Preparation and *In Vitro* Release Behavior of Urapidil Hydrochloride Loading into Microspheres Based on Poly(L-lactide)

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ABSTRACT: Microencapsulation of the antihypertensive drug urapidil hydrochloride was investigated as a means of controlling drug release and minimizing or eliminating local side effects. Poly(L-lactide) (PLLA) microspheres were prepared using an alternative oil-in-water (O/W) solvent-evaporation method such as the O/W cosolvent solvent-evaporation method and O/W with various electrolytes added to the aqueous phase method. The surface morphology and the size of the microspheres were observed by scanning electron microscope. Meanwhile, the drug loading efficiency of microspheres and the *in vitro* release of urapidil hydrochloride from microspheres were performed. The release study indicated that the urapidil hydrochloride-PLLA microspheres exhibited better sustained release capacity, and the kinetics of urapidil hydrochloride-PLLA microspheres *in vitro* release could be described by the Higuchi equation. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2317–2321, 2010

Key words: urapidil hydrochloride; poly(L-lactide) (PLLA); microsphere; loading efficiency

INTRODUCTION

Urapidil hydrochloride is a potent antihypertensive compound without serious side effects. Its action is mainly due to a postsynaptic α-receptor antagonism that inhibits the vasoconstrictive action of catecholamines and reduces blood pressure by decreasing peripheral vascular resistance. Urapidil hydrochloride also has an agonistic effect on central 5-HT1A receptors and lowers blood pressure by preventing the stimulation of baroreceptors. The majority of adverse events occurring during urapidil hydrochloride therapy are mild and transient, usually subsiding after long-term treatment. The most common events reported during oral or intravenous therapy are dizziness, nausea, and headache. Adverse events associated with intravenous urapidil hydrochloride are usually due to a rapid decrease in blood pressure.[1] Therefore, the need of a prolonged and controlled release for urapidil hydrochloride warrants a thorough study of the microencapsulation of urapidil hydrochloride in polymeric microspheres.

Poly(L-lactide) (PLLA) is a biodegradable and biocompatible polymer, with resultant environmental advantages among synthetic polymers. It could be applied as controlled-release devices.[2] The aim of this article was to study the microencapsulation of urapidil hydrochloride in PLLA via solvent-evaporation methods. The oil-in-water (O/W) method is one of the simplest techniques to prepare microspheres and has the advantages of efficient incorporation of lipophilic drugs, the ability to prepare a wide range of particle sizes, the less use of organic solvent, and the ready resuspension in water without aggregation. However, the O/W solvent-evaporation method may not be the best for water-soluble drugs, such as urapidil hydrochloride, due to the low loading efficiency caused by the partitioning of drug into the continuous phase.[3] To reduce the partitioning of urapidil hydrochloride into the continuous phase, alternative methods such as the O/W cosolvent solvent-evaporation method (O/W-S method) and O/W solvent evaporation with various electrolytes added to the aqueous phase method were studied in this article. In addition, the release kinetics of microspheres was analyzed according to various theoretical models.

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TABLE I
Characteristics of PLLA Used in this Work

IR absorption (cm^{-1})	C=O	1758
	C—O	1186
^{1}H-NMR	OH	7.29
	C*H*—CH_3	5.18
	CH—C*H*$_3$	1.59
^{13}C-NMR	C=O	169.20
	C—O	68.61
	CH_3	16.24
GPC	$M_n = 1.1 \times 10^5$	

MATERIALS AND METHODS

Materials

Urapidil hydrochloride was kindly supplied by Xi'an Lijun Pharmaceutical Co., Ltd. Polyvinyl alcohol (PVA-217) was purchased from Shanghai Lisheng Chemical Company and stannous octanoate was purchased from Sigma Chem. (USA). Dichloromethane, NaCl, Na_2SO_4, NaBr, $NaClO_4$ were all of ACS grade. L-lactide (Purac) was both recrystallized twice from ethyl acetate and dried at room temperature in vacuo (0.4 mbar) over P_2O_5 for 48 h. The melting point was 95°C.

Polymer synthesis

Well-purified L-lactide was kept in evacuated sealed tubes containing 0.03% (w/w) stannous octanoate. The polymerization was carried out in a silicone oil bath at 180°C for 6 h. The solid polymer residue in the tube was dissolved in chloroform and then precipitated repeatedly from a large quantity of ethanol at room temperature. The purified polymer thus obtained was white and fibrous in appearance. The results of its ^{1}H-NMR, ^{13}C-NMR analysis, IR measurement, and molecular weight obtained by gel permeation chromatography are listed in Table I.

