Biodegradable Membranes for the Controlled Release of Progesterone. 1. Characterization of Membrane Morphologies Coagulated from PLGA/Progesterone/DMF Solutions

MYEONG-JIN HAN

Department of Chemical Engineering, Kyungil University, 33 Buhori, Kyungbuk 712-701, Korea

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ABSTRACT: Biodegradable membranes containing progesterone as a drug were prepared from ternary, poly(*d,l*-lactide-co-glycolide)/progesterone/dimethylformamide, solutions. The homogeneous solutions, after cast on glass plates, were solidified to result in a solid membrane structure by three different solvent-removal processes: solvent evaporation under vacuum, solvent extraction via immersion into the nonsolvent bath, or vapor exposure at high humidity condition. Impregnation characteristics of progesterone in the prepared membranes varied significantly, depending on the removal processes used. When a cast solution was solidified by exposure at the environment of 70% relative humidity, progesterone was separated from a membrane structure with the morphology of flake-like shapes, and thermal analysis of the prepared membrane showed the clear, endothermic peak of the drug. Vitrification of a cast solution by solvent evaporation under vacuum induces both the uniform drug dispersion in the polymer matrix, with the drug forming spherical structures, and the strong interaction between the drug and the matrix, as identified by a broadened melting endotherm of the drug. When coagulated at thermodynamic nonequilibrium conditions through rapid exchange between dimethyformamide and water, the cast solution film results in a membrane structure consisting of the drug distributed nonuniformly in the polymer matrix. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 75: 60–67, 2000

Key words: poly(*d,l*-lactide-co-glycolide); progesterone; biodegradable polymer; membrane; coagulation

INTRODUCTION

Recently, extensive research has been done to develop efficient drug delivery systems. The research in new delivery system has been picked up by some demands such as the improvement of drug safety and efficacy, the development of drug release patterns, and the increase of efficiency of expensive drugs. One of the delivery methods developed is the controlled release system, which can be applied for maintaining the therapeutic range of a drug for a prolonged period with the reduction of oscillation of drug levels, but without repetitive administrations.[1,2] In addition, the controlled release system can make it possible to deliver a drug to a specific location or to preserve the body-sensitive medications, such as proteins. Therefore, the controlled release systems are widely studied for applying to the clinical, novel drug delivery, which can help health of human beings as well as of livestock.[3]

One of the common attempts to prepare a controlled release formulation is to utilize a polymeric membrane or a microsphere containing a

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therapeutic agent.[4–10] Polymers commonly used as a drug carrier are the biodegradable, biocompatible polyesters such as poly(*d,l*-lactide-*co*-glycolide) (PLGA) and polylactide (PLA), which can be degraded into unharmful products excreted via the kidney.[11] Drugs impregnated in a polymeric carrier formed by proper preparation protocols, are released for playing a therapeutic role, after being administered into target body compartments. In the controlled release system containing biodegradable polymers as a carrier, the drug release from a carrier matrix normally is influenced both by the drug diffusion through polymer network and by the erosion of the carrier matrix.[12,13] Therefore, the drug release rate is a function of the morphological properties of a carrier as well as the physicochemical properties of drug and matrix polymer, including molecular weights of both drug and polymer, a monomer ratio in the matrix polymer, and an interaction between the constituents of the system. On the other hand, the morphology of a polymer carrier such as the porosity—pore size, pore volume and distribution—can be affected by a carrier-preparing method, and the impregnation of a agent loaded in the carrier is also influenced by the preparation procedure.[14] Therefore, to control drug release rates for the controlled release system, both the morphological structures of a drug carrier and the ensuing impregnation characteristics of drug in the carrier have to be understood and determined, along with the physicochemical properties of constituents in the system.

The preparation of biodegradable drug carriers can be obtained by solidifying a homogeneous or heterogeneous polymeric solution containing a specific drug. A homogeneous solution, in general, includes a solvent-miscible drug dissolved in the solution, and a heterogeneous one consists of a solvent-immiscible drug emulsified in the polymer solution.[15,16] One of the most common solidification process is to evaporate solvent from a solution consisting of polymer, drug, and solvent. In this process a polymeric solution changes into a solid structure via vitrification due to the increase of polymer concentration in the system, if the polymer is uncrystallizable.

