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COMMUNICATION

pH-Sensitive Release of Indomethacin Using Lactan–Acetate Microspheres

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ABSTRACT

Hydrophobic lactan acetate was prepared from hydrophilic lactan gum by chemical modification and it was used for a pH-sensitive drug delivery system. Lactan acetate microspheres were prepared by the solvent evaporation method. The size of the microspheres prepared from lactan acetate was below 5 μm. The drug loading efficiencies of microspheres were approximately 70 and 80% at the initial amount of drug 40 and 80 mg, respectively. The microsphere showed pH-sensitive swelling behavior. The swelling capacity of microspheres at pH 7.4 was much greater than that at pH 1.2. The pH of the medium significantly influenced the in vitro release rate; the rate at pH 7.2 was approximately 10 times faster than that at pH 1.2. A pH-sensitive drug release pattern was due to the escape of drug from the microsphere that disintegrated after swelling.

INTRODUCTION

A stimuli-sensitive drug delivery system is one of the novel approaches in effective drug delivery and may overcome the tolerance problems and undesirable side effects that occur with constant delivery rate, minimize the physiological pattern of hormonal concentration, and supply drugs on demand (1). Stimuli-sensitive materials in a drug delivery system allow changes to occur in response to changes in environmental conditions, such as temperature, pH, and electric field (2–4). In particular, pH-sensitive materials can be used to protect the acid-labile drugs in the stomach.

Since Einsenberg and colleagues reported on the phase transition of pH-sensitive polymers (5), numerous researchers have devoted significant effort to the development and mechanistic evaluation of pH-sensitive drug delivery systems (6,7). Yuk et al. reported on a pH-sensitive drug delivery system using o/w emulsion (8).

Indomethacin (IND) is one of the most potent nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of rheumatoid arthritis, osteoarthritis, and acute

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gouty arthritis. Indomethacin acts by suppressing prostaglandin synthesis in the tissues via inhibition of cyclooxygenase activity, as do various NSAIDs. Even though indomethacin is very potent, its usage may be curtailed by the adverse effects that are frequently associated with the gastrointestinal tract and the central nervous system (9).

Recently, the use of polysaccharides as matrices for controlled release has received considerable attention and in several cases the use of hydrophilic polysaccharides was proposed since their ingestion never produced adverse dietary, physiological, or toxic effects in animals or humans. Albin et al. reported slow-release indomethacin formulations based on polysaccharides (10). Santucci et al. reported that gellan is suitable for the formulation of sustained-release beads (11).

Lactan gum (an anionic galactomannan), produced from the facultative anaerobic bacterium *Rahnella aquatilis*, was first investigated by Flatt et al. (12). Lactan gum is composed of mannose, galactose, and galacturonic acid with an approximate molar ratio of 5:3:2 and containing no organic acid modifying groups. The weight average molecular weight of the gum is approximately 7×10^6 (12,13).

The viscosity of aqueous lactan gum solution is stable over a pH range of 2–11, and is particularly stable in alkaline environments. Aqueous solutions of lactan gum at pH 5–11 show excellent thermostability, retaining at least 80% of the original viscosity after heating at 121°C for 15 min (12).

In this study, we prepared the hydrophobic lactan acetate from the hydrophilic lactan gum by chemical modification. We also prepared the microspheres using lactan acetate and measured the swelling capacity related to the surrounding pH. We studied the drug release of microspheres for the pH-sensitive drug delivery system.

MATERIALS

IND and dichloromethane (DCM) were purchased from Sigma Chemical Co. (St. Louis, MO). Formamide and pyridine (chemical grade) were purchased from Junsei Chemical Co. (Japan). Acetic anhydride was purchased from Lancaster Synthetic Co. (England). Lactan gum was obtained from fermentation of *Rahnella aquatilis*.

METHODS

Lactan Gum Purification

Lactan gum was purified from fermentation broth. It was precipitated from the broth by addition of ethanol (75% v/v) and then equilibrated for 24 hr at 4°C. The precipitate was recovered by centrifugation at 10,000 × *g* for 20 min. The precipitate was redissolved with deionized water. Cells were removed by centrifugation at 15,000 × *g* for 1 hr and followed by decantation of supernatant. The gum was then reprecipitated by the addition of ethanol (90% v/v).

