Preparation and Characterization of Polylactic Acid Microspheres Containing Water-Soluble Anesthetics with Small Molecular Weight

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Polylactic acid (PLA) microspheres containing water-soluble anesthetics (phenobarbital sodium, pentobarbital sodium, procaine hydrochloride, lidocaine hydrochloride, or dibucaine hydrochloride) with low molecular weight were prepared using a water-in-oil-in-water (w/o/w) emulsion solvent evaporation method, and the optimization of preparative conditions was performed. In the microencapsulation of sodium salt anesthetics (phenobarbital sodium, pentobarbital sodium) into PLA matrix, the addition of NaCl (10% (w/v)) into the external aqueous phase and the choice of comparatively high concentration of PLA (16% (w/v)) in CH_2Cl_2 significantly improved drug loading efficiency compared to the case when no additives were added to the external aqueous phase, or low PLA concentration (4% (w/v)) in CH_2Cl_2 was employed in the manufacturing process. The loading efficiency of hydrochloride salt anesthetics (procaine hydrochloride, lidocaine hydrochloride, or dibucaine hydrochloride) in PLA microspheres, in constract, was dramatically improved using the alkalic buffer solution as the external aqueous phase. The mean volume diameter was obtained in the region of 20—30 μm and normal distribution for prepared microspheres in all cases. PLA microspheres containing 10% (theoretical) of various anesthetics exhibited so-called burst releases, but, some portion of the loaded anesthetics still remained in the microspheres at 7 d.

Key words polylactic acid; microsphere; sustained-release; anesthetic; w/o/w emulsion; solvent evaporation method

Polylactic acid (PLA) microspheres have been investigated as a long-acting, injectable drug delivery system for the past 15 years. A wide range of synthetic drugs as well as biologicals such as enzymes, hormones, and proteins have been microencapsulated. These microspheres were manufactured by various techniques, *e.g.* solvent evaporation,[1,2] phase separation[3-5] using non-solvent addition or solvent partition. The most simplest and commonly employed method is the oil-in-water (o/w) emulsion solvent evaporation, and commonly employed. Nevertheless, the major problem with this system is its poor encapsulation efficiency of water soluble compounds. Bodmeier and McGinity[6] reported that water soluble drugs such as theophylline, caffeine, and salicylic acid could not be entrapped within microspheres using this o/w system. More recently, a novel water-in-oil-in-water (w/o/w) multiple-emulsion solvent evaporation process was developed and a soluble active hormone, leuprolide acetate, was efficiently incorporated into poly(lactide-co-glycolide) (PLGA) microspheres, and successfully used as a once-a-month injectable delivery system.[7,8] This w/o/w multiple-emulsion solvent evaporation process has the capability of encapsulating various types of water-soluble compounds; yet, there have been few reports of encapsulated water-soluble compounds with low molecular weights in PLA microspheres. We therefore microencapsulated five anesthetics as salts (phenobarbital sodium, pentobarbital sodium, procaine hydrochloride, lidocaine hydrochloride, and dibucaine hydrochloride) in PLA microspheres using a w/o/w multiple-emulsion solvent evaporation method and determined their optimal preparative conditions which gave high loading efficiency. The *in vitro* release characterization of obtained microspheres was also undertaken.

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Experimental

Chemicals PLA (M.W. = 58000) was purchased from Medisorb Technologies (Cincinnati, OH, U.S.A.). Phenobarbital sodium, pentobarbital sodium, procaine hydrochloride, lidocaine hydrochloride, and dibucaine hydrochloride (see Fig. 1) were purchased from Sigma Co. (St. Louis, MO, U.S.A.). Polyvinyl alcohol (PVA; 87—89% hydrolyzed, M.W. = 85000—146000) was supplied by Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). NaCl was from Wako Chemicals (Osaka, Japan). Other reagents are all of special reagents grade.