Solvent extraction via the exchange between solvent and nonsolvent is another popular solidification method that can lead to rapid polymer precipitation, owing to fast compositional variation. This method can entail demixing or phase separations in a solution, and, subsequently, porous polymer structures are likely to be induced. In general, a relatively dense structure formed by solvent evaporation is compared with a porous one prepared by solvent extraction. On the other hand, the previous study has shown that in the preparation of a polysulfone membrane carrier containing an inorganic fluor, the impregnation characteristics of the inorganic agent, as well as the morphology of the carrier, are significantly changed depending upon the solidification processes used.[14]

To study the effect of solidification processes of a cast solution film on the membrane formulation for a controlled release system, PLGA and progesterone can be selected as model ingredients for carrier and drug, respectively. Progesterone, as an endogenous hormone, plays an important role in the preparation of the uterus for control of pregnancy. It can be used for medicines of oral contraceptive as well as for veterinary application, such as the prevention of pregnancy loss in horses.[3] For efficient therapeutic results, it is desirable to prepare an oral or parenteral system that can be delivered at a consistent rate over an extended period, with the prevention of the abscesses, inflammation, or scars caused by repetitive, intramuscular administrations of that drug.

To obtain an effective controlled delivery system holding progesterone, several researchers have tried to formulate biodegradable polymer carriers containing the drug and to analyze drug release profiles from the carriers.[3,17–20] Even though some promising results have been reported in the formulation of a controlled release system, further research is needed to optimize the delivery system. As a basic study for optimizing the progesterone-loaded membrane, the present paper describes the relationships between the solvent removal processes from a PLGA/progesterone/solvent mixture and the resulting structure of formulations.

EXPERIMENTAL

Membrane Preparation

PLGA 50/50 (M_w: 58,000–68,000, Resomer RG 505) was obtained from Boehringer Ingelheim, Ingelheim, Germany. Dimethylformamide (DMF) and progesterone were reagent grades and used as received from Aldrich Chemical. Casting solutions consisted of either PLGA/DMF (2 g/10.5 mL) or PLGA/progesterone/DMF (2 g/0.5 g/10.5 mL). At 25°C and 70% relative humidity (RH), the pre-

pared homogeneous solutions were cast to 0.20-mm clearance gap on glass plates at the same temperature as the solution.

The cast solution films were solidified using one of three different solvent-removal techniques. The vitrification of a cast film was obtained by vacuuming the cast film in a vacuum oven at 25°C. After 24 h, the corresponding solidified film was detached from a glass plate and was vacuumed for additional 3 weeks in the oven. The immersion precipitation involved the immersion of a cast solution film into a water bath of 25°C. After 24 h in the bath, the solidified film was removed from the bath, followed by placing in a vacuum oven. The vapor exposure coagulation entailed exposing a freshly cast film for 48 h in the environment of 25°C and 70% RH and then the film was lifted off the glass plate. The solidified film was also kept in a vacuum oven.

DSC Test

DSC experiments were carried out using a SETARAM (Model DSC92). Samples of 10 mg were heated at a scanning rate of 10°C/min. All samples including the prepared membranes were vacuum-dried for at least 3 weeks at 25°C before being tested.

SEM

Top surfaces and cross sections of the solidified membranes were observed by using scanning electron microscopy (SEM 151, Philips). Samples were freeze-fractured under cryogenic condition using liquid nitrogen and coated with gold before being tested.

