Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 303 (2005) 153–159
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

New drug salt formation in biodegradable microspheres

Ahmad Al-Maaieh[a,*], Douglas R. Flanagan[b]

[a] *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[b] *Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242, USA*

Received 30 March 2005; received in revised form 4 June 2005; accepted 6 June 2005
Available online 25 August 2005

Abstract

Purpose. To investigate the effects of inorganic salts in the external phase of an oil-in-water (O/W) emulsion method during microsphere preparation.
Methods. An O/W emulsion method was used to prepare poly(D,L-lactic acid) microspheres containing quinidine sulfate. Different inorganic salts were used in the external phase during microsphere preparation. Microsphere drug loading was determined by UV and the drug salt anions inside the microspheres were determined by ion chromatography.
Results. New drug salts were formed during encapsulation in the microspheres when salts with non-common anions to the drug salt were used. Drug loading increased when $NaClO_4$ or NaSCN were used. The fraction of drug as the new salt in microspheres increased non-linearly with the salt concentration in the external phase, however, the fraction of drug as the new encapsulated salt was linearly related to drug loading. Drug loading decreased and new salt fraction increased with increasing organic solvent volume or with decreasing cosolvent polarity.
Conclusions. Introducing salts containing non-common anions to the drug salt employed in the external phase of O/W emulsion microsphere method leads to new salt formation. The extent of new drug salt formation is affected by salt levels added, cosolvent type and polymer concentration.

Keywords: Microsphere loading; Inorganic salt; Salt formation; Ion chromatography; Biodegradable microsphere

1. Introduction

An oil-in-water (O/W) emulsion with internal phase evaporation is one of the simplest methods for preparing biodegradable polymeric microspheres. This method has the advantage of efficient incorporation of lipophilic drugs. However, hydrophilic drugs are poorly entrapped by the O/W method. This is due to drug partitioning to the external aqueous phase during emulsification and polymer hardening. Recently, we reported the effect of adding salts to the external phase during microsphere preparation by an O/W method (Al-Maaieh and Flanagan, 2001). A salt (Na_2SO_4) containing a common anion to the drug (quinidine sulfate)

* Corresponding author. Tel.: +1 317 277 3680; fax: +1 317 277 2403.
E-mail address: al-maaieh_ahmad@lilly.com (A. Al-Maaieh).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.06.029

decreased microsphere quinidine sulfate loading even though the drug's aqueous solubility was depressed by the common-ion effect. On the other hand, salts containing non-common anions to the drug (e.g., NaSCN or $NaClO_4$) increased drug loading. In this work, the effect of non-common anions on quinidine loading into biodegradable microspheres is further investigated. We intend to determine possible mechanisms for increasing drug loading. Since these salts ($NaClO_4$ and NaSCN) decrease quinidine aqueous solubility, one possible mechanism is that these salts in the external aqueous phase of the O/W emulsion slow quinidine distribution from the emulsified polymer solution droplets into the external aqueous phase, resulting in higher microsphere drug loading. Another possibility is that the increase in drug loading with $NaClO_4$ or NaSCN is due to the formation of new, less soluble quinidine salts inside the microspheres. In this investigation, ion chromatography is used to investigate the type and level of the anions present inside the microspheres in order to explain the increase in quinidine loading.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (PLA, i.v. of 0.51 dl/g in $CHCl_3$ at 30 °C) was obtained from Birmingham Polymers. Quinidine sulfate (QS) was purchased from Sigma. Polyvinyl alcohol (PVA, 9–10 K, 80% hydrolyzed), 1-propanol and 1-pentanol were obtained from Aldrich. Ethanol (USP, dehydrated, 200 proof) was purchased from Pharmco. 1-Butanol was obtained from Fisher Scientific. Dichloromethane (DCM), NaCl, NaBr, $NaClO_4$, NaSCN and Na_2SO_4 were all of ACS reagent grades.