Preparation of microspheres

An O/W solvent-evaporation method was used to prepare microspheres. One hundred fifty milligrams of PLLA was dissolved in the organic solvent (3 mL) that was either dichloromethane alone or dichloromethane with ethanol. Urapidil hydrochloride was then dissolved in PLLA solution. The ratio of drug : PLLA was 1 : 2 (w/w). The external phase was the 0.9% (w/v) solution of polyvinyl alcohol in distilled water (30 mL). Inorganic salts were added to the external aqueous phase that the concentration of PVA was kept 0.9% (w/v). The organic phase was emulsified with the external aqueous phase (1600 rpm for 1 min). The dispersion was then mixed on a magnetic stirrer for 4 h under room temperature to harden the microspheres. The microspheres were then separated by filtration, washed with deionized water, vacuum dried, weighed, and stored in a vacuum desiccator.

Loading efficiency

The drug loading efficiency of the microspheres was determined by first dissolving an exactly weighed amount of microspheres (50 mg) in dichloromethane (1 mL) and then diluting the mixture with ethanol (9 mL). After removing PLLA, the UV absorbance of this solution was then determined at 268 nm (urapidil hydrochloride) using an UV-2501PC spectrophotometer. The measured absorbance was then converted to the amount of urapidil hydrochloride based on a standard calibration curve ($C = 14.684A + 0.2129$), which was previously constructed using the UV-2501PC spectrophotometer on mixture of dichloromethane and ethanol (1 : 9) containing a known amount of urapidil hydrochloride each. Finally, the percentage loading efficiency of the microspheres was calculated as follows: loading efficiency (%) = 100 × (recovered milligram of urapidil hydrochloride per milligram of PLLA)/(charged milligram of urapidil hydrochloride per milligram of PLLA).

Morphology

The morphology and surface characteristics of the microspheres were examined with a scanning electron microscope (SEM, S-570). To assess the surface morphology, samples were mounted onto a metal stub using double-sided adhesive tape, vacuum-coated with a gold film and directly analyzed by SEM.

In vitro release studies

A total of 50 mg microspheres was transferred to a dialysis bag that contain 10 mL phosphate buffer (pH 7.4) and then the dialysis bag was put into measuring flask that contains 90 mL phosphate buffer acting as the dissolution medium. The flask was closed tightly and rotated at 100 rpm/min at 37°C. At scheduled time intervals, 10 mL of the dissolution medium was collected. The remaining dissolution medium was immediately replenished with fresh phosphate buffer to maintain the original volume. Based on the UV-2501PC absorbance at 268 nm, the urapidil hydrochloride content was measured, from which the release rate of urapidil hydrochloride could be determined.

TABLE II
Effects of PLLA/Urapidil Hydrochloride Ratio on Drug Loading Efficiency

PLLA/ urapidil HCl (g g^{-1})	1 : 2	1 : 1	2 : 1	3 : 1	5 : 1
Loading efficiency (%)	1.22	2.77	8.53	6.83	7.19

RESULTS AND DISCUSSION

Optimization of drug loading efficiency

Effect of the drug : polymer ratio

Using O/W solvent-evaporation method, the effects of the ratio of drug : polymer on drug loading efficiency were shown in Table II. When the ratio of PLLA : drug varied from 1 : 2 to 5 : 1, the urapidil hydrochloride loading efficiency increased first (from 1.22 to 8.53%) and then decreased slightly (from 8.53 to 7.19%). These results indicated that the urapidil hydrochloride levels in the feed must be low enough to obtain higher drug loading efficiency. Because the therapeutic level of drugs is 30 mg for a single dose of oral urapidil, the O/W solvent-evaporation method was unsuitable for the preparation of water-soluble urapidil hydrochloride microspheres with potential therapeutic application. To reduce the partitioning of urapidil hydrochloride into the external aqueous phase, the O/W-S method and O/W solvent evaporation with various electrolytes added to the aqueous phase method were used.