RESULTS AND DISCUSSION

Formulations of solid, polymeric carriers can be obtained by the conversion of a polymer solution to a solid structure by either solvent extraction due to solvent–nonsolvent exchange or solvent evaporation. In a formulation system including PLGA, the solid–liquid phase separation or crystallization can be excluded from possible solidification mechanisms of a cast film, because of the amorphous property of PLGA. Therefore, when coagulated at an isothermal state, PLGA in a solution can precipitate via the vitrification due to the build-up of polymer concentration or via the phase separation due to a nonsolvent addition in the solution. The former can be induced by solvent evaporation under vacuum and the latter by a nonsolvent in-diffusion into a system. The diffusion-induced solidification process, entailing the phase separation, can be designed to solidify a polymer solution via two different routes: immersion precipitation into the nonsolvent bath and exposure to nonsolvent vapor. As shown in the previous articles, morphologies of the polymeric membranes prepared by the diffusion-induced precipitation are markedly different, depending on the solvent-removal conditions.[14,21]

Prior to preparing drug-loaded membranes, PLGA membranes were fabricated by solidifying the binary solutions consisting of PLGA and DMF. When exposed at the environment of 70% RH, a cast, binary PLGA solution (PLGA/DMF: 2 g/10.5 mL) is demixed within 15 min, as indicated by the feature change of the film from transparency to cloudy. The further exposure results in the formation of an opaque membrane film. On the contrary, when a freshly cast binary solution is immersed into a water bath, the cast film changes into a white membrane structure shortly after the immersion. These opaque or white structures of the prepared membranes are distinguished from the transparent feature of the film that is formed by the vitrification via solvent evaporation under vacuum.

As shown in Figure 1, the homogeneously dense polymer structure in the vitrified PLGA film, prepared by the solvent evaporation under vacuum, is compared to either the cell-like structure in the membrane prepared by the water vapor exposure or the finger-like structure in the membrane coagulated by the immersion precipitation. The finger-like or macrovoid structures are commonly found in the so-called phase inversion membranes, prepared by the immersion of a polymeric solution into a nonsolvent bath. The voids in the cell-like membrane structure represent the polymer-lean phase that is nucleated, resulting in the liquid–liquid phase separation in a nascent film. The homogeneous cell shapes, in the membrane prepared by the vapor exposure technique, indicate that the coagulation process is operated near the thermodynamic equilibrium conditions. In contrast, the asymmetric and macrovoid structure, found in the membrane prepared by the immersion precipitation, reveals that a cast PLGA solution is coagulated under nonequilibrium conditions. As shown in Figure 2 (top) surfaces of the membranes prepared by either the solvent removal under vacuum or the

immersion precipitation show the dense structure. On the contrast, the membrane prepared by the vapor exposure technique has a porous surface structure, with large round pores.

