a diffusional type as seen in Fig. 9. Moreover, the time required to reach the stationary state release was also extended as a result of surface crosslinking from 15 hours for conventional microparticles to about 24 hours for surface crosslinked microparticles.

To ensure that the reduction in burst release was due to polymerization of additional crosslinkers, either EGDMA or Tri(EG)DMA, and not just due to spraying of the crosslinker over the PLGA microparticles, three different release rate studies were conducted in comparison. One set consisted of the original microparticles, another with the microparticles sprayed with the crosslinker but not polymerized, and the third set consisted of the sprayed and UV crosslinked crosslinkers. All three sets were analyzed for their release rate profiles in a comparative study. The release rates evidently showed that the release profile for the microparticles that were only sprayed either with the EGDMA or Tri(EG)DMA crosslinker and not polymerized was not very different from that for the original microparticles. Whereas the release profile of the microparticles that were sprayed with the crosslinkers and polymerized showed a clear reduction in burst release from 61% of the drug released from original particles down to about 7% (about one-eighth) of the drug from surface crosslinked microparticles. Moreover, the time to reach the stationary state release was also extended from 10 hours in the case of the original microparticles to 120 hours in the case of the surface crosslinked microparticles. These observations are clear from the release profiles shown in Fig. 11. Moreover, the fact that the drug release profile from the particles that were only sprayed with the crosslinkers and photoinitiator but not crosslinked was not very different from the original microparticles, suggests that the uncrosslinked crosslinkers and photoinitiator did not destabilize or degrade the drug in any way.

In another set of experiments where seven different sets were analyzed simultaneously to study the effect of multiple layers of surface crosslinked EGDMA and Tri(EG)DMA, the release profiles in the case of both the crosslinkers showed further reduction in burst release with every additional layer of the surface crosslinked polymer. Moreover, the release profile from the microparticles that were sprayed and crosslinked with three layers show almost no burst release with a gradual and uniform increase in the drug release with time, until reaching a stationary state value. The time required to reach the stationary state release was observed to be extended. These observations are evident from the

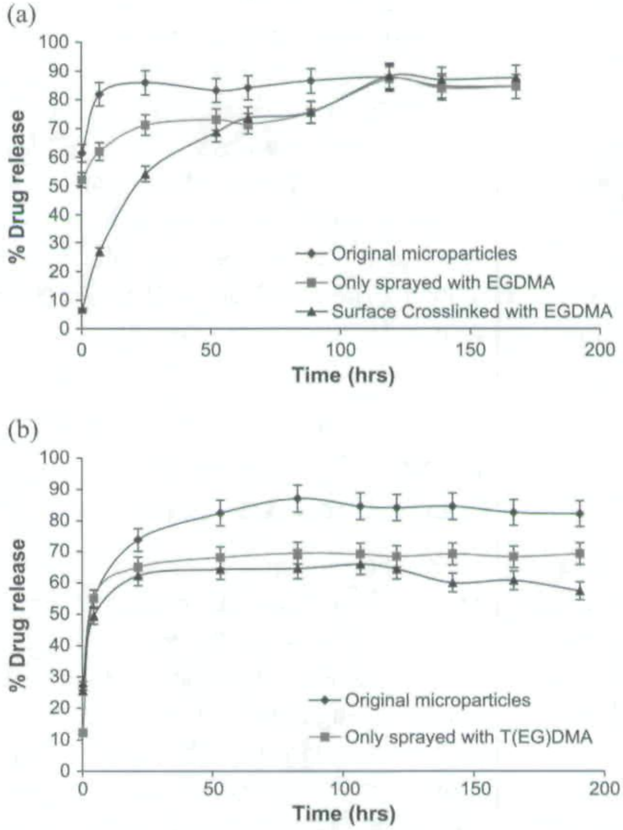


FIGURE 11 Dexamethasone Release Profile From Original Microparticles, Microparticles Only Sprayed But Not Crosslinked, and Surface Crosslinked Microparticles Using (a) EGDMA, and (b) Tri(EG)DMA. NaCl was Used in the Aqueous Medium of the o/w Emulsion During the Synthesis of These Microparticles. Quantity of Microparticles = 35 mg.

release rate profiles shown in Fig. 12. For all the cases in Figs. 11 and 12, the microparticles were formulated in the presence of NaCl in the aqueous solution. Hence, the particles had better drug encapsulation. Even though the drug release profiles in these cases were different from those in Fig. 8, what is more Fig. 12 significant is the comparison of the release profiles between those from the original microparticles and surface crosslinked ones in each case.

