Drug Development and Industrial Pharmacy, 31:667–675, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500216394

Taylor & Francis
Taylor & Francis Group

Alginate-Based In Situ Gelling Suspensions and Emulsions Comprising N^4-Alkyloxycarbonyl Derivatives of Cytosine: Zero-Order Release and Effect of Physicochemical Properties

Karsten Petersson and Claus Larsen
Department of Pharmaceutics, The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark

ABSTRACT As an alternative to incorporation of various excipients, N^4-alkyloxycarbonyl-cytosine derivatives possessing various physicochemical properties and cytosine regeneration rates have been examined to modify release rate and kinetics from in situ gelling alginate formulations, e.g., liquid formulations that gel in acidic gastric juice and release the entrapped derivative or parent cytosine. Linear relationships were obtained between the release rate constants and the square root of the solubility for suspension formulations. Calculated diffusion coefficients were observed to be similar for suspension and solution formulations; however, for in situ gelling emulsion formulations, diffusivity correlated linearly to log P. Zero-order release of parent cytosine was observed from in situ gelling suspensions of the poorly soluble acid-labile N^4-adamantyloxycarbonyl-cytosine prodrug.

KEYWORDS In situ gelation, Prodrug, Sodium alginate, Cytosine derivatives, *Helicobacter pylori*, Oral drug delivery, Effect of physicochemical properties, Zero-order release kinetic

INTRODUCTION

Helicobacter pylori, a spiral bacterium colonizing the gastric mucus, is recognized to be a major cause of gastric ulcer (Hopkins et al., 1996). To eradicate *H. pylori*, conventional antibiotics are generally used. However, the treatment often fails and recurrent infections are common. Antibiotic resistance is an emerging issue (Perri et al., 2003). In light of the fact that pathogenic bacteria may develop resistance towards conventional antibiotics, nucleobase-containing compounds such as cytosine nucleosides (Isono, 1991) and peptide nucleic acids (Good & Nielsen, 1998) might constitute a potential

Address correspondence to Karsten Petersson, Ph.D. Pharm., Pharmaceutical Development, LEO Pharma, 55 Industriparken, DK-2750 Ballerup, Denmark; Fax: +45-7226-3662; E-mail: karsten.petersson@leo-pharma.com

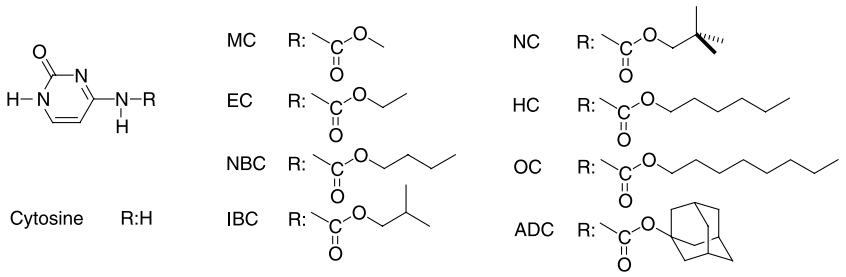

FIGURE 1 **Chemical Structures of Cytosine and the N^4-Alkyloxycarbonyl-Cytosine Derivatives.**

therapeutic alternative. The antibiotics used to eradicate *H. pylori* are in general administered in the form of conventional tablets or capsules. Thus, drug concentration at the target site is mainly dependent on drug transport/diffusion from the blood to the gastric lumen (exsorption). In contrast, transport of an antibiotic from the lumen into the mucus through the mucus layer has been suggested to be more effective in treatment of *H. pylori* infections (Kimura et al., 1995). By employing a drug delivery system exhibiting prolonged gastric retention and drug release, the therapeutic effect of antibiotics might be improved. The ability of alginate formulations to exhibit prolonged gastric residence time (potentially due to mucoadhesive properties) and subsequently sustained drug release in the stomach is well documented (Kubo et al., 2003; Miyazaki et al., 2000, 2001; Murata et al., 2000; Whitehead et al., 1998) with gastric residence times up to 6 hours (Whitehead et al., 1998). Furthermore, gastric retention of alginate formulations might be enhanced by incorporation of olive oil or gas-producing agents such as $CaCO_3$ and $NaHCO_3$ based on buoyant properties of such systems (Choi et al., 2002; Murata et al., 2000).