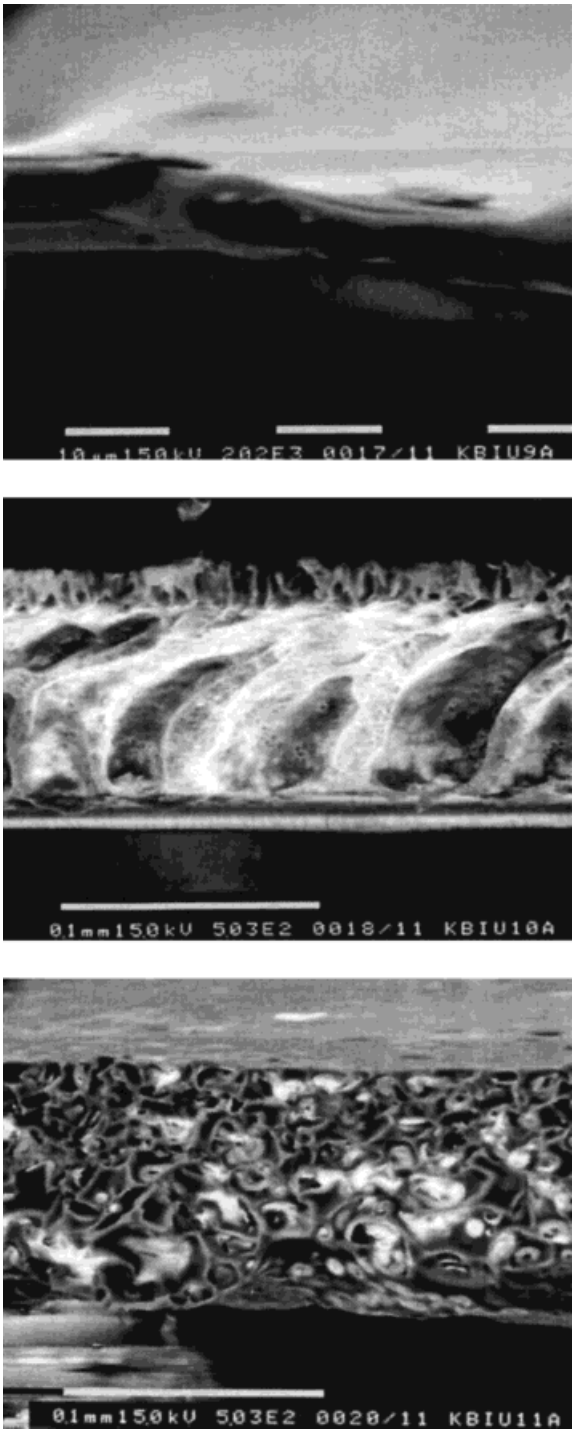

Figure 1 Scanning electron micrographs of cross sections of membranes without progesterone; membranes are prepared by vacuuming (top), immersion precipitation (middle), and vapor exposure (bottom).

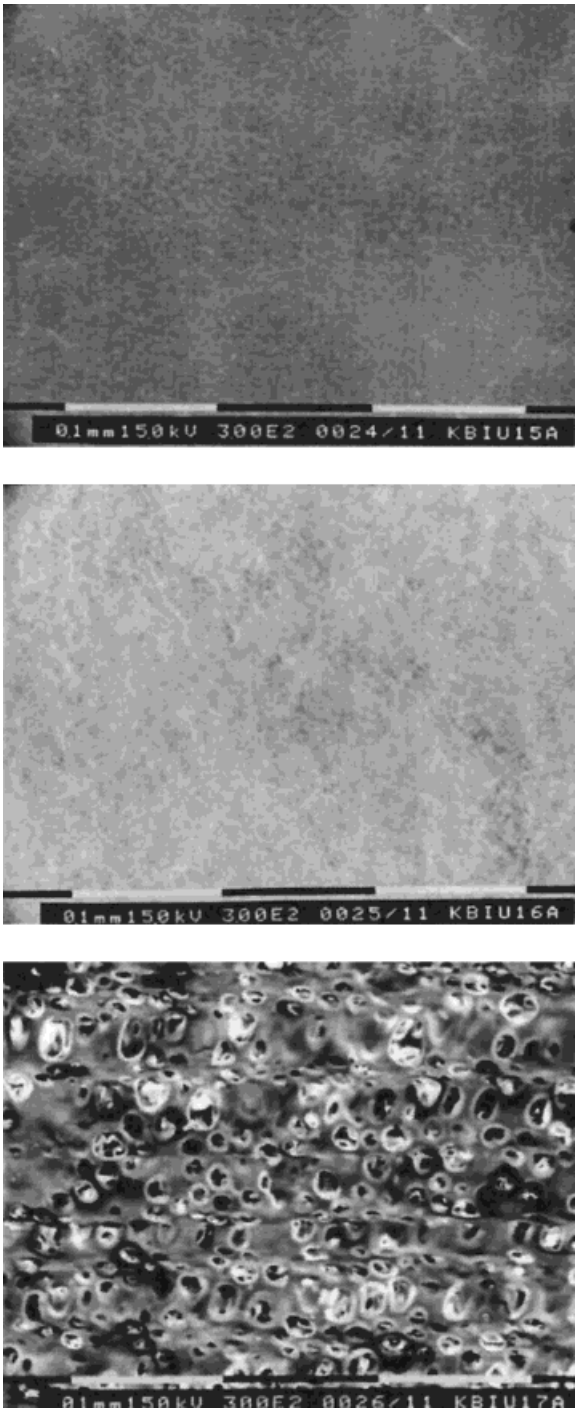

Figure 2 Scanning electron micrographs of top surfaces of membranes without progesterone; membranes are prepared by vacuuming (top), immersion precipitation (middle), and vapor exposure (bottom).