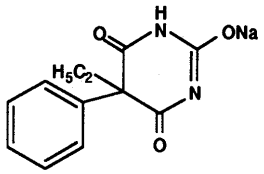
Preparation of PLA Microspheres Containing Anesthetics A w/o/w multiple-emulsion solvent evaporation method was adopted as shown in Fig. 2; previous methods[9,10] were also modified and used. Firstly, 22.2 mg of the drug was dissolved in 50 μl water. The solution was emulsified with 1.25—5.0 ml of methylene chloride containing 200 mg of PLA for 1 min using an ultrasonic disruptor (UD-200; Tomy Seiko Co., Ltd., Tokyo, Japan). This w/o emulsion was poured into 200 ml of 0.5% PVA solution. Emulsification was continued using a homogenizer (NS-60; Nichionirikakikai Co., Ltd., Tokyo) at 3000 rpm for 1 min. NaCl as an additive was added to the 0.5% PVA solution as the external aqueous phase if necessary. This dispersion was gently agitated in a 500 ml beaker on a stirring plate containing an 1.5 inch stirring bar for 5 h at room temperature. The microspheres were collected by centrifugation at 3000 rpm for 10 min. The obtained microspheres were filtered, washed with water and freeze-dried (FD-1; Tokyo Rikakikai Co., Ltd., Tokyo) for at least 12 h.

Loading Test The actual load was determined by placing about 10 mg of PLA microspheres into 1 ml of methylene chloride, extracting anesthetics using 10 ml of the 0.1 N HCl (phenobarbital sodium, pentobarbital sodium) or pH 10 phosphate buffer (procaine hydrochloride, lidocaine hydrochloride, and dibucaine hydrochloride). The drug concentration in the extracted medium was determined using HPLC. Twenty microliters of extracted sample was injected onto a chromatograph (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD -10AV), an integrator (Shimadzu C-R6A) and reversed phase C8 column (Asahipak ODP506D, 6.0 × 150 mm, Showa Denko, Tokyo, Japan). For sodium salts (phenobarbital, pentobarbital), the mobile phase employed was 0.1% phosphoric acid : acetonitrile = 3 : 2. The mobile phase for hydrochloride salts (procaine, lidocaine, dibucaine) was 0.1% phosphoric acid : acetonitrile : methanol = 87 : 10 : 3. The wavelength was set at 240 nm for sodium salts and 290 nm for hydrochloride salts, respectively. The flow rate was maintained at 1.5 ml/min, and the column was operated at 40 °C in all cases. The load-

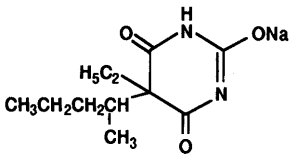
ing was calculated from the weight of the initial microspheres and the amount of drug incorporated.

In Vitro **Drug Release** Various microspheres containing 1.0 mg of drug were suspended in 5 ml phosphate buffered saline (PBS) (pH 7.4) in a 20 ml of glass tube and shaken horizontally at 75 rpm at 37 °C using a shaker (EP-1, Taiyo Co., Ltd., Tokyo, Japan). The sample was picked up at determined intervals, and its concentration was determined by the HPLC method described above.

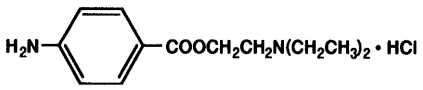
Microsphere Morphology Microsphere morphology was observed using a scanning electron microscope (SEM) (Akashi WS 250, Tokyo).



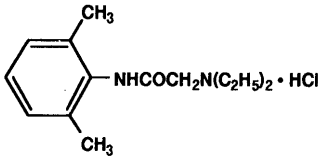
Phenobarbital sodium (M.W. 254.4)



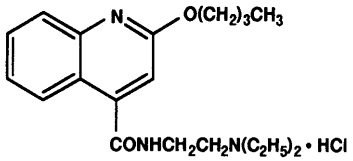
Pentobarbital sodium (M.W. 248.3)



Procaine hydrochloride (M.W. 272.8)



Lidocaine hydrochloride (M.W. 270.8)



Dibucaine hydrochloride (M.W. 379.9)

Fig. 1. Chemical Structures of Anesthetics Used

Results and Discussion

Effect of NaCl Concentration in the External Aqueous Phase, and Oil Phase Volume on Loading Efficiency of Phenobarbital Sodium into Microspheres The formation of a stable w/o/w emulsion seems essential to entrap the water-soluble drugs into PLA microspheres efficiently. In previous studies,[11,12] we demonstrated that presence of NaCl in the external aqueous phase and smaller internal aqueous phase formed a stable w/o/w emulsion, and thereby dramatically improved loading efficiency of water-soluble drugs into PLA microspheres. In this experiment, simultaneous manufacturing conditions were adopted. The volume of the internal aqueous phase was fixed at 50 μl and NaCl was added to the external aqueous phase (0—10% (w/v)). Phenobarbital sodium was selected since the drug has been used as a water-soluble model compound in the literatures.[13] The theoretical phenobarbital sodium loading % in microspheres was 10%, and PLA concentration in CH_2Cl_2 was 4% (w/v). Nonetheless, the loading efficiency of obtained PLA microspheres was very low (under 5%; see Fig. 3).