The polysaccharide gum was then dissolved in deionized water to achieve a final gum concentration of 1–2 g/l. Any remaining low molecular weight solutes and salts were removed by the following method. The gum solutions were ultrafiltrated at a constant concentration which was maintained by the cumulative addition of five volumes of deionized water. The solutions were then concentrated to a final concentration of 5–8 g/liter by ultrafiltration. A Millipore Pellicon tangential-flow plate system fitted with a 0.5-ft^2 100,000 MW cutoff PTHK, polysulfone-type filter (Millipore Corp., Bedford, MA) was used in the ultrafiltration step. Finally, the deionized material was lyophilized and ground to a coarse white powder.

Preparation of Lactan Acetate from Lactan Gum

Lactan acetates were prepared as follows: 2 g of lactan gum was suspended in 20 ml of formamide and dissolved by vigorous stirring at 50°C. A 60-ml volume of pyridine and 150 ml of acetic anhydride were added and the mixture was stirred at 54°C for 48 hr. Lactan acetate was obtained after reprecipitation from 200 ml of water, with a modification of the method of Motozato et al. (14). Prepared lactan acetate was identified using FTIR spectroscopy (Nicolet 520P).

Preparation of Lactan Acetate Microsphere and Drug Loading Procedure

To prepare lactan acetate microspheres and drug loading procedure, 40 mg of lactan acetate was dissolved in 5 ml of DCM and subsequently 40–80 mg of IND was added. Then, the solution was stirred at room temperature and solubilized frequently. To form microspheres, the solution was dropped slowly into 50 ml of double-distilled water and vigorously stirred to evaporate the solvent for 2 hr at room temperature, and the residual solvent was removed entirely by evaporation using an evaporator for 30 min. IND-loaded microspheres was harvested by centrifugation at 3000 × *g* for 10 min and then they were freeze-dried to obtain microsphere samples.

For measurement of drug loading content, freeze-dried samples of lactan acetate microspheres were sus-

pended into methanol and vigorously stirred for 30 min and sonicated for 10 min. The resulting solution was centrifuged with 12,000 × *g* for 20 min and supernatant was taken for measurement of drug concentration using a UV spectrophotometer (Shimadzu UV-1201) at 319 nm.

Observation by Scanning Electron Microscope (SEM)

The morphology of the microspheres was observed using an SEM (JSM 5400, Jeol, Japan). A freeze-dried sample was placed on a graphite surface and coated with gold/palladium using an Ion Sputter (Jeol, JFC-1100). Coating was performed at 20 mA for 4 min. Observation was performed at 25 kV.

Swelling Measurment

Swelling was measured as a function of pH. The lactan acetate pellets, equilibrated with distilled and deionized water, were dried in a vacuum oven at room temperature until no detectable weight change was observed. The dried pellets were placed in the aqueous media (two different pH values, 1.2 and 7.4) until equilibrium was reached. Generally, equilibrium was reached within 12 hr. The swelling (defined as the weight of water uptake per unit weight of dried gel) was calculated by measuring the weight of swollen pellets until weight changes were within 1% of previous measurment. This method is a modification of the method of Yuk et al. for swelling measurement of o/w emulsions (8).

In Vitro Release Studies

The release experiment was carried out as follows: IND-loaded microspheres and 1 ml phosphate buffered saline (PBS, 0.15 M, pH 7.4) or HCl solution (0.1 M, pH 1.2) were put into a dialysis tube and then the tube was introduced into a vial with 10 ml PBS or HCl solution. At specific time intervals, medium was taken and replaced with fresh medium. The concentration of the released IND was determined by a UV spectrophotometer (Shimadzu UV-1201) at 319 nm.

RESULTS AND DISCUSSION

Because lactan gum is a hydrophilic polysaccharide, it is necessary to change lactan gum into hydrophobic gum for use in a drug delivery system. We prepared

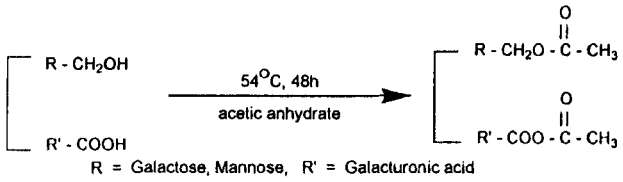


Figure 1. Preparation of lactan acetate from lactan gum by acetylation.

hydrophobic lactan acetate, with a substituted proton ion at O-6 mannose, galactose, and galacturonic acid (composition sugars of lactan gum) into acetyl group (Fig. 1). The lactan acetate was analyzed with FTIR spectra (Fig. 2). The sample showed increase or creation of the functional group of acetate as C=O in carbonyl compounds (wave number 1753 cm^{-1}), CH_3 deformation (wave number 1381 cm^{-1}), and O–C=O in carboxylic acid (wave number 599 cm^{-1}).