With the drug loaded, the morphologies of solidified membranes are not markedly different from those of membranes prepared from the binary solutions of PLGA and DMF (Fig. 3); how-

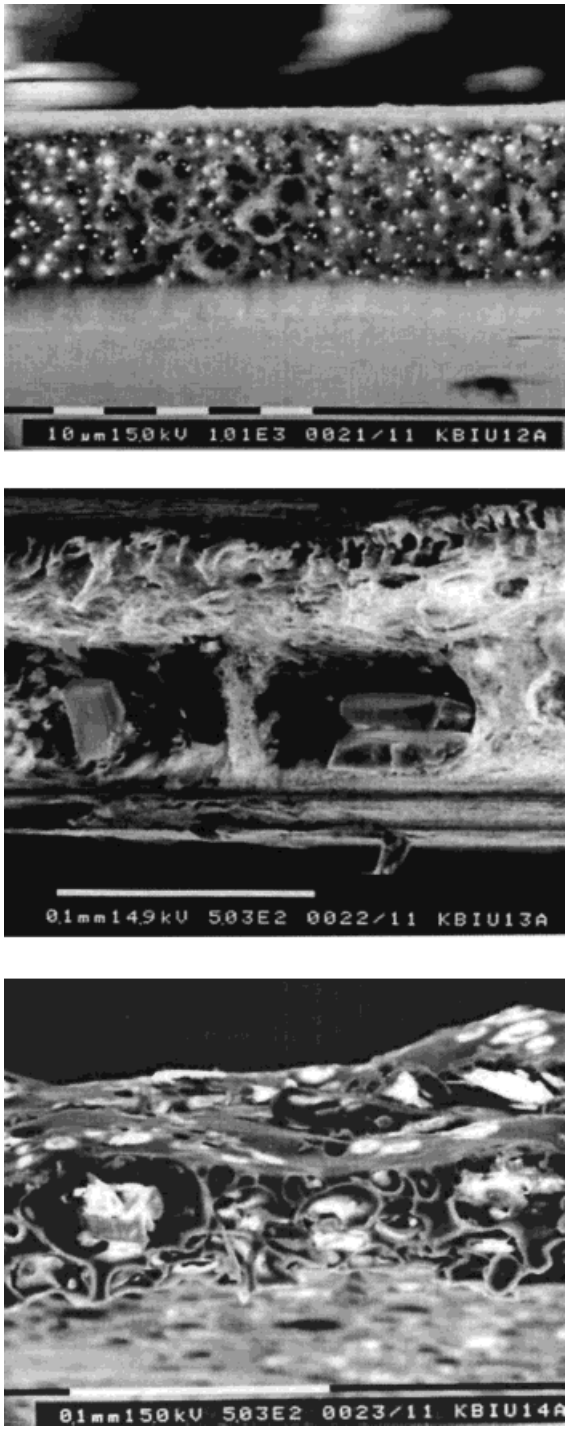

Figure 3 Scanning electron micrographs of cross sections of membranes with progesterone; membranes are prepared by vacuuming (top), immersion precipitation (middle), and vapor exposure (bottom).

ever, the drug's physical states in the membranes are significantly changed, depending on the solvent-removal processes used. In the membrane prepared by the solvent evaporation under vacuum, the drug is distributed evenly with the spherical shapes in the membrane structure. In contrast, the morphologies of the membranes prepared by either the vapor exposure technique or the immersion precipitation show the drug distributed nonuniformly in the PLGA matrices. The drug separated from the membrane polymer and localized inside the cell structure in the membrane is observable in the micrograph (Fig. 3, bottom) of the membrane prepared by the vapor exposure process. In the membrane prepared by the immersion precipitation, the drug impregnated in the substructure beneath the skin region can be found (Fig. 3, middle).