The factors that govern the type of release rate from a drug-encapsulated polymer microsphere system are M_w of the polymer (Eliaz & Kost, 2000; Murakami et al., 2000; Song et al., 1997), size of the microspheres (Anderson & Shive, 1997), drug loading (Mandal, 1998), drug distribution in the polymer matrix, etc. The factors affecting the drug distribution within the polymer matrix, which in turn would determine type of release rate, are the polymer-to-drug ratio and the steps involved in the process of hardening of the polymer microspheres. In this study, the polymer-to-drug ratio used was 5:1 and the particles were dried using the lyophilization process. For such ratios, with lyophilization, there is greater likelihood that the drug would get encapsulated in small pockets within the polymer matrix and would release in bulk quantities due to channeling effects. A monodispersed encapsulation with gradual drug release over a longer period of time could be expected with higher polymer-to-drug ratios.

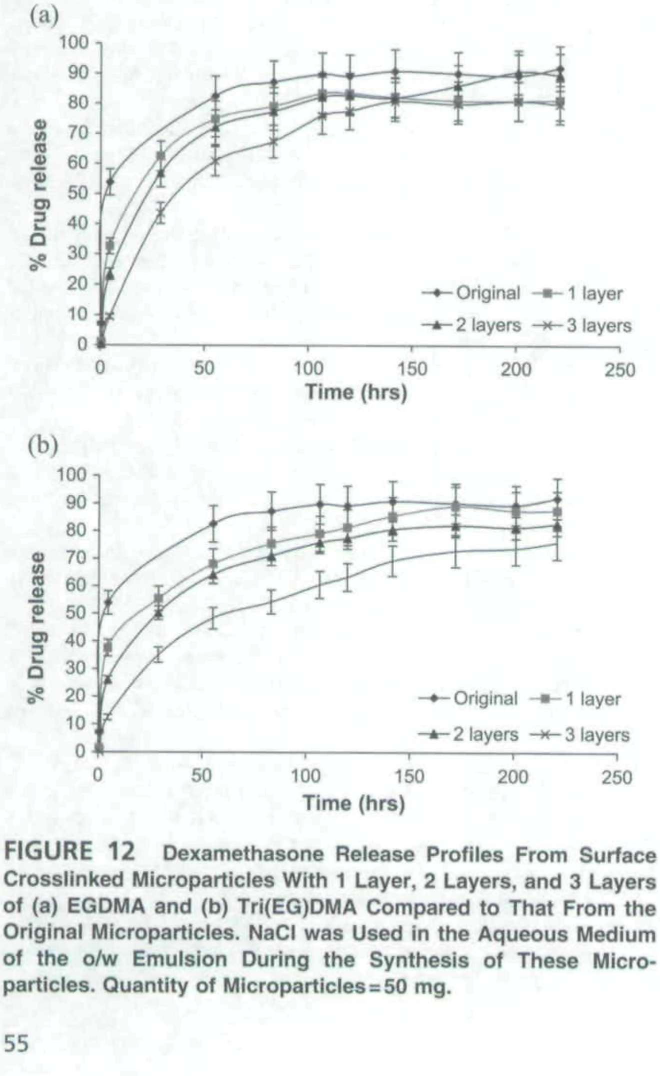


FIGURE 12 **Dexamethasone Release Profiles From Surface Crosslinked Microparticles With 1 Layer, 2 Layers, and 3 Layers of (a) EGDMA and (b) Tri(EG)DMA Compared to That From the Original Microparticles. NaCl was Used in the Aqueous Medium of the o/w Emulsion During the Synthesis of These Microparticles. Quantity of Microparticles = 50 mg.**

CONCLUSION

Dexamethasone-encapsulated PLGA microparticles were produced in the size range of 30–40 μm using the solvent evaporation technique. Dexamethasone phosphate-loaded PLGA microparticles were produced in the size range of 100–120 μm using the phase separation/coacervation method. The surface crosslinking of these microparticles creates a diffusional barrier that can significantly reduce the initial burst release of both dexamethasone and dexamethasone phosphate from the PLGA microparticles. The surface crosslinking can also extend the time for stationary state release. Multiple layers of these barriers can further help in reducing the burst release and prolonging the stationary state drug release rate.

ACKNOWLEDGMENTS

Authors thank Dr. W. R. Ravis for technical advice and Dr. M. E. Miller for assistance with SEM and CLSM analyses.

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