2.2. Microsphere preparation

Poly(D,L-lactic acid) (PLA) microspheres were prepared at ambient temperature by emulsifying an organic solution (0.6 ml) of PLA (200 mg) in 0.5% (w/v) aqueous PVA solution (100 ml). The drug (quinidine sulfate, 10 mg) was suspended in the organic polymer solution and added salts were dissolved in the aqueous PVA solution prior to emulsification. A VirTiShear homogenizer was used for emulsification (1500 rpm, 1 min) of this PLA/drug dispersion in the aqueous PVA solution. The resulting O/W emulsion was then magnetically stirred for 2 h to harden the microspheres. The microspheres were then filtered, washed and dried under vacuum. The organic solvent was either pure DCM or DCM:alcohol (5:1, v/v).

2.3. Microsphere drug content

Drug-containing microspheres were dissolved in DCM. Distilled water (DW) was used to extract the drug from the organic phase. A sample of the aqueous extract was analyzed by suppressed ion chromatography to determine drug salt anions. The UV absorbance of an acidified extract was measured (HP 8450A diode array spectrophotometer, 345 nm) to determine quinidine content. Drug loading and drug salt content were calculated from these results.

2.4. Ion chromatography

Ion chromatography was used to investigate the type/content of drug salts inside the microspheres. A Dionex® IonPac® AS12A and a Dionex® Micromembrane Suppressor Model AMMS-II were used as the exchange and the suppressor columns, respectively. The eluent was acetonitrile:12.5 mM Na_2CO_3 (45:55) at 1.5 ml/min and the regenerant solution was 50 mN H_2SO_4 at 8.0 ml/min. Samples (20 μl) were injected into a Dionex® QIC Analyzer with a conductivity detector at a conductivity setting of 10 μS.

2.5. Quinidine distribution

The distribution of quinidine was studied by mixing 5.0 mL of DCM with 5.0 mL of aqueous QS solution containing 0.05 M of either Na_2SO_4, NaCl, NaBr, NaSCN or $NaClO_4$. The two phases were mixed at 25 °C for 2 h and then left without shaking for 30 min at 25 °C before the aqueous phase was sampled, acidified (few drops of 1 M HCl) and assayed for quinidine content by UV spectroscopy at 345 nm. The equilibrium quinidine concentration in the DCM phase was calculated by mass balance.

3. Results and discussion

The loading of water-soluble drugs inside biodegradable microspheres prepared by the O/W emulsification method is generally poor. This is due to drug loss from the internal phase by distribution to the external aqueous phase. This drug distribution to the external aqueous phase can take place immediately after emulsification across the liquid interface between the emulsified polymer solution and the external aqueous phase and can last as long as the emulsified phase is fluid. The loss of the organic solvent from the emulsified polymer solution droplets by evaporation or partitioning to the external phase and subsequent evaporation leads to increasing polymer concentration in the emulsified droplets. Eventually, the polymer precipitates first at the droplet periphery and later inside the suspended particles resulting in hardened microspheres. Since drug loss across the solid barrier formed by polymer precipitation will be much slower than diffusion across a liquid interface, drug loss from the internal phase occurs mainly before polymer precipitation.

In our previous work, we found that $NaClO_4$ and NaSCN depress quinidine aqueous solubility significantly (Al-Maaieh and Flanagan, 2001). This was found to be due to the formation of new, less soluble salts (e.g., quinidine perchlorate). Energy dispersive X-ray analysis (EDX) and ion chromatography characterization at the end of solubility studies indicated that the solid phase in equilibrium with a saturated solution were those of the new quinidine salts (e.g., quinidine perchlorate or quinidine thiocyanate) (unpublished results). Because of the ability of these salts ($NaClO_4$ and NaSCN) to depress quinidine aqueous solubility, we used them in the external phase of an O/W emulsification method to improve microsphere drug loading. Fig. 1 shows the effect of using either of $NaClO_4$ or NaSCN in the external phase on quinidine loading into PLA microspheres. It can be seen that loading increases about two fold with these added salts. At higher salt concentrations drug loading reached a plateau.