These impregnation phenomena indicate that the drug reacts differently to the dynamic fluctuation, caused by compositional changes, in the process of solidification. It may be explained by the following theory. As the vacuum drying is kept for evaporating the solvent, both PLGA and progesterone are precipitated because of their own solubility limits within DMF. While being precipitated, the localized drug forms a spherical shape for reducing the surface energy, with the highly concentrated polymer phase hampering the drug migration through its structure. On the other hand, if the liquid–liquid phase separation happens with nonsolvent addition, both the nucleated PLGA-lean phase and the PLGA-rich phase continue to grow until the polymer-rich phase vitrifies, while inducing the dynamic fluctuation in the polymeric solution. During the growth, the drug is expelled from the polymer-rich phase and localized in the PLGA-lean phase. As shown in Figure 4 (bottom), the drug is separated from the polymer matrix and exists at the localized pores that represent the polymer-lean phase.

Here, we notice that some drug crystallites are larger than the PLGA cell sizes in the membrane, forming the inhomogeneous membrane morphology. This inhomogeneity, due to the drug crystallites piercing the polymer-rich phases, is distinguished from the homogeneous cell structure of the membrane prepared from a polymer solution containing an inorganic agent, where the agent is localized in the polymer-lean phase, maintaining the homogeneous structure.[14] Therefore, it is assumed that during the growth of the two separated phases, the drug crystallites in the polymer-lean phase continue to grow with penetrating and opening up the polymer-rich phase, until the poly-

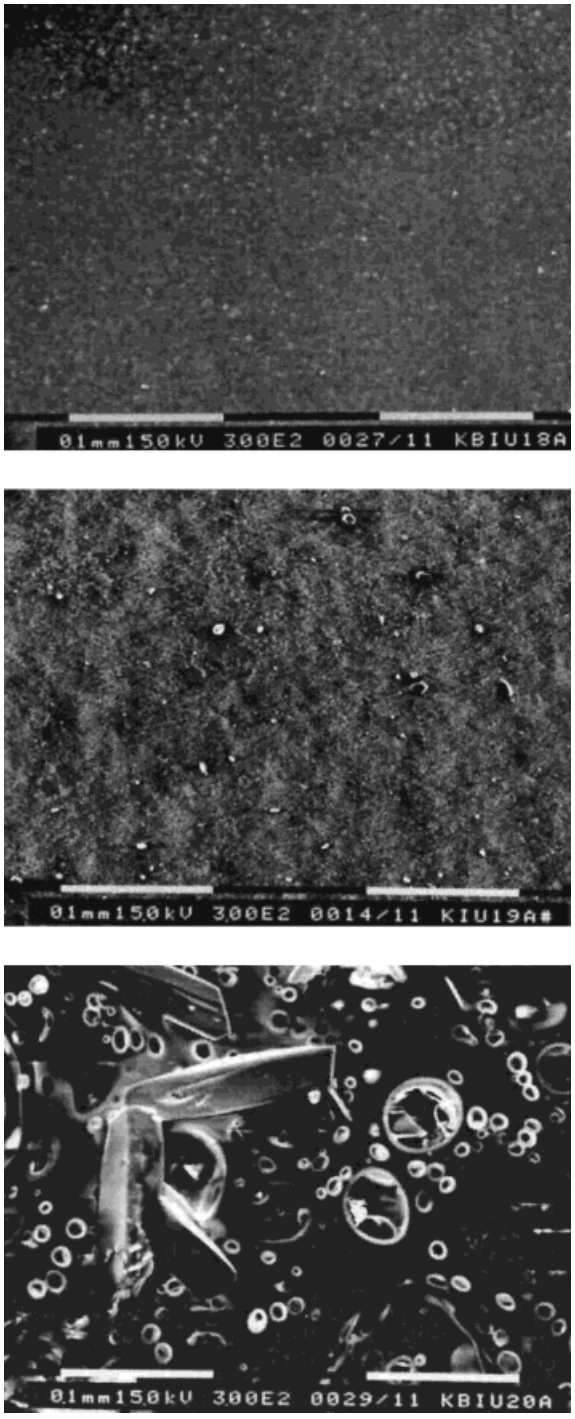

Figure 4 Scanning electron micrographs of top surfaces of membranes with progesterone; membranes are prepared by vacuuming (top), immersion precipitation (middle), and vapor exposure (bottom).

mer concentration in the polymer-rich phase can endure the penetration force of crystallites.