Effect of the water-miscible cosolvent

Use of a more water-miscible cosolvent (such as alcohol) has been shown to improve hydrophilic drug loading in microspheres prepared by an O/W method. When alcohol was used as the cosolvent in the O/W-S method, the alcohol diffused to the external aqueous phase because of its hydrophilicity, polymer concentration in the emulsified droplets increases and the organic solution viscosity increases. Alcohol diffusion continues until enough alcohol has left the droplets to cause polymer precipitation. The diffusion of a more water-miscible cosolvent from emulsified polymer-drug solution droplets to the external aqueous phase during microspheres preparation is faster than the other organic solvent.[4,5] As a result, polymer solution viscosity increases more rapidly and polymer droplet hardening occurs earlier. The formation of the solid shell limited the diffusion of urapidil hydrochloride to the external aqueous phase and microspheres loading efficiency could be improved. This is evident in this work when comparing the drug loading efficiency results for dichloromethane or mixture of dichloromethane and ethanol (EtOH) (Table III). When EtOH is added the drug loading efficiency increased from 8.53 to 30.52%.

As the ratio of EtOH : $CHCl_3$ increased from 0.5 to 1, the urapidil hydrochloride loading efficiency decreased (from 30.52 to 6.81%) because the diffusion of EtOH to the external aqueous phase inevitably entailed the concurrent loss of urapidil hydrochloride from the internal organic phase due to the high solubility of urapidil hydrochloride in EtOH. The addition of EtOH, which significantly alter the surface tension between the water and oil, reduced the average size of microsphere.

Effect of the electrolyte salts

Salt added to the aqueous phase in microsphere preparation can affect drug loading efficiency by affecting the aqueous solubility of the drug. Depressing drug aqueous solubility decreases drug loss to the external phase, leading to higher drug loading efficiency.

In general, drug aqueous solubility is depressed by the addition of a salt that has a common ion to the drug salt. This is known as the common ion effect.[5] Drug salt solubility can be expressed by a solubility product constant, K°_{sp}. The solubility produce of the drug salt, M_zX_y, can be described as follows:

$$M_zX_y \rightleftharpoons zM^{+y} + yX^{-z}$$

$$K^{\circ}_{sp} = ({}^{\alpha}M^{+y})^z({}^{\alpha}X^{-z})^y = (M^{+y})^z(X^{-z})^y(\gamma_{\pm})^{z+y}$$

$$= K_{sp(app)}(\gamma_{\pm})^{z+y}$$

where "α" is the activity, $\gamma_{\pm}$ is the mean ionic activity coefficient, M^{+y} and X^{-z} are the molar concentrations of the cation and the anion, respectively. Addition of a salt containing the common ion, X^{-z}, depresses the drug salt's aqueous solubility to satisfy the K°_{sp} relationship. Thus, urapidil hydrochloride solubility was expected to decrease in solutions containing NaCl or KCl. This was seen in our work (Table IV). The urapidil hydrochloride loading efficiency increased as the adding of NaCl or KCl.

In all other solutions containing uncommon ions to urapidil hydrochloride, the urapidil hydrochloride loading efficiency increased. This may be due to either a salting out of urapidil hydrochloride from the

TABLE III
Effects of the Amount of Ethanol on Drug Loading Efficiency

$CHCl_3$/ethanol (v/v)	3.0/1.0	2.5/0.5	2.0/1.0	1.5/1.5	1.0/2.0
Loading efficiency (%)	8.53	13.01	30.52	6.81	–

PLLA could not be dissolved in $CHCl_3$ when $CHCl_3$/ethanol (v/v) was 1.0/2.0.

TABLE IV
Effects of the Kind of Salt on Drug Loading Efficiency

Salt	KCl	Na_2SO_4	NaCl	$NaClO_3$	$KClO_3$
Loading efficiency (%)	35.52	33.83	33.93	10.66	11.96

The ratio of PLLA : drug was 2 : 1.

aqueous salt solutions or to new salt formation between the protonated urapidil hydrochloride and the uncommon anion.

Potassium salt could protect the vascular through suppression of salt-induced oxidative stress. The antioxidant mechanism of potassium salt might involve mainly its counteracting action against salt-induced NADPH oxidase activation, a harmful effect of excess salt. So a few KCl that remained on microspheres is beneficial to hypertension patient. When the concentration of KCl is 0.4%, the drug loading was the highest (Table V).