The ability of $NaClO_4$ and NaSCN to increase quinidine microsphere loading can be attributed to two possible mechanisms. Since $NaClO_4$ and NaSCN lower the quinidine aqueous solubility, they may depress the rate at which quinidine distributes to the external aqueous phase during microsphere preparation. On the other hand, loading may increase because these salts may lead to the formation of new, less soluble quinidine salts inside the microspheres. In order to investigate which of these two possible mechanisms is operative, microspheres were analyzed by both UV spectroscopy (quinidine content) and ion chromatography (anion content). Fig. 2 shows the amount of sulfate inside the microspheres (expressed as μmol SO_4^{2-}/mg microspheres) as a function of $NaClO_4$ concentration in the external phase during microsphere preparation. It can be seen (Figs. 1 and 2) that even though $NaClO_4$ results in higher quinidine loading, the amount of sulfate inside the microspheres does not increase accordingly. Since

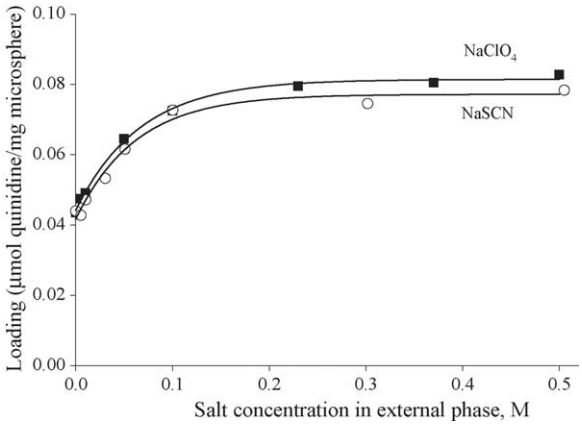

Fig. 1. Quinidine loading in PLA microspheres using different $NaClO_4$/NaSCN concentrations in the external phase.

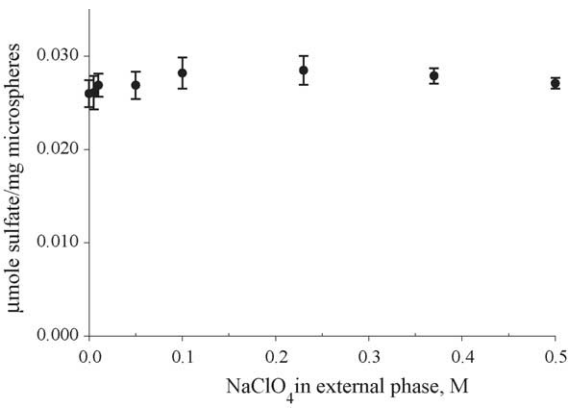

Fig. 2. Sulfate loading in PLA microspheres using different $NaClO_4$ concentrations in the external phase.

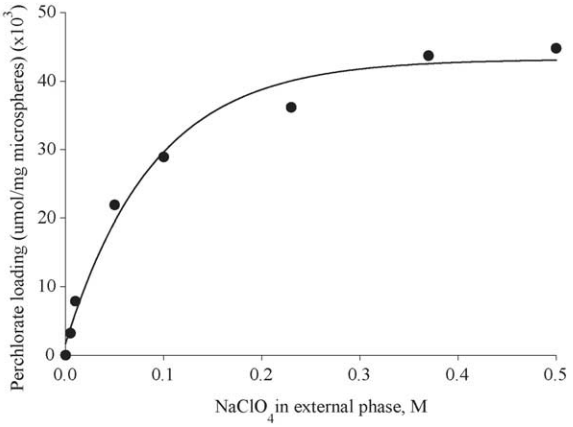

Fig. 3. Perchlorate loading in PLA microspheres as a function of $NaClO_4$ concentration in the external phase.

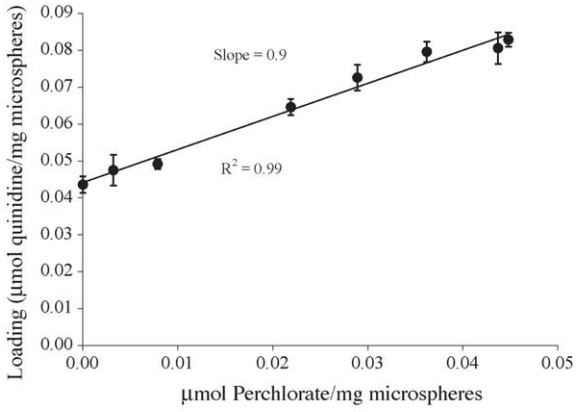

Fig. 4. Quinidine loading vs. perchlorate loading in PLA microspheres.

quinidine was added to the internal phase during microsphere preparation as quinidine sulfate, the additional entrapped quinidine is a result of added $NaClO_4$ and not because more drug sulfate salt is inside the microspheres.