From DSC curves of the raw materials, PLGA and progesterone, it can be determined that PLGA exhibits an endothermic peak around 59°C due to the glass transition T_g, and an endothermic peak is observed for progesterone at 133°C, corresponding to its melting transition. As shown in Figure 5, for the DSC curves of membranes prepared without drug, there is no significant difference between the prepared membranes. However, the endothermic peak of the membrane prepared by the vacuum coagulation is slightly lower and broadened, compared to that of the PLGA polymer. This phenomenon indicates that even though the depression of glass transition is not dominant, the membrane still has some residual DMF, in spite of the extended vacuuming.

Figure 6(a) shows the thermogram of the sample that is loaded with raw PLGA and progesterone at the same ratio as that in the casting solution. Even though the weight of progesterone (2 mg) is much less than that of PLGA (8 mg), the endothermic peak for the drug is more definite than that for PLGA. This result indicates that if progesterone is separated from the matrix of a PLGA membrane and distributed evenly in the matrix, the endothermic shape of a membrane containing the separated drug has to be similar to this curve. Therefore, the DSC curves for the

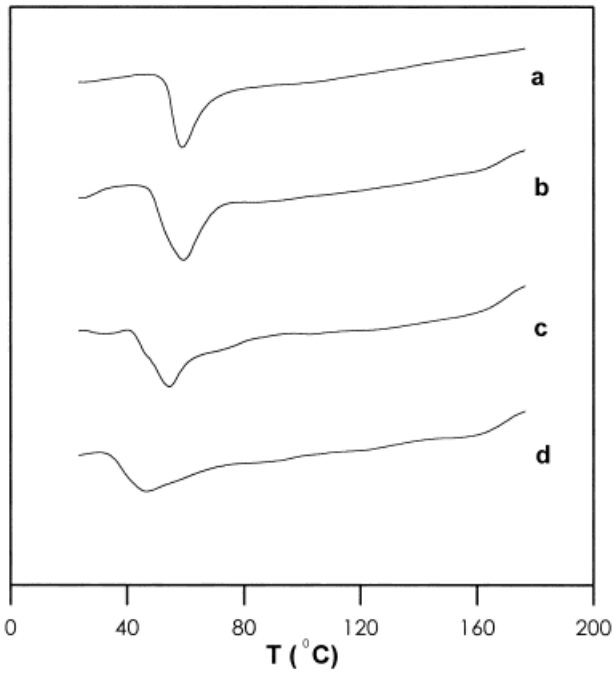

Figure 5 DSC thermograms of raw PLGA (a) and membranes without progesterone; membranes are prepared by immersion precipitation (b), vapor exposure (c), and vacuuming (d).