TABLE V
Effects of the Amount of KCl on Drug Loading Efficiency

KCl concentration (mol)	0.01	0.05	0.1	0.4	0.7
Loading efficiency (%)	18.88	22.18	23.36	35.52	28.60

Morphology and particle size

Morphological analysis was performed by SEM. Typical SEM micrographs, presented in Figure 1, illustrate that microspheres from O/W solvent-evaporation method were characterized by a smooth and dense surface without visible pores [Fig. 1(a)]. The average diameter of the drug-containing microspheres was 20 μm, with 86% of the particles within the size range of 15–25 μm.

Release studies

Urapidil hydrochloride release profiles from microspheres with 20 μm size and 29.94% drug loading efficiency in pH 7.4 phosphate buffer were shown in Figures 2 and 3, respectively. The microspheres exhibited a burst release within 3 h, which could be attributed to some drug crystals spread over or situated near the periphery of particles. After 3 h, the drug release rate was relatively constant. Compared with urapidil hydrochloride powder, prolonged release of urapidil hydrochloride from PLLA microspheres was observed. It was possible to sustain the release of urapidil hydrochloride over 20 days. The favorable results suggested that PLLA would be more useful biodegradable polymeric carriers for drug delivery, especially for drugs requiring a long period of sustained release.

The release mechanism of urapidil hydrochloride from microspheres was investigated. Drug release kinetics from microspheres has often been described by three kinetics models: a zero-order model, a first-order model, and the Higuchi model. The zero-order model and the first-order model pertained to the reservoir-type system and the amount of encapsulated drug and the fraction of shell in microspheres dictated the correct model type. The Higuchi model pertained to the monolithic-type system which

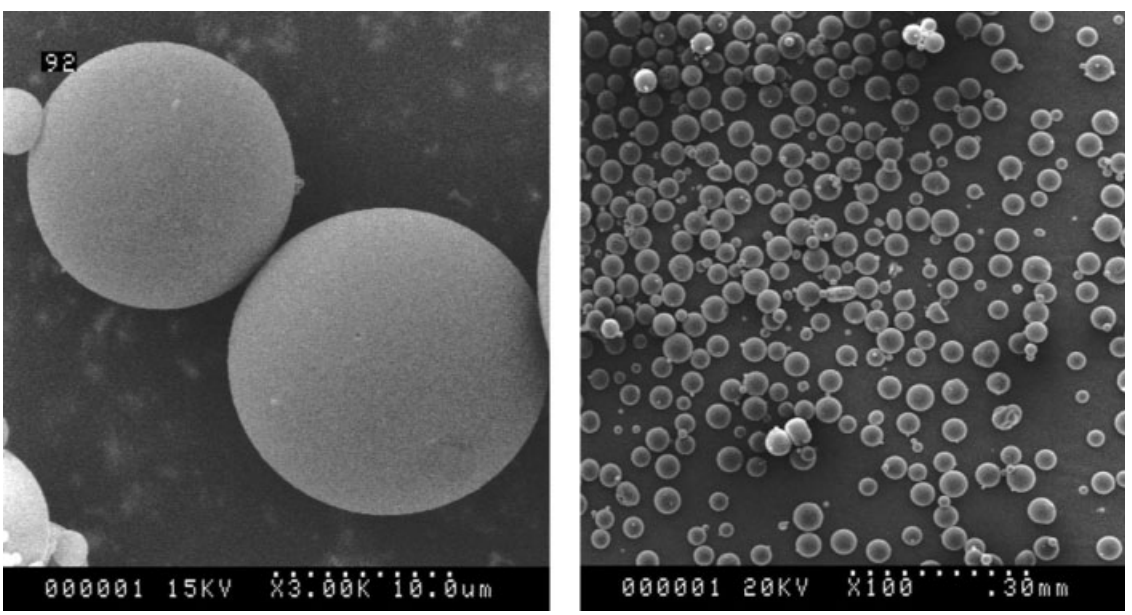

Figure 1 Scanning electron micrograph of urapidil hydrochloride-PLLA microspheres.