Ion chromatography indicates that when $NaClO_4$ or NaSCN were used, perchlorate or thiocyanate salts were found inside the microspheres. Entrapped perchlorate increased with higher $NaClO_4$ concentrations in the external phase (Fig. 3). The shape of the plot (Fig. 3) is similar to that in Fig. 1 where loading increased with $NaClO_4$ concentration and leveled off at higher $NaClO_4$ concentrations. Moreover, entrapped quinidine correlates linearly with microsphere perchlorate content with a slope of 0.9 (Fig. 4). This indicates that each additional micromole of quinidine entrapped (per milligram microsphere) is a result of an equimolar perchlorate entrapment inside the microspheres. This strongly supports the proposal that additional entrapped quinidine with added $NaClO_4$ occurs as quinidine perchlorate.

In order for a new quinidine salt to be formed inside the microspheres, anion transfer across the interface during microsphere preparation is necessary. Ion and solute transfer across interfaces has been observed and characterized by several investigators (Ghanem et al., 1970; Schweighofer and Benjamin, 1995; Benjamin, 1996; Berny et al., 2000). During microsphere preparation, the solute/ion transfer across the interface between the external and the internal phases is limited by the rate of polymer precipitation. Solute/ion transfer across a solid/liquid interface would be much slower than that across a liquid/liquid interface.

Since O/W emulsion variables like the organic solvent volume and the use of cosolvents in the internal phase affect the kinetics of polymer precipitation, we studied their effects on the drug loading and new salt formation. Also, the effect of adding $NaClO_4$ to the external phase at different times during microsphere preparation following organic phase emulsification was also studied. The effect of different non-common anions in the external phase was also studied since different anions may have different effects on drug loading, new salt formation or polymer precipitation. The results of these studies are outlined below.

3.1. Organic solvent volume effect

The organic solvent volume affects the speed of PLA precipitation. Thus, at smaller organic solvent volumes, the initial polymer concentration is higher and the organic phase is more viscous such that it should take less time for sufficient organic solvent loss for polymer precipitation to occur forming a barrier to drug loss. This behavior should lead to higher drug loading at smaller organic solvent volumes as Fig. 5 shows.

However, with smaller organic solvent volumes there will also be less time available for ion transfer across the interface before polymer precipitation occurs. Thus, smaller organic solvent volumes will allow shorter amounts of time during which new drug

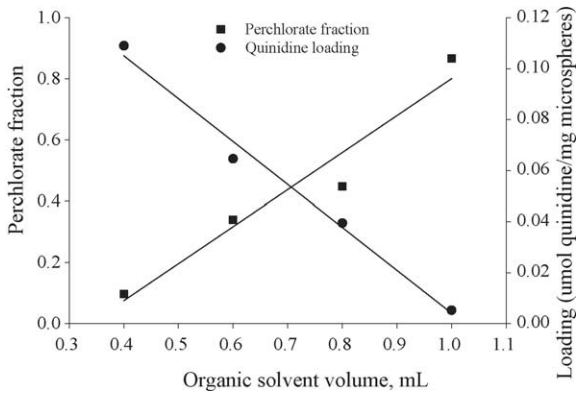

Fig. 5. Quinidine loading and perchlorate fraction in PLA microspheres using different organic phase volumes.