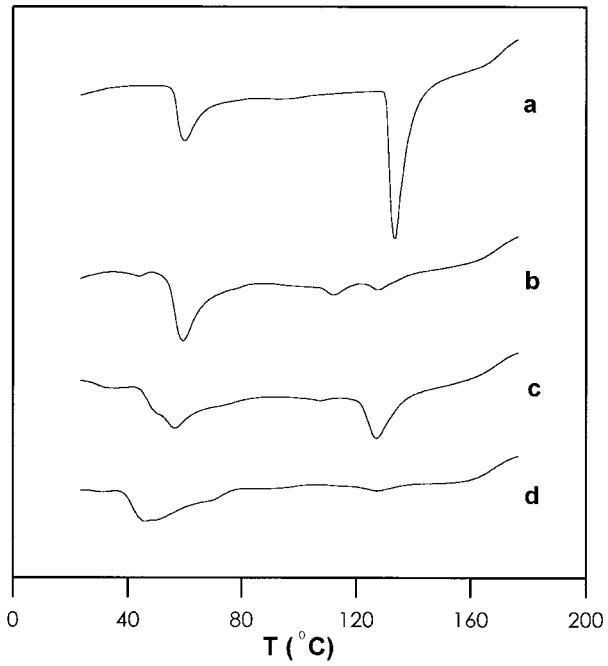

Figure 6 DSC thermograms of a sample loaded with PLGA/progesterone (8 mg/2 mg) (a) and membranes with progesterone; membranes are prepared by immersion precipitation (b), vapor exposure (c), and vacuuming (d).

drug-loaded membranes are compared to this endothermic peak.

With the drug loaded, both the T_g of PLGA around 54–58°C and the melting endotherm of progesterone near 127°C are found at all membranes. However, endothermic peaks for progesterone show significantly different shapes depending on the solvent-removal processes. In particular, in the membrane prepared by the solvent evaporation under vacuum, an endothermic peak for the impregnated drug is not clearly observable in the thermogram (Fig. 6[d]), even though the T_g of PLGA is detected in that curve. From this event, it can be said that the progesterone in the membrane may exist either in an amorphous state or in the state of a strong interaction with PLGA matrix. By contrast, the endothermic peak for the membrane prepared by the water vapor exposure is clear around 127°C (Fig. 6[c]), and the curve is similar to that of the separately loaded sample. This result reveals that the drug is separated from the polymer matrix, as also proven from SEM pictures. On the contrary, even though the melting endotherm of progesterone is found for the membrane prepared by the immersion precipitation, the peak is not as clear as that for the membrane prepared by the vapor exposure process (Fig. 6[b]). This phenomenon indicates that melting of the progesterone not separated from the PLGA matrix is inhibited by the interaction with the matrix polymer. Therefore, we can assume that the drug near the skin region of a cast solution does not have enough time to respond the dynamic fluctuation during the initial exchange between solvent and nonsolvent, and, subsequently, the drug precipitates along with PLGA aggregates.

From the above results, we conclude that progesterone's physical status in a membrane is affected significantly by solvent-removal conditions of a cast solution, even though we can not define the variations on the chemical and biological properties of the drug. The solvent extraction method, inducing the fast coagulation of a cast solution, has an advantage of less solvent residue in the carrier compared to the solvent evaporation under vacuum. However, the extraction method causes nonuniform drug distribution in the carrier matrix, compared to the vacuum evaporation method, which results in relatively even distribution of the drug. This kind of variation of the impregnation characteristics is attributed to the phase separation behavior of the constituents. Therefore, when we apply the solvent extraction method for the coagulation of a cast film, we must investigate carefully the possible change in the drug's physical status. Also, the significantly different impregnation characteristics of the drug in the prepared membranes indicate that the release profile of the drug can not be a simple function of time, when the membranes are fabricated by the solvent extraction method. The overall results indicate that to analyze the controlled delivery precisely, the physical status of drug as well as the drug loading itself in the carrier matrix must be evaluated.

CONCLUSION

The drug, progesterone, impregnated in a PLGA membrane carrier differs in its physical status, such as the shape, distribution, and molecular interaction with the carrier matrix, depending on the type of solvent-removal conditions for solidifying a homogeneous casting solution. When the phase separation and solidification of PLGA solution film is induced by the rapid polymer collapse via the immersion precipitation, the drug dissolved in the casting solution is impregnated non-

uniformly in the resulting membrane matrix. The thermal analysis of the membrane indicates that the melting of a portion of the drug is inhibited by the interaction with the PLGA matrix. The vitrification of a cast solution by solvent evaporation results in both the uniform distribution of the drug in the polymer matrix and the strong interaction between the drug and the matrix. In contrast, the membrane prepared by vapor exposure holds the drug, which is separated completely from the PLGA matrix. The crystallized drug shows the clear endothermic peak at the melting point.

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