One reason for this low loading efficiency seemed to be the lower molecular weight of phenobarbital sodium (M.W. = 254) compared to the soluble dyes (brilliant blue; M.W. = 793, acid yellow; M.W. = 534) as described in the previous papar[11]; this might cause leakage of the drug from the internal to the external aqueous phase through PLA solution phase dissolved in CH_2Cl_2. When PLA concentration increased from 4 to 8 or 16% (the corresponding CH_2Cl_2 volume was 2.5 or 1.25 ml, respectively) in the presence of NaCl, the loading efficiency was dramatically improved. Especially, when 1.25 ml, the smallest CH_2Cl_2 volume (PLA concentration was 16% (w/v)) was employed in the presence of 5 or 10% (w/v) of NaCl in the external aqueous phase, loading efficiency of almost 80% was obtained as shown in Fig. 3. High PLA concentration (16% (w/v)) in CH_2Cl_2 may prevent the drug leakage from the internal aqueous phase to the external phase due to the barrier function of PLA solution phase dissolved in CH_2Cl_2. But the mean volume diameter became larger as the PLA concentration increased in CH_2Cl_2 as shown in Table 1. Normal distributions were obtained for mean volume diameter of obtained microspheres, and average values of the mean

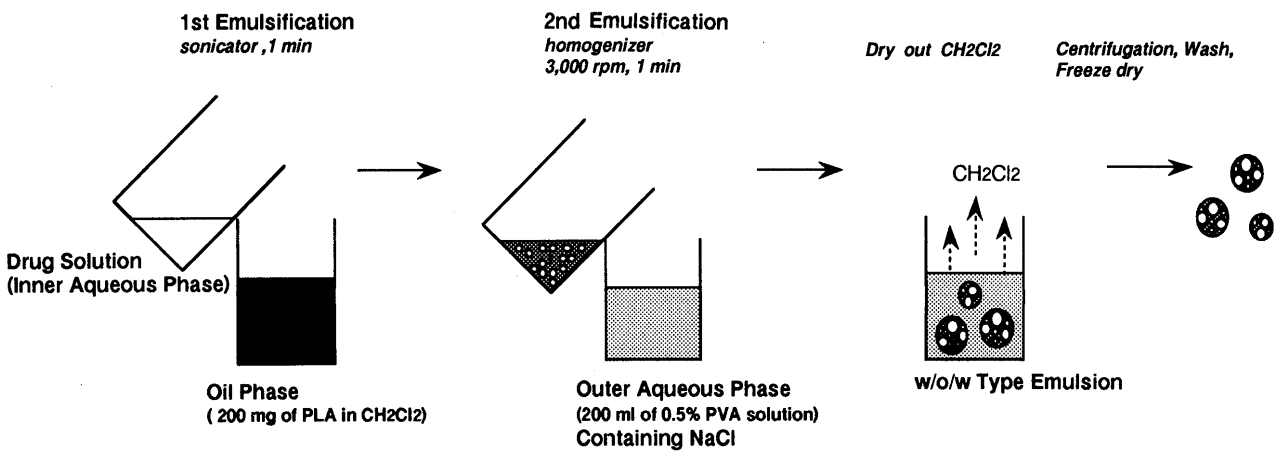


Fig. 2. Preparation Scheme for PLA Microspheres

volume diameter and standard deviation were also summarized. Each size distribution was not sharp, and the scale of standard deviation for mean volume diameter was almost 30% that of mean volume diameter. The increase in diameter may be caused by high viscosity of the PLA solution phase dissolved in CH_2Cl_2 during the manufacturing process.

A typical scanning electron microphotograph of prepared PLA microspheres containing phenobarbital sodium (the case when 1.5 ml of CH_2Cl_2 was employed) is shown in Fig. 4. A comparatively smooth surface of the microspheres was confirmed.