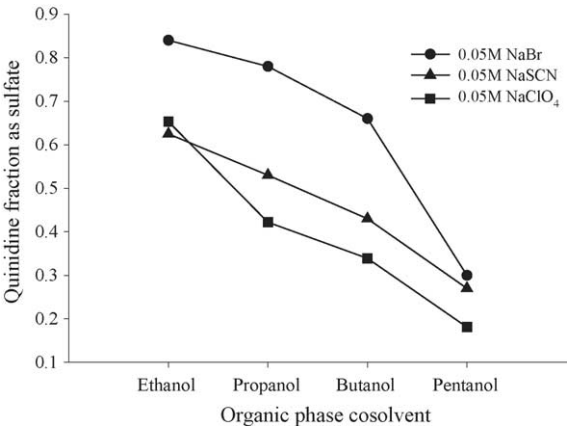

Fig. 6. Sulfate fraction in PLA microspheres using different alcohol cosolvents (1:5 ratio to DCM) with 0.05 M of different salts added to the external phase.

salt formation can take place. This leads to a smaller fraction of the entrapped drug inside the microsphere being present as the new salt at smaller organic solvent volumes and this can also be seen in Fig. 5.

3.2. Use of cosolvents in the organic phase

We previously studied the effect of EtOH as a cosolvent during microsphere preparation by an O/W method (Al-Maaieh and Flanagan, 2001). EtOH (1:5 EtOH:DCM) was sufficient to dissolve QS in the organic solvent resulting in a uniform microsphere drug distribution, leading to smoother release profiles with a smaller burst effect (Al-Maaieh and Flanagan, 2001). Microsphere drug loading also increased with EtOH because EtOH partitions faster to the external aqueous phase leading to more rapid polymer precipitation in the emulsified droplets. To further investigate new drug salt formation inside microspheres, EtOH and other short chain alcohols (propanol, butanol and pentanol) were used as cosolvents with DCM (1:5, v/v) in the internal phase of the O/W emulsion during microsphere preparation. The effect of 0.05 M of NaBr, NaSCN or $NaClO_4$ on the fraction of loaded quinidine present as the sulfate salt inside the microspheres using different alcohols as cosolvents is shown in Fig. 6. Longer chain alcohols are less hydrophilic which leads to internal phase hardening at a slower rate. This allows more time for new drug salt formation leading to lower sulfate fraction in the microspheres with longer chain alcohols (Fig. 6).

3.3. Effect of time of addition of $NaClO_4$

The effect of adding $NaClO_4$ to the external phase at different times during microsphere preparation was studied. During microsphere preparation, the internal organic phase was emulsified into the aqueous phase by homogenization for 1 min. Then, the emulsion was magnetically stirred until the microspheres hardened. An aliquot (5.0 mL) of 1.0 M $NaClO_4$ was added at different times (0, 2, 6 or 10 min.) after the homogenization step to the external phase during microsphere preparation giving a final concentration of 0.048 M of NaClO4. Two controls were also studied: a positive control used 5.0 mL of 1 M $NaClO_4$ added *before* homogenization; and a negative control used 5.0 mL of DW added instead of $NaClO_4$. Table 1 lists the perchlorate salt fraction of entrapped quinidine and total quinidine loading. It can be seen (Table 1) that $NaClO_4$ is most effective on both quinidine loading and new salt fraction when it is added prior to the internal phase emulsification (positive control). No significant effects on loading were observed when $NaClO_4$ was added after the homogenization step and new salt formation occurred to only a small degree when $NaClO_4$ was added immediately following the homogenization.

The effect of $NaClO_4$ on both microsphere loading and new salt fraction of entrapped drug was expected to decrease if it was added at later times during microsphere preparation. The results in Table 1, however, indicate that $NaClO_4$ is really only effective when

Table 1
Effect of $NaClO_4$ (0.048 M) addition time on quinidine fraction present as the perchlorate and on total quinidine loading

Time (min)[a]	Quinidine perchlorate fraction	Total quinidine loading[b]
Positive control[c]	0.34 (0.01)[e]	0.065 (0.002)
0	0.04 (0.06)	0.048 (0.001)
2	0	0.049 (0.004)
6	0	0.048 (0.002)
10	0	0.050 (0.004)
Negative control[d]	0	0.044 (0.005)

[a] Time between the end of the homogenization step and the addition of $NaClO_4$ to the external phase.
[b] Loading expressed as μmol quinidine/mg microspheres.
[c] $NaClO_4$ added to the external phase prior to the homogenization step.
[d] No $NaClO_4$ added.
[e] Value in parentheses is standard deviation ($n = 2$).

added during the initial homogenization step since it is much less effective at any later times. This indicates that vigorous shearing during homogenization of the emulsion is important for any salt effect to occur. Also, this indicates that the emulsified droplets harden quickly at the liquid/liquid interface after the initial emulsification step.