The morphological structure of microspheres was examined with SEM. The shape of IND-loaded microspheres was a complete sphere. The size of the IND-loaded microspheres was smaller than 5 μm (Fig. 3). The loaded drug content ratio and drug loading efficiency of the lactan acetate microspheres were studied (Table 1). For 40 mg IND, the loaded drug content ratio and drug loading efficiency were 41 and 69%. For 80 mg indomethacin, the loaded drug content ratio and drug loading efficiency were approximately 62 and 82%, respectively.

The major factor for controlling the release pattern was the swelling behavior of the microspheres that was affected by surrounding pH. The pH-sensitive swelling behavior of lactan acetate pellets was measured to assess the feasibility of pH-sensitive drug delivery system using lactan acetate microspheres (Fig. 4). The swelling

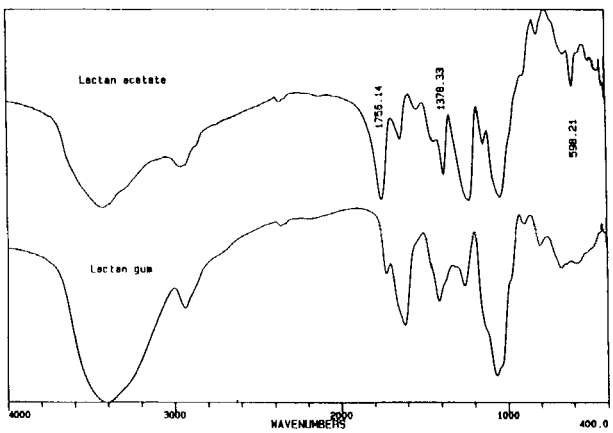


Figure 2. Comparison of FTIR spectra of lactan gum and lactan acetate.

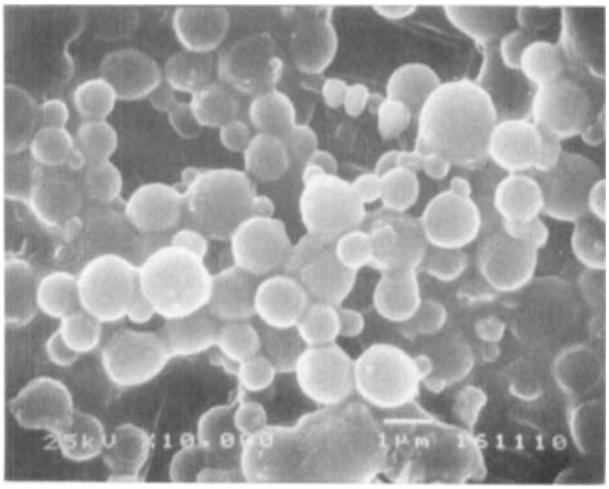


Figure 3. Photograph of lactan acetate microspheres by SEM (×10,000).

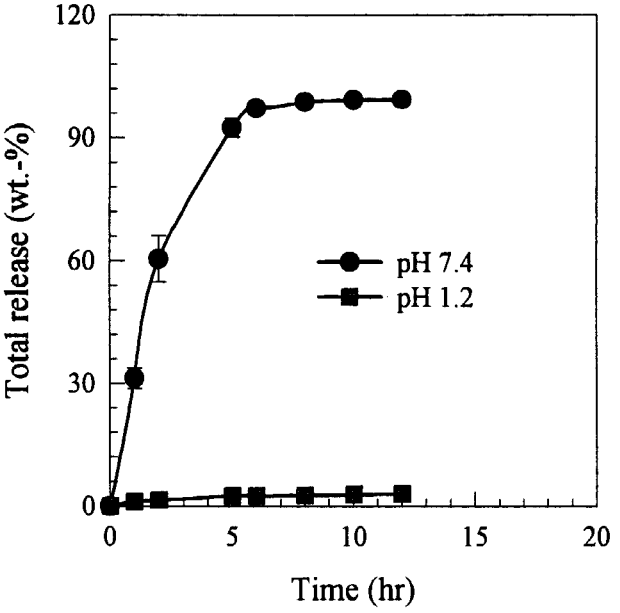


Figure 5. Release of indomethacin from lactan acetate microspheres (drug loading: 41wt%) against pH.