Optimization of Manufacture of PLA Microspheres Containing Other Anesthetics Microencapsulation was performed simultaneously in other anesthetics. The volume of the internal aqueous phase was fixed to 50 μl and PLA concentration in CH_2Cl_2 was fixed at 16% (w/v). The theoretical drug loading was 10%. Ten percent (w/v) of NaCl was added to the external aqueous phase if necessary. Table 2 represents the effect of various preparative conditions on loading efficiency of various drugs in PLA microspheres. The effect of the external aqueous phase pH on the loading efficiency of various drugs is summarized in the absence or the presence of 10% (w/v) of NaCl in the external aqueous phase. In the case of pentobarbital sodium, smaller CH_2Cl_2 volume (1.25 ml) and the addition of NaCl significantly enhanced loading efficiency (76.2%) as true of phenobarbital sodium. In contrast, in the preparation of PLA microspheres containing hydrochloride salts (procaine hydrochloride, lidocaine hydrochloride, dibucaine hydrochloride), comparatively poor loading efficiencies were observed (42.8, 16.0, 4.3% (w/v), respectively), when the same manufacturing conditions were employed. Most of these hydrochloride salts are in anionic form at pH 5 of purified water which we used as the external aqueous phase. This fact suggests that high solubilities of these compounds to purified water (pH 5) used as the external aqueous phase might be one reason for low loading. Therefore, we adopted pH 7.4 Tris buffer solution as the external aqueous phase in the presence or the absence of NaCl (10% w/v); even in the absence of the NaCl, usage of pH 7.4 Tris buffer solution as this phase improved loading efficiency of hydrochloride salts significantly. Table 2 shows the data when 50 mM pH 7.4 Tris buffer solution was used as the external aqueous phase. Usage of 100 mM or 150 mM pH

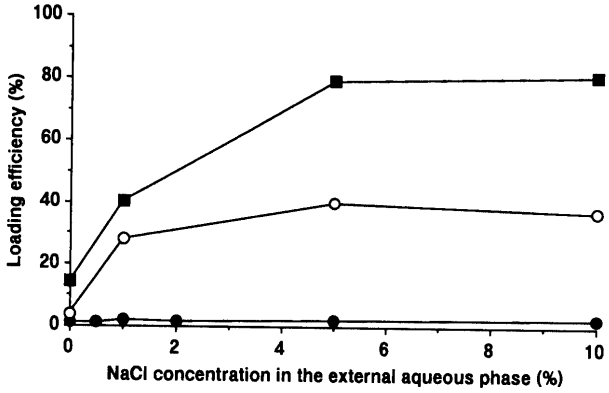


Fig. 3. The Effect of the Oil Phase Volume on Loading Efficiency of Phenobarbital Sodium in PLA Microspheres Theoretically Loaded with 10% of Phenobarbital Sodium

Key: theoretically loaded (●), 5 ml; (○), 2.5 ml; (■), 1.25 ml. The data was the average of two experiments.

Table 1. Arithmetic and Mean Volume Diameters for Prepared Microspheres

Oil phase volume (ml)	Arithmetic mean diameter (deviation; δ_g) (μm)	Mean volume diameter (deviation; δ_v) (μm)
1.25	23.0 (9.1)	27.3 (9.4)
2.5	16.4 (6.3)	20.5 (6.6)
5.0	12.0 (3.8)	13.8 (4.1)

The mean diameter represents the average of two experiments.

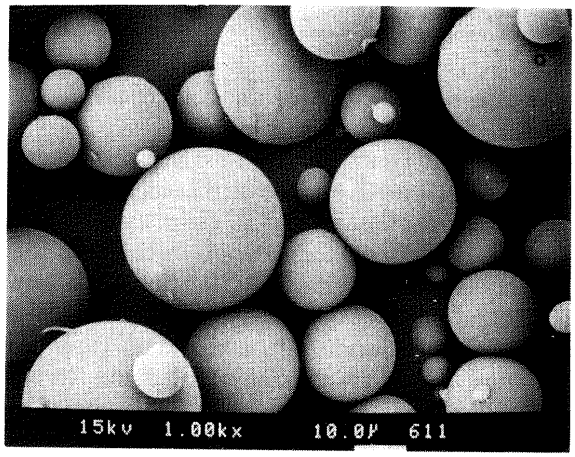


Fig. 4. Scanning Electron Micrograph of PLA Microspheres Theoretically Loaded 10% of Phenobarbital Sodium, (1.25 ml of CH_2Cl_2 was Employed in the Preparation Process)