3.4. Effect of different non-common anions

The effect of different sodium salts on quinidine distribution between aqueous/organic phases was studied. Different anions increase quinidine's distribution coefficients ($D_{O/W}$) between DCM and water to different degrees (Table 2). Thiocyanate and perchlorate are much more effective in increasing quinidine distribution into DCM than the other investigated anions (Table 2). This may be related to their ability to form ion-pairs with the drug (Schill et al., 1965). The effect

Table 2
Quinidine distribution coefficients ($D_{O/W}$) between dichloromethane and water as a function of added salts in the aqueous phase at 25 °C

Salt[a]	$D_{O/W}$	Standard deviation[b]
None	0.22	0.066
Na_2SO_4	0.25	0.045
NaCl	0.47	0.049
NaBr	1.48	0.095
NaSCN	10.50	1.17
$NaClO_4$	6.34	0.51

[a] 0.05 M.
[b] $n = 2$.

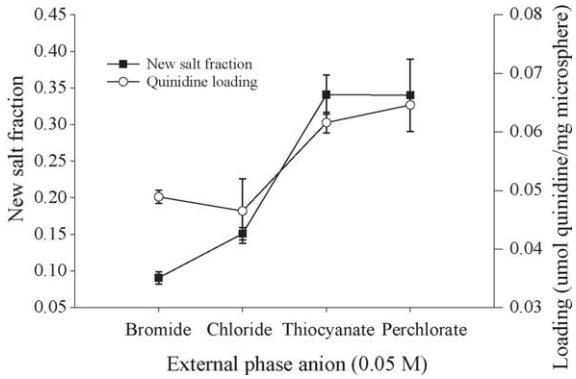

Fig. 7. Quinidine loading and new salt fraction in PLA microspheres using different sodium salts (0.05 M) in the external phase.

of 0.05 M sodium bromide, chloride, thiocyanate or perchlorate on quinidine loading and new drug salt fraction in microspheres is shown in Fig. 7. It can be seen that the anions that were most effective in increasing quinidine distribution (perchlorate and thiocyanate) were also the most effective in increasing microsphere loading and forming new drug salts inside the microspheres (Fig. 7).

4. Conclusions

This research has improved our understanding of salt effects on microsphere entrapment properties for a cationic drug and shown the possibility of new drug salt formation inside the microspheres. With the O/W emulsion method for preparing biodegradable microspheres, we have shown that non-common anions in the external phase lead to partial new drug salt formation inside microspheres. Drug loading and extent of new drug salt formation depend on anion type and concentration. Drug loading decreases and the extent of new salt formation increases with increasing organic solvent volume and with decreasing organic cosolvent polarity. We have also demonstrated that ion chromatography is an effective tool for investigating drug salt types in microspheres.

References

Al-Maaieh, A., Flanagan, D.R., 2001. Salt and cosolvent effects on ionic drug loading into microspheres using an O/W method. J. Control. Release 70, 169–181.

Benjamin, I., 1996. Chemical reactions and solvation at liquid interfaces: a microscopic perspective. Chem. Rev. 96, 1449–1475.

Berny, F., Shurhammer, R., et al., 2000. Distribution of hydrophilic, amphiphilic and hydrophobic ions at a liquid/liquid interface: a molecular dynamics investigation. Inorg. Chim. Acta 300, 384–394.

Ghanem, A.H., Higuchi, W.I., Simonelli, A.P., 1970. Interfacial barriers in interphase transport II: influence of additives upon the transport of diethylphthalate across the hexadecane–gelatin–water interface. J. Pharm. Sci. 59, 232–237.

Schill, G., Modin, R., Persson, B., 1965. Extraction of amines as complexes with inorganic anions. Acta Pharm. Suecica 2, 119–136.

Schweighofer, K., Benjamin, I., 1995. Transfer of small ions across the water/1,2-dichloroethane interface. J. Phys. Chem. 99, 9974–9985.