of lactan acetate pellets at pH 7.4 was much greater than that at pH 1.2. Yuk et al. reported that pH-sensitive drug release pattern was due both to the diffusion of drug from the capsule network and to the escape of drug from the surface, which underwent the disintegration after swelling depending on the chemical composition of the capsule network and the pH of release media. Under acidic conditions (pH 1.2), carboxylic groups were protonated and lactan acetate microspheres were

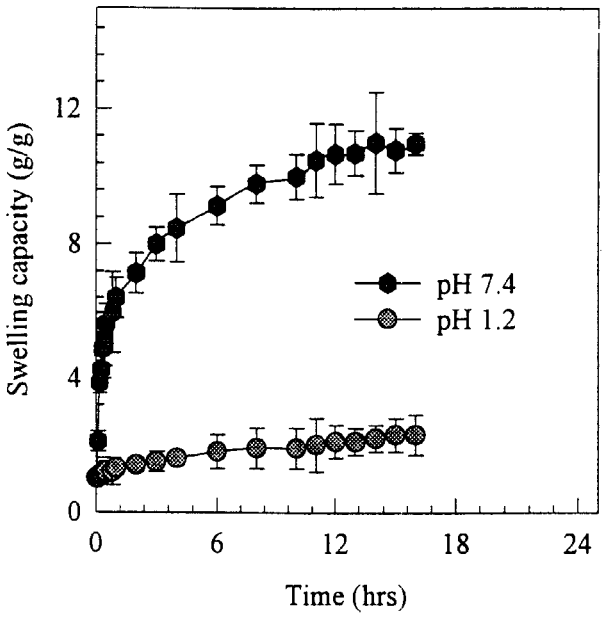


Figure 4. Time courses of swelling of lactan acetate at pH 1.2 and 7.4.

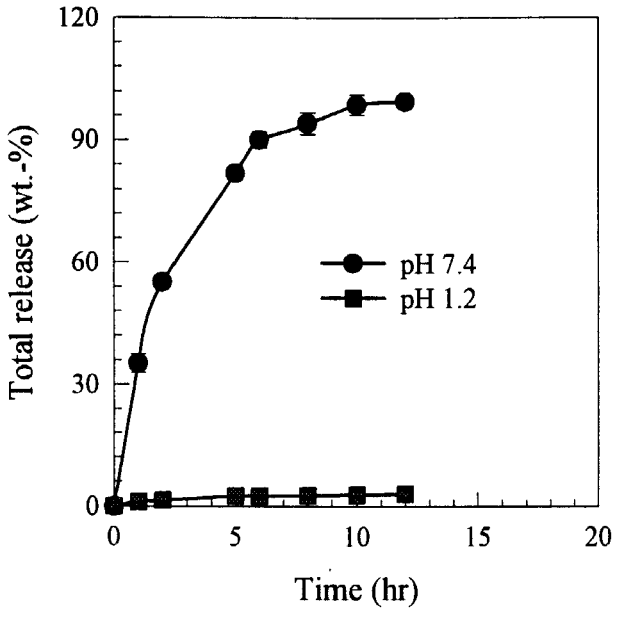


Figure 6. Release of indomethacin from lactan acetate microspheres (drug loading: 62wt%) against pH.

Table 1

Indomethacin Loading Content and Loading Efficiency in Lactan Acetate Microspheres

	Initial Amount of Drug (mg)	Drug Loading (wt%) ± SD	Loading Efficiency (wt%) ± SD
Lactan acetate	40	41 ± 4.3	69 ± 4.5
	80	62 ± 6.3	81 ± 6.7

deswelled. Under neutral conditions (pH 7.4), however, the concentration of negatively charged carboxylic group in lactan acetate microspheres increased. This result was due to a drastic increase in swelling.