Table 2. Effect of NaCl in the External Aqueous Phase on Loading Efficiency of Various Drugs in the PLA Microspheres

	pH of the internal aqueous phase	pK_a of the drug	Theoretical loading (%)	Loading efficiency (%)			
				External aq. phase: pH 5		External aq. phase: pH 7.4	
				NaCl–0%	NaCl–10%	NaCl–0%	NaCl–10%
Phenobarbital sodium	10.76	7.3, 11.8	10	14.2	81.1	—	—
Pentobarbital sodium	11.47	8.2, 12.7	10	25.4	76.2	—	—
Procaine hydrochloride	5.28	9.0	10	42.8	29.8	62.1	64.1
Lidocaine hydrochloride	3.89	7.4	10	16.0	11.9	75.3	81.5
Dibucaine hydrochloride	4.15	8.5	10	4.30	3.50	70.4	74.1

Loading efficiencies represent the average value of two experiments.

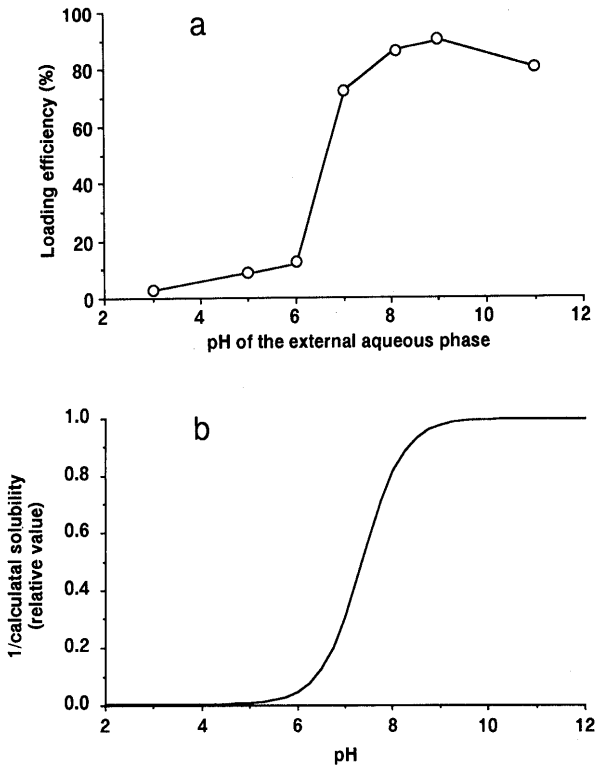


Fig. 5. (a) Effect of the External Aqueous Phase pH on Loading Efficiency of Lidocaine Hydrochloride in the PLA Microspheres; (b) Effect of pH on Lidocaine Hydrochloride Solubility (Theoretical Line)

The components of various buffers were as follows: pH 3.0, citric acid–Na_2HPO_4; pH 5.0, purified water; pH 6.0, citric acid–Na_2HPO_4; pH 7.0, citric acid–Na_2HPO_4; pH 8.0, citric acid–Na_2HPO_4; pH 9.0, Tris–HCl; pH 11.0, Na_2HPO_4–NaOH. The data was the average of two experiments.

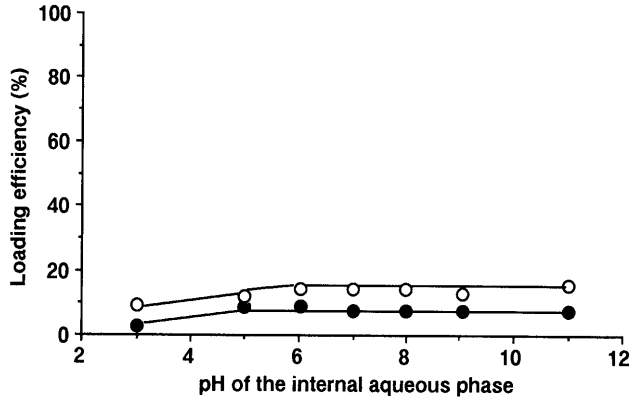


Fig. 6. Effect of the Internal Aqueous Phase pH on Loading Efficiency of Lidocaine Hydrochloride (○) or Dibucaine Hydrochloride (●) in the PLA Microspheres

The components of various buffers were as follows: pH 3.0, citric acid–Na_2HPO_4; pH 5.0, purified water; pH 6.0, citric acid–Na_2HPO_4; pH 7.0, citric acid–Na_2HPO_4; pH 8.0, citric acid–Na_2HPO_4; pH 9.0, Tris–HCl; pH 11.0, Na_2HPO_4–NaOH. The data was the average of two experiments.