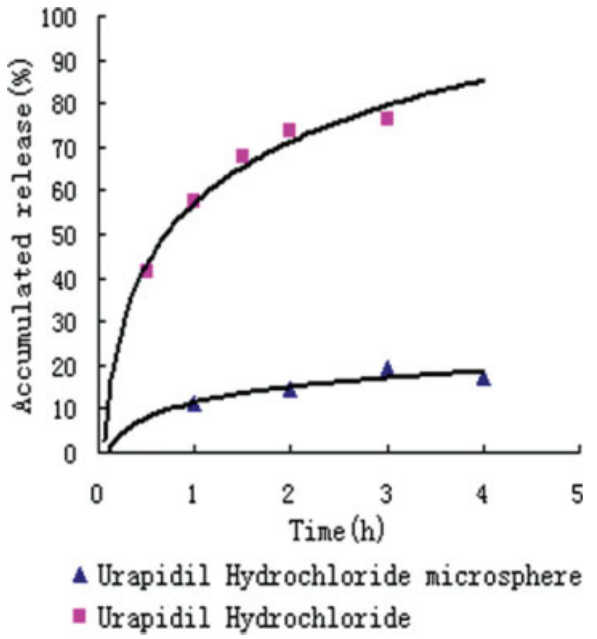

Figure 2 Comparison of the release curve of urapidil hydrochloride microspheres and urapidil hydrochloride *in vitro* (5 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

meant the drug was uniformly distributed over the entire microspheres.

The release constants (k) and the correlation coefficients (r) based on the three release kinetics models are summarized in Table VI. The model with a larger correlation coefficient was judged to be a better model for the release data. Urapidil hydrochloride release from PLLA microspheres agreed better with the Higuchi model. It might be the drug granules dispersed inside the polymer matrix and the release rate and pattern of drug from the PLLA matrix was mainly dependent on diffusion mechanism.[6] As the molecular weight of PLLA microspheres had no decrease over 16 days at 37°C,[7] the drug release corresponded to a Fickian diffusion mechanism.

TABLE VI
Regression Equation of Urapidil Hydrochloride Microspheres

Zero-order equations	First-order equations	Higuchi equations
$Q = 3.4588t + 24.519$, $r = 0.9165$	$\mathrm{Ln}(1 - Q) = -0.1069t - 0.1212$, $r = 0.9396$	$Q = 20.68t^{1/2} + 1.5157$, $r = 0.9739$

Q, accumulated release; t, time; r, correlation coefficient.

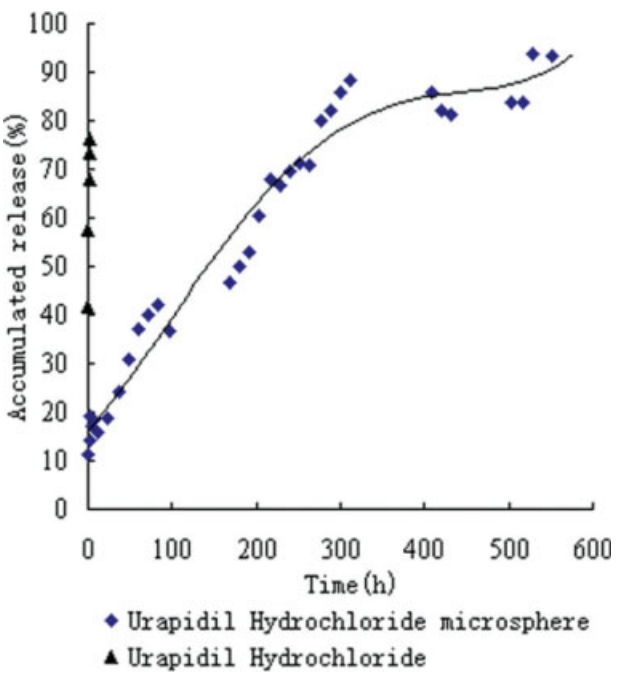

Figure 3 Comparison of the release curve of urapidil hydrochloride microspheres and urapidil hydrochloride *in vitro* (600 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

This study showed that the water-soluble drug urapidil hydrochloride microspheres with high drug loading efficiency were successfully prepared by the O/W-S method and O/W solvent evaporation with various electrolytes added to the aqueous phase method. Adding ethanol to the organic phase could increase drug loading efficiency and result in a uniform microspheres drug distribution. The drug loading efficiency was 30.52% when the volume ratio of ethanol and trichloromethane was 2 : 1. Adding various electrolytes in different concentrations to the aqueous phase could also increase drug loading efficiency. Salts affect microsphere drug loading efficiency by changing the aqueous solubility of both the drug and the organic solvent. KCl depressed drug solubility to the highest extent, resulting in microspheres with high drug loading efficiency. The highest drug loading efficiency of microspheres was 35.52% when KCl concentration was 0.4% (mol). *In vitro* release studies in phosphate buffer solution (pH 7.4, 37°C) revealed that microspheres of PLLA could sustain the release of urapidil hydrochloride over 20 days.

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