IND release experiments were performed at two drug content ratios and at two pH conditions for 12 hr. Fig. 5 shows the release pattern of microspheres at drug loading content ratio of 41 wt%. At pH 1.2, carboxylic groups of lactan acetate were protonated and the lactan acetate microsphere was deswelled. Therefore, it is possible that drug is not released from the microsphere. However, at pH 7.4 the overall release rate increased because the swelling of the microsphere was increased as a result of the ionization of carboxylic groups at neutral pH. At drug loading of 62 wt%, the drug release pattern was similar to that of 41 wt% (Fig. 6). Fig. 7 shows the release of drug from microspheres on the repeated pH change of aqueous media (pH 1.2–7.4–1.2–7.4 for 2 hr, respectively). The drug release was similar to that of pH 1.2 and 7.4. At initial pH of 1.2 for 2 hr drug release was not observed

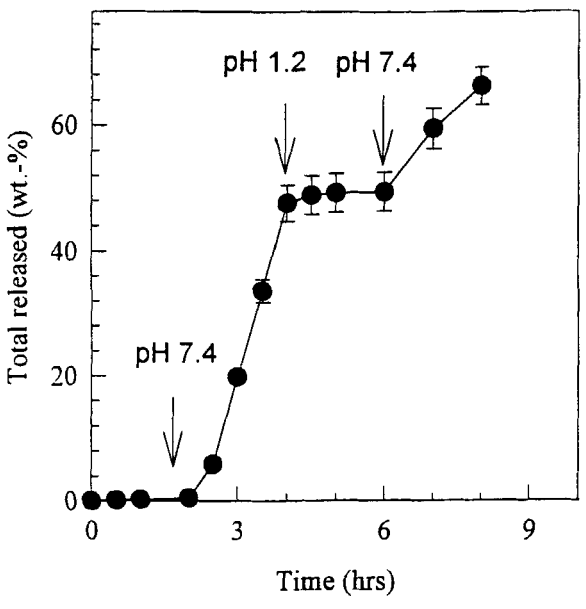
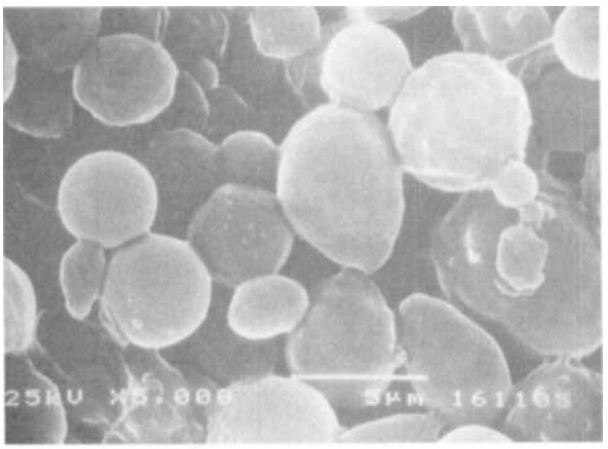


Figure 7. Release of indomethacin from lactan acetate microspheres against repeated pH change (1.2–7.4–1.2–7.4).



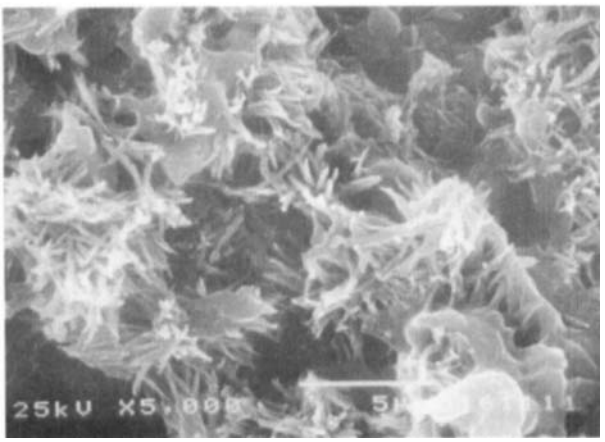


Figure 8. Photoghaphs of lactan acetate microspheres by SEM after drug release at pH 1.2 (a) and pH 7.4 (b) for 24 hr.

from microspheres and after pH change of aqueous media (to 7.4) for 2 hr, drug was quickly released from microspheres. Again, with a pH change of aqueous media (pH = 1.2), release of drug was stopped and then at pH 7.4, release of drug was started again. After drug release at pH 1.2 and 7.4, microspheres were observed by SEM (Fig. 8). The microspheres at pH 1.2 maintained a sphere shape. The microspheres at pH 7.4, however, disintegrated.

CONCLUSIONS

Lactan acetate microspheres showed swelling behavior under acidic conditions because of the protonation of the carboxylic group and swelling under neutral conditions because of the ionization of the carboxylic group. The drug was not released from the microspheres at pH 1.2. At pH 7.4, however, the overall release rate increased because the swelling of microspheres was increased as a result of the ionization of carboxylic groups. Therefore, lactan acetate is a useful material for pH-sensitive drug delivery system.

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