Sodium alginate is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues linked by 1,4-glycosidic linkages (Kubo et al., 2003; Miyazaki et al., 2000, 2001). The M and G monomers have pK_a values of 3.38 and 3.65, respectively (supplier's information). Sodium alginate is soluble in water but insoluble in acid (Zatz & Woodford, 1987). Relatively low concentrated solutions of the polymer form hydrogel matrices in acidic solutions and in the presence of bi- or polyvalent cationic crosslinkers (Ca^{2+}, poly-L-lysine) (Gonzalez-Ferreiro et al., 2002; Kubo et al., 2003; Miyazaki et al., 2000, 2001). Alginates have constituted the main matrix component in many experimental systems. However, only relatively few studies have dealt with the potential utility of alginates to form in situ gelling systems, e.g., liquid formulations that form gels in contact with an aqueous acidic environment. To this end, 1% (w/v) sodium alginate solutions have been shown to form gels in contact with acidic gastric juice (Zatz & Woodford, 1987) accompanied by an internal source of Ca^{2+} ions (Kubo et al., 2003; Miyazaki et al., 2000, 2001), in neutral gastric juice containing preadministered Ca^{2+} ions (Katayama et al., 1999) and finally in lachrymal fluid that contains Ca^{2+} ions (Cohen et al., 1997). Drug release from gel-forming sodium alginate liquid formulations has been investigated in case of ampicillin (Katayama et al., 1999), cimetidine (Miyazaki et al., 2001), paracetamol (Kubo et al., 2003), pilocarpine (Cohen et al., 1997), and theophylline (Miyazaki et al., 2000; Zatz & Woodford, 1987).

The nucleobase cytosine is, per se, devoid of pharmacological activity. However, in the present study it has been used as a model compound for antibiotics containing cytosine substructures. As an alternative to alterations in drug release from alginate formulations accomplished by admixing of various excipients, the aim of the present study was to modify drug release rates and kinetics from liquid in situ gelling formulations based on sodium alginate by employing recently synthesized cytosine derivatives (Petersson et al., 2004) (Fig. 1) exhibiting various physicochemical properties and cytosine regeneration rates.

MATERIALS AND METHODS

Materials

Cytosine and *N*,*N*,-dimethylacetamide (DMA) were purchased from Bie & Berntsen, Copenhagen, Denmark. N^4-methyloxycarbonyl-cytosine (MC), N^4-ethyloxycarbonyl-cytosine (EC), N^4-n-butyloxycarbonyl-cytosine (NBC), N^4-isobutyloxycarbonyl-cytosine (IBC),

TABLE 1 In Situ Gelling Sodium Alginate Formulations Prepared

Formulation		Amount of test compound per volume of formulation tested	Concentration of test compound (μmol/mL)	Compounds loaded
Suspensions: 1% Sodium alginate containing the test compounds in suspension	I	36 μmol (8–10 mg) per 3.6 mL	10	IBC[a], NC[a], HC and OC
	II	36 μmol (10 mg) per 36 mL	1.0	ADC[b]
Emulsions: 30% Castor oil in 1% sodium alginate, containing the test compounds in solution	III	2.5 μmol (0.4–0.6 mg) per 36 mL	0.07	MC, EC, IBC, NC, and cytosine
Solutions: 1% Sodium alginate containing the test compounds in solution	IV	3.6 μmol (0.6–0.8 mg) per 36 mL	0.10	MC[c], EC, NBC, IBC and NC
	V	36 μmol (4 mg) per 36 mL	1.0	Cytosine

[a]Also, suspension formulations of IBC and NC with various initial amount loaded [36–144 μmol (8–32 mg) per 3.6 mL] were prepared.
[b]Also, suspensions of ADC [36 μmol (10 mg) per 36 mL] without sodium alginate were prepared.
[c]Also, solution formulations of MC with various initial concentration [3.6–36 μmol (0.6–6 mg) per 36 mL], content of DMA (0–20%) and sodium alginate (0.5–1.5%) were prepared.

N^4-neopentyloxycarbonyl-cytosine (NC), N^4-hexyloxycarbonyl-cytosine (HC), N^4-octyloxycarbonyl-cytosine (OC), and N^4-adamantyloxycarbonyl-cytosine (ADC) were synthesized as previously reported (Petersson et al., 2004). Sodium alginate (Protanal LF200DL) was a gift from FMC BioPolymer, Drammen, Norway. Castor oil was obtained from Sigma-Aldrich, Copenhagen, Denmark. All other reagents were of analytical grade. Demineralized water was used throughout the study.

Preparation of Liquid In Situ Gelling Formulations

Suspensions

To 3.0 mL of a relatively concentrated solution of the test compound in DMA (50 mM) 150 mg sodium alginate was added while stirring with a magnetic stir bar. Twelve mL of water was added, and the dissolution of sodium alginate was accompanied by precipitation of the test compound. Sodium alginate dissolved readily since DMA worked as a wetting agent. A similar dissolution of sodium alginate upon addition of water was also observed in the absence of test compound. The precipitated test compound was uniformly distributed throughout the viscous formulation as assessed visually. Air formed when mixing water and DMA was allowed to separate from the formulation by standing. Formulations containing the test compounds in suspension consisted of 1% (w/v) aqueous sodium alginate solution containing 20% (v/v) DMA. The incorporated test compounds, amount loaded, and volumes of liquid formulations tested are given in Table 1 entries I and II. A suspension without sodium alginate was prepared by adding water to a DMA solution of ADC.

Emulsions

Sodium alginate (1.0 g) was suspended in 20 mL DMA solution of the respective test compound (0.50 mM). By addition of 80 mL water under stirring, viscous solutions were formed. Air formed when mixing was allowed to separate from the formulation by standing. Forty-two mL castor oil was added and viscous emulsions were formed after stirring overnight with a magnetic stir bar. Emulsions containing the compounds in solution consisted of 30% (v/v) castor oil and 70% (v/v) [1% (w/v) aqueous sodium alginate solution containing 20% (v/v) DMA]. The incorporated test compounds, amount loaded, and volumes of liquid formulations tested are given in Table 1, entry III.

Solutions

Sodium alginate (1.5 g) was suspended in 30 mL DMA solution of the respective test compound (0.50 mM). By addition of 120 mL water under stirring

with a magnetic stir bar, viscous solutions were formed. Air formed when mixing water and DMA was allowed to separate from the formulation by standing. Unless otherwise stated, the composition of the investigated formulations containing dissolved test compound was 1% (w/v) aqueous sodium alginate solution containing 20% (v/v) DMA. The incorporated test compounds, amount loaded, and volumes of liquid formulations tested are given in Table 1, entries IV and V. Also, 1% sodium alginate solutions without DMA were prepared by dissolving sodium alginate in water by heating to 60°C. After cooling to room temperature, an aqueous solution of MC was added to the polymer solution.

In Vitro Release

Release of the compounds from the gels was examined using the paddle method (Eur. Ph. 4th dissolution test apparatus) at 37°±0.5°C according to previous studies (Katayama et al., 1999) with modifications. Five-hundred mL of the release medium (0.1 M HCl, pH 1.1) was placed in the vessel and preheated to 37°±0.5°C. The liquid formulation was placed in a petri dish [36 mL in a dish (6.9 × 1.2 cm) or 3.6 mL in a dish (2.8 × 0.9 cm)]. A piece of woven gauze made from stainless steel wire (0.30 mm in diameter and having mesh apertures of 1.00 mm) was placed on top of the petri dish. The petri dish was then placed at the base of the dissolution vessel using three-legged stainless steel tweezers, and stirring of the dissolution medium was initiated (50 rpm). At appropriate time intervals samples were withdrawn for HPLC analysis and replaced with fresh release medium. In the cases of cytosine and ADC, the samples were added to an equal volume of 1 M phosphate buffer, pH 7.4. The cumulated amount of drug released, Q, (corrected for sampling) into the aqueous phase was calculated according to Eq. 1:

$$Q = V_s \sum_{n=1}^{n} C_{n-1} + V_m C_n \quad (1)$$

where V_s and V_m are the volumes of sample taken and release medium, respectively, and C_n is the drug concentration in the sample n. Release experiments were performed in triplicate.

HPLC Analysis

The HPLC system consisted of a Merck Hitachi L-7100 pump, a Merck Hitachi L-7200 auto sampler, and a Merck Hitachi L-7480 fluorescence detector operating at Ex 288 nm/Em 350 nm when detecting the derivatives and a Merck Hitachi L-7400 UV-detector operating at 266 nm for detection of cytosine. Reversed-phase chromatography was carried out using a Phenomenex Aqua C_{18} column (150 × 4.60 mm i.d.; 5 μm particles) equipped with a C_{18} precolumn (4 × 3.0 mm i.d.) (Supware, Copenhagen, Denmark). The flow

TABLE 2 Diffusion Coefficients and Log $P_{octanol\text{-}water}$ and Solubility (C_s) Data for Cytosine and the N^4-Alkyloxycarbonyl-Cytosine Derivatives at 37±0.5°C (Experiments Performed in Triplicate)

	× $10^3 D$ (cm^2 min^{-1}) (S.E.M.)					
Compound	Suspensions[a]	Emulsion[b]	Solutions[c]	log $P_{pH\ 7.4}$[d]	$C_{s,pH1.1}$ (μM)[d]	$C_{s,pH7.4}$ (μM)[d]
Cytosine	n.d.	3.69 (0.38)	1.69 (0.04)	−1.5	n.d.	102624
MC	n.d.	0.99 (0.17)	1.18 (0.11)[e]	−0.55	n.d.	1843
EC	n.d.	0.71 (0.08)	1.09 (0.13)	−0.085	2377	199
NBC	n.d.	n.d.	1.23 (0.14)	1.1	983	147
IBC	1.07 (0.01)[f]	0.46 (0.05)	1.21 (0.02)	1.1	1670	235
NC	1.49 (0.10)[f]	0.30 (0.03)	1.29 (0.17)	1.5	886	122
HC	n.c.	n.d.	n.d.	2.3	34	4
OC	n.c.	n.d.	n.d.	3.3	0.7	0.3
ADC	n.d.	n.d.	n.d.	2.9	n.d.	19

[a]Formulation 1% Sodium alginate containing the test compounds in suspension (Table 1, entries I and II).
[b]Formulation 30% castor oil in 1% sodium alginate, containing the test compounds in solution (Table 1, entry III).
[c]Formulation 1% sodium alginate containing the test compounds in solution (Table 1, entries IV and V).
[d]From Petersson et al., 2004.
[e]Formulation not significantly affected by variations of the initial concentration (3.6–36 μmol in 36 mL gel matrix), content of DMA (0–20%) or sodium alginate (0.5–1.5%).
[f]Formulation not significantly affected by variation of the initial amount loaded (36–144 μmol in 3.6 mL gel matrix). n.c. not calculated due to the relatively small amounts released. n.d. not determined.

rate was set at 1 mL/min^{-1}. Mobile phase systems of 10–80% (v/v) methanol in 0.1% (v/v) phosphoric acid were used with the methanol content adjusted for each compound to provide retention times of 3–8 min. In the case of cytosine, 0.02 M phosphate buffer, pH 7.4, was used as the mobile phase.

RESULTS

Preliminary Characteristics of Formulations

In preparation of the formulations, DMA was used as a solubilizer for the poorly soluble derivatives. Interestingly, this cosolvent might also act as a wetting agent due to the observation that sodium alginate suspended in DMA dissolved immediately by addition of water in contrast to the slow dissolution process taking place in pure water. In the preparation of the suspension formulations, the test compounds were initially dissolved in DMA. Upon addition of water, sodium alginate dissolved parallel to precipitation of the test compounds. The viscous mixtures formed provided physical stabilization of the uniform suspensions with no sedimentation observed upon storage for 2 weeks at room temperature. Similarly, the emulsions were to some extent stabilized by sodium alginate, though creaming was observed after 24 hours at room temperature. Both systems were easily reconstituted by shaking. Transferring the three formulation types to the acidic release medium resulted in alginic acid gel structures. In this study the release experiments were carried out on freshly prepared formulations without further physical characterization of the formed preparations.

Effect of Solubility on Release

Previous studies of drug release from alginate-based matrix systems indicate that the drug release is governed by drug diffusion in the hydrated polymer matrix (Kubo et al., 2003; Miyazaki et al., 2001). The mechanism indicates that drug release may be influenced by physicochemical properties of the drug molecules. In order to investigate a potential relationship between drug solubility and drug release rate, four cytosine derivatives previously shown to cover a relatively broad range of solubilities (Table 2; 0.7 to 1670 μM, pH 1.1; 37°C) were selected. Figure 2a shows the release profiles of IBC, NC, HC, and OC from in situ gelling sodium alginate formulations containing the test compounds in suspension (Table 1, entry I). It appears that the time-dependent cumulative amount of substance released decreases with increasing chain length of the alkyl substituent. The fastest release was observed for gels loaded with IBC followed by NC, with 18 and 15 μmol released after 360 minutes, respectively. In contrast, HC and OC release was relatively slow with less than 5 and 1 μmol released, respectively, in this period of time. During the release experiments less than 4% of the various

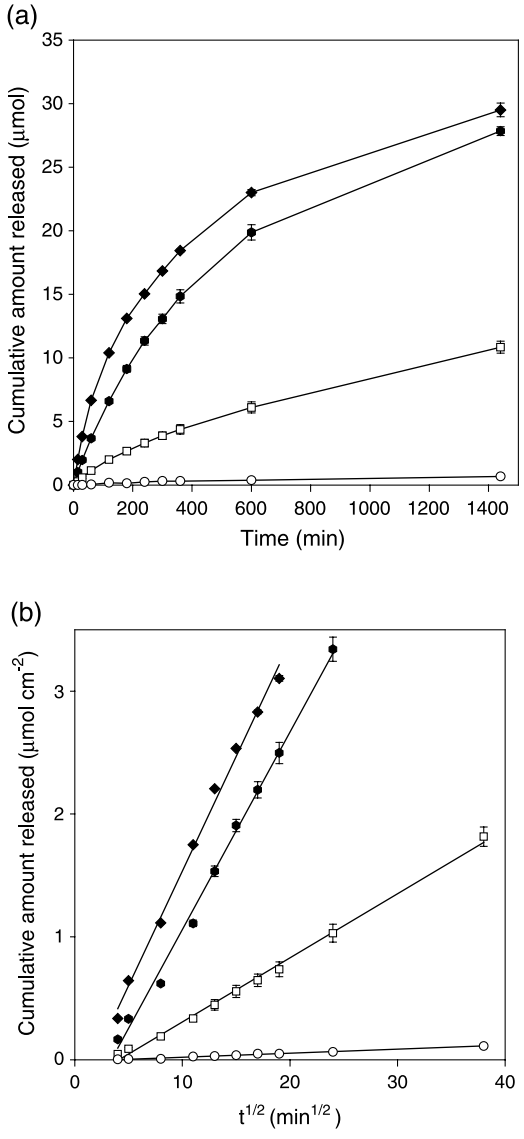

FIGURE 2 **Release of IBC (◆), NC (⬢), HC (□), and OC (○) from In Situ Gelling Suspensions Plotted as (a) Cumulative Release as a Function of Time and (b) Cumulative Release Per Unit Area as a Function of Square Root Time. Each Value is the Mean ± S.E.M.**

derivatives were hydrolyzed (Petersson et al., 2004), suggesting that calculations may be done from the present release data. The curvature of cumulative drug release profiles suggests that the instantaneous drug release rate decreases with time for all the formulations. For drugs dispersed in a semi-solid polymeric matrix, the Higuchi model for ointment bases containing drugs in suspension (Higuchi, 1961; Peppas, 1985) might be applied to describe drug release mechanism. Accordingly, the cumulative amount Q of drug released per unit area from gels is proportional to the square root of time t:

$$Q = \sqrt{2ADC_{s,gel}t} \qquad (2)$$

where D is the diffusion coefficient of the drug molecule, A is the initial amount of drug, and $C_{s,gel}$ is the drug solubility in the vehicle. Equation 2 is derived for systems where the amount of drug present per unit volume is substantially greater than $C_{s,gel}$ (Higuchi, 1961). Thus, the amount of test compound loaded into the gels was at least five times the saturation solubility at pH 1.1 (37°C). The observed linear relationships between Q and $t^{1/2}$ for the matrices (Fig. 2b) demonstrate the adequacy of using the Higuchi model and that (within the time period investigated) the release of the derivatives from the in situ gelled suspensions is consistent with a diffusion mechanism (Peppas, 1985). The results also indicate that the gels do not swell or disintegrate during the experiments (Peppas, 1985). Assuming identical solubilities of the respective compounds in the gel vehicle formed ($C_{s,gel}$) and in the release medium ($C_{s,pH1.1}$), the diffusion coefficients D can be calculated from the slopes of the linear plots presented in Fig. 2b (Table 2). By employment of different initial amounts of IBC and NC (Table 1, entry I), it was observed that the initial drug load had no significant effect on the diffusion coefficient and that the effect of the polymer on compound solubilities was negligible (data not shown). Likewise, 70% sorbitol has been reported to have virtually no effect on the solubility of benzocaine, phenytoin, and diazepam (Rubino & Yalkowsky, 1987). The diffusion coefficients are in favorable agreement with those obtained for in situ gelling sodium alginate formulations containing the test compounds in solutions (Table 1, entry IV) (Table 2 calculated from Eq. 3, see later) and consistent with previous reports on solution-based calcium-alginate systems (Kubo et al., 2003; Miyazaki et al., 2001). The consistency between the systems implies that the dispersed substance [0.25% (w/v)] provides no significant physical hindrance to diffusion of drug molecules.

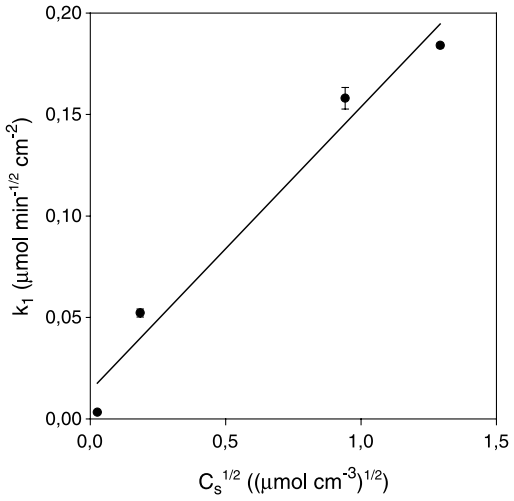

FIGURE 3 **The Correlation Between the Release Rate Constants, k_1, for the Release from In Situ Gelling Suspension and the Square Root of the Solubility, $C_{s,pH1.1}^{1/2}$ of IBC, NC, HC, and OC. Each Value is the Mean ± S.E.M. ($R^2 = 0.97$).**

For drug release from matrix systems comprising solid drug, the solubility of drug in the hydrated polymeric environment constitutes an important factor governing drug release rates (Higuchi, 1961; O'Connor & Schwartz, 1993), since the concentration gradient between the gel and the bulk solution is the driving force for the diffusion process. This phenomenon was observed in the present study and is consistent with observations previously reported for systems based on microcrystalline cellulose (O'Connor & Schwartz, 1993). Figure 3 shows the relationship between the Higuchi release rate constants (k_1), calculated from the slopes of the straight line relationships depicted in Fig. 2b and the square root of the solubility, $C_{s,pH1.1}^{1/2}$ (pH 1.1; 37°C) (Table 2). The obtained reasonably linear relationship demonstrates that the release rates of the derivatives correlate well with solubility and thus the data are consistent with Eq. 2. This empirical relationship may be useful for estimation of compound release rates for similar in situ gelling suspensions based solely on knowledge of the solubility of particular derivative.

Effect of Lipophilicity on Release

Previous studies have shown that alginate-based matrix systems with incorporated vegetable oils may

possess buoyant properties in the gastric fluid, with both buoyancy and drug release rates being dependent on the amount of oil added (Murata et al., 2000). However, for a certain drug molecule the desirable buoyancy of the drug formulation might not necessarily afford an appropriate drug release rate. In order to investigate the relationship between drug lipophilicity and drug release rate, four cytosine derivatives possessing different lipophilicities (Table 2; log P range −0.55 to 1.5) were investigated. In Fig. 4a the release profiles of MC, EC, IBC, and NC from in situ gelling sodium alginate castor oil emulsions containing the dissolved derivative (Table 1, entry III) are shown. An apparent decline in rate of drug release with increasing lipophilicity of the test compound was found. For example, after 360 minutes, approximately 1.6, 1.4, 1.1, and 0.98 μmol derivative were released from the matrices loaded with MC, EC, IBC, and NC, respectively. The results are consistent with results obtained for systems based on silicon oil (Steffansen et al., 1996) and demonstrate that manipulation of lipophilicity can be used to modify drug release rates. The concave release profiles obtained indicate that drug release rate decrease with time. Accordingly, the release data might be analyzed according to the Higuchi procedure valid for drug release from semisolid vehicles containing drugs in solutions (Higuchi, 1962). Thus, the correlation between the cumulative amount Q of drug released per unit surface area [up to 60% release (Peppas, 1985)] and time t might be given by:

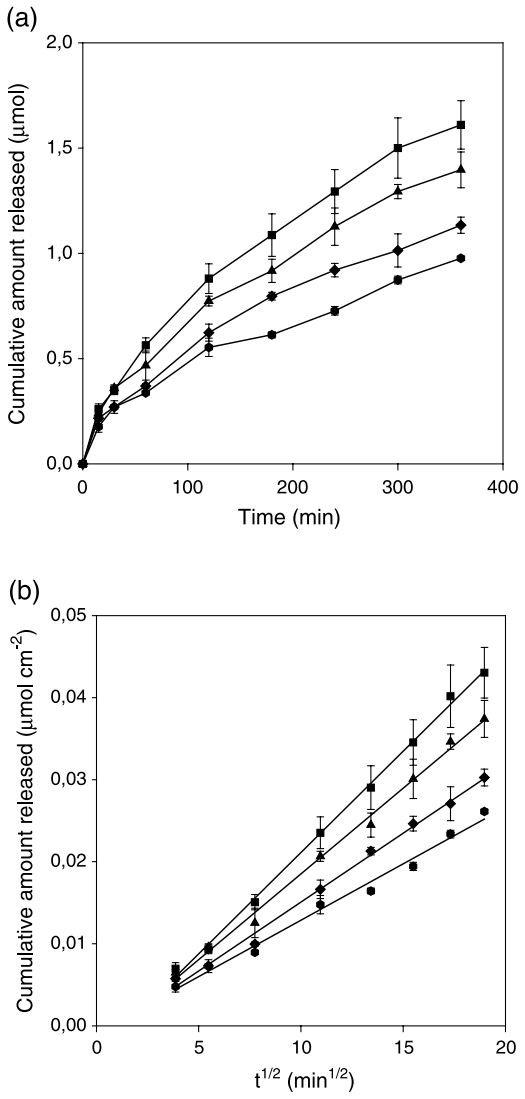

FIGURE 4 **Release of MC (■), EC (▲), IBC (◆), and NC (⬢) from In Situ Gelling Emulsions Plotted as (a) Cumulative Release as a Function of Time and (b) Cumulative Release Per Unit Area as a Function of Square Root Time. Each Value is the Mean ± S.E.M.**

$$Q = 2C_0\sqrt{\frac{Dt}{\pi}} \tag{3}$$

where D and C_0 are the diffusion coefficient of the drug molecule and the initial drug concentration in the vehicle, respectively. Linear relationships between Q and $t^{1/2}$ were observed for all the formulations tested (Fig. 4b), demonstrating that the release of MC, EC, IBC, and NC from the in situ formed gels fits well to the Higuchi model consistent with diffusion controlled drug release (Kubo et al., 2003; Miyazaki et al., 2001; Peppas, 1985). Diffusion coefficients calculated from the slopes of the line segment are presented in Table 2. The effect of prodrug lipophilicity on release rate from lipid drug formulations, in general, has been reported (Larsen et al., 2001; Sloan & Wasdo, 2003; Steffansen et al., 1996). In the present study, derivatization has been shown to affect drug affinity to the delivery system as well as diffusion rate within the formulation. According to Table 2, the diffusion coefficient for NC is lowered three-fold as compared to that of MC. In contrast, fairly identical diffusion coefficients were found in the cases of MC, EC, NBC, IBC, and NC for in situ gelling sodium alginate formulations containing the test compounds in solutions (Table 1, entry IV) (Table 2).

A reasonably linear relationship was obtained by plotting the diffusion coefficients against the corresponding log $P_{(octanol\text{-}water)}$ values (Fig. 5), indicating that diffusion coefficients are inversely related to lipophilicity expressed by the partition coefficient. The employment of log $P_{octanol\text{-}water}$ values (Table 2)

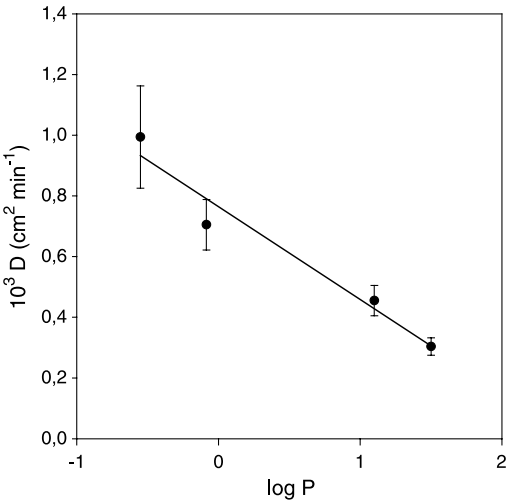

FIGURE 5 **The Relationship Between the Diffusion Coefficients Obtained from In Situ Gelled Emulsions and Log P of MC, EC, IBC, and NC. Each Value is the Mean ± S.E.M. ($R^2 = 0.96$).**

appears acceptable since linear free energy relationships for partition coefficients obtained in octanol-water and oil-water systems, respectively, have been established (Collander, 1951; Hansch & Leo, 1979; Larsen et al., 2002). Thus, the relationship between derivative diffusion coefficients and log P values may be used to adjust compound release rates from in situ gelling emulsions exhibiting appropriate buoyancy.

Zero-Order Release of Parent Cytosine

Antibacterial effect of compounds containing cytosine substructures [such as antisense molecules (Good & Nielsen, 1998)] may, by virtue of binding to the ribosome, be based on bacteriostatic mechanisms (Brock et al., 1994). Thus, employment of a zero-order drug release formulation may result in maintenance of a static effect on bacteria cell growth (Colombo et al., 2000). Preliminary studies have indicated prolonged cytosine release from in situ formed alginic acid gels loaded with ADC compared to gels loaded with equimolar amounts of parent cytosine (Petersson et al., 2004). Figure 6 shows the release profiles of ADC and cytosine from in situ gelling suspensions containing the acid-labile, poorly soluble ADC prodrug (Table 1, entry II). It is seen that parent cytosine is the main compound released while only minute amounts of ADC appeared in the release medium, although parallel appearance of ADC and cytosine in the release medium are observed for the first 30 minutes. The minute amounts of ADC present in the release medium indicate that ADC conversion to cytosine predominantly takes place inside the gel. Hence, the overall cytosine release profile may most likely be the result of consecutive reactions involving dissolution of ADC within the gels, followed by hydrolysis of ADC to parent cytosine and finally release of cytosine from the gel. The R^2 value of the linear regression line, drawn through the data points for cytosine release, was greater than 0.99 and the points were randomly distributed for all the formulations studied. Thus, the correlation between the cumulative amount Q of cytosine released per unit surface area from ADC-loaded gels and time t is given by:

$$Q = k_0 t \tag{4}$$

where the zero-order release rate constant k_0 (1.25×10^{-9} mol cm^{-2} min^{-1}) is obtained from the slope of the regression line (Peppas, 1985) (per unit area of the gel) (Fig. 6). Since ADC exhibits enhanced lability in acidic solutions with a half-life of 41 minutes at pH 1.1 and 37°C (Petersson et al., 2004), the prolonged release of cytosine from ADC-loaded gels may be ascribed to the low solubility of the ADC prodrug (Table 2), creating a small concentration gradient (gel to bulk solution). In comparison, relatively fast release was observed from both in situ gelling solutions containing equimolar amounts of parent cytosine as well as from equimolar suspensions

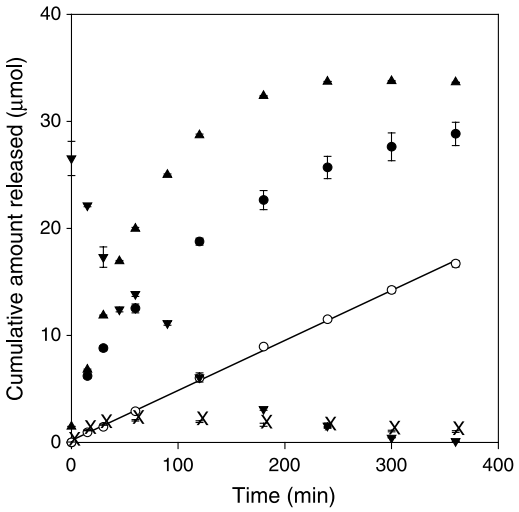

FIGURE 6 **Release of ADC (X) and Cytosine Formed by Hydrolysis of ADC (○) from ADC Prodrug Loaded In Situ Gelling Suspensions. For Comparison Release Data from an In Situ Gelling Solution of Cytosine (●) and the Release of ADC (▼) and Cytosine (▲) from a Suspension of ADC Prodrug (Without Sodium Alginate) are Included. Each Value is the Mean ± S.E.M.**

of ADC in the absence of sodium alginate (Table 1, entries II and V) (Fig. 6). These results illustrate the principle of combined formulations based on prodrugs and in situ gelling systems exhibiting zero-order drug-compound release.

CONCLUSION

By combination of relatively stable cytosine derivatives and in situ gelling sodium alginate formulations, release rates were correlated to compound solubility in the case of in situ gelling suspensions, and diffusion coefficients were correlated to compound lipophilicity in the case of in situ gelling emulsions. Release of parent cytosine fitted zero-order release kinetics when the acid-labile and poorly soluble ADC prodrug was formulated as in situ gelling suspension formulations.

ABBREVIATIONS

MC	N^4-methyloxycarbonyl-cytosine
EC	N^4-ethyloxycarbonyl-cytosine
NBC	N^4-n-butyloxycarbonyl-cytosine
IBC	N^4-isobutyloxycarbonyl-cytosine
NC	N^4-neopentyloxycarbonyl-cytosine
HC	N^4-hexyloxycarbonyl-cytosine
OC	N^4-octyloxycarbonyl-cytosine
ADC	N^4-adamantyloxycarbonyl-cytosine
DMA	*N*,*N*,-dimethylacetamide

REFERENCES

Brock, T. D., Madigan, M. T., Martinko, J. M., & Parker, J. (1994). *Biology of Microorganisms*. New Jersey: Prentice-Hall, Inc., 349–356.

Choi, B. Y., Park, H. J., Hwang, S. J., & Park, J. B. (2002). Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents. *International Journal of Pharmaceutics*, *239*, 81–91.

Cohen, S., Lobel, E., Trevgoda, A., & Peled, Y. (1997). Novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *Journal of Controlled Release*, *44*, 201–208.

Collander, R. (1951). The partition of organic compounds between higher alcohols and water. *Acta Chemica Scandinavica*, *4*, 774–780.

Colombo, P., Santi, P., Bettini, R., Brazal, C. S., & Peppas, N. A. (2000). Wise, D. L. (Ed.). *Handbook of Pharmaceutical Controlled Release Technology*. New York: Marcel Dekker Inc., 183–209.

Gonzalez-Ferreiro, M., Tillman, L. G., Hardee, G., & Bodmeier, R. (2002). Alginate/poly-L-lysine microparticles for the intestinal delivery of antisense oligonucleotides. *Pharmaceutical Research*, *19*, 755–764.

Good, L., & Nielsen, P. E. (1998). Inhibition of translation and bacterial growth by peptide nucleic acid targeted to ribosomal RNA. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 2073–2076.

Hansch, C., & Leo, A. (1979). *Substituent Constants for Correlation Analysis in Chemistry and Biology*. New York: Wiley.

Higuchi, T. (1961). Rate release of medicaments from ointment bases containing drugs in suspension. *Journal of Pharmaceutical Sciences*, *50*, 874–875.

Higuchi, W. I. (1962). Analysis of data on the medicament release from ointments. *Journal of Pharmaceutical Sciences*, *51*, 802–804.

Hopkins, R. J., Girardi, L. S., & Turney, E. A. (1996). Relationship between *Helicobacter pylori* eradication and reduced dudenal and gastric ulcer recurrence: a review. *Gastroenterology*, *110*, 1244–1252.

Isono, K. (1991). Current progress on nucleoside antibiotics. *Pharmacology & Therapeutics*, *52*, 269–286.

Katayama, H., Nishimura, T., Ochi, S., Tsuruta, Y., Yamazaki, Y., Shibata, K., & Yoshitomi, H. (1999). Sustained release liquid preparation using sodium alginate for eradication of *Helicobacter pylori*. *Biological & Pharmaceutical Bulletin*, *22*, 55–60.

Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., & Yoshida, Y. (1995). A 1-h topical therapy for the treatment of *Helicobacter pylori* infection. *American Journal of Gastroenterology*, *90*, 60–63.

Kubo, W., Miyazaki, S., & Attwood, D. (2003). Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. *International Journal of Pharmaceutics*, *258*, 55–64.

Larsen, S. W., Thomsen, A. E., Rinvar, E., Friis, G. J., & Larsen, C. (2001). Effect of drug lipophilicity on in vitro release rate from oil vehicles using nicotinic acid esters as model prodrug derivatives. *International Journal of Pharmaceutics*, *216*, 83–93.

Larsen, D. B., Parshad, H., Fredholt, K., & Larsen, C. (2002). Characteristics of drug substances in oily solutions. Drug release rate, partitioning and solubility. *International Journal of Pharmaceutics*, *232*, 107–117.

Miyazaki, S., Kubo, W., & Attwood, D. (2000). Oral sustained delivery of theophylline using in-situ gelation of sodium alginate. *Journal of Controlled Release*, *67*, 275–280.

Miyazaki, S., Kawasaki, N., Kubo, W., Endo, K., & Attwood, D. (2001). Comparison of in situ gelling formulations for the oral delivery of cimetidine. *International Journal of Pharmaceutics*, *220*, 161–168.

Murata, Y., Sasaki, N., Miyamoto, E., & Kawashima, S. (2000). Use of floating alginate gel beads for stomach specific drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *50*, 221–226.

O'Connor, R. E., & Schwartz, J. B. (1