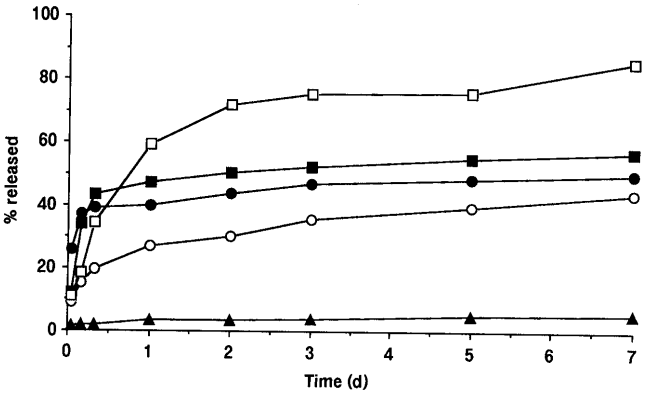


Fig. 7. Release Profiles of Various Drugs from PLA Microspheres

Key: (●), phenobarbital sodium; (○), pentobarbital sodium; (■), procaine hydrochloride; (□), lidocaine hydrochloride; (▲), dibucaine hydrochloride. Microspheres containing 1.0 mg of drug were suspended in 5 ml PBS (pH 7.4). The data was the average of two experiments. The mean volume diameters for microspheres used in the present study were in the range of 20—30 μm in all drugs.

7.4 Tris buffer solution showed the same results (data not shown).

Addition of NaCl into Tris buffer as the external aqueous phase in the preparative process further increased loading efficiency to some though not a great extent. Further experiments were conducted to confirm the effect of the external aqueous phase pH on loading efficiency of hydrochloride salts. We selected lidocaine hydrochloride as a model hydrochloride salt, and the effect of pH of external aqueous phase was examined in detail. As shown in Fig. 5a, low loading efficiency (30%) was obtained at pH 3.0, while above pH 7.0 the loading efficiency was dramatically increased. This observed pH-loading efficiency profile in Fig. 5a almost coincided with the theoretical pH-reversed solubility profile (normalized line) in Fig. 5b, also suggesting the importance of the external aqueous phase pH in the preparation process. Heya *et al.*[14] reported that the internal aqueous phase pH affected loading efficiency of the drug. Therefore, we prepared PLA microspheres by adjusting the internal aqueous phase pH from 3.9 to 7.4 using lidocaine hydrochloride and dibucaine hydrochloride; no changes or improvement in loading efficiency was not observed, however (Fig. 6). In our experiments, the external aqueous phase pH and the stability of the boundary between PLA surface and the external aqueous phase seemed important. The comparatively high PLA concentration (16% (w/v) in the present study) seemed essential to prevent the leakage of small molecular weight compound. This information could be important for microencapsulation of highly water-soluble compounds with low molecular weight using the w/o/w emulsion solvent evaporation process.

Release of Drugs from Microspheres Figure 7 showed the release profiles from PLA microspheres containing various anesthetics. In two hydrochloride salts (procaine, lidocaine), comparatively fast release of drug from microspheres were observed. As reported in the article by Heya *et al.*,[14] which incorporation of a citric acid into internal aqueous phase gave rise to a burst release of weakly based compound from PLGA microspheres. The report suggested that hindrance induced by the electrical interaction between PLGA (negative charge) and the weakly based compound (positive charge) in the internal aqueous phase might be one reason for the burst release. The simultaneous electrical hindrance between PLA and procaine or lidocaine as a weak base compound might have happened in the present case.

On the contrary, two sodium salts (phenobarbital, pentobarbital) showed a more restricted release than hydrochloride salts even though burst releases were observed to some extent.

The release of dibucaine from PLA microspheres was restricted to a low level, and showed an exceptional release profile. Although the exact reason was not ascertained, the comparatively larger molecular weight of dibucaine seems to be one reason; another possibility might be the interaction between anionic carboxyl group of PLA and cationic group of dibucaine molecules as an amino group as described previously.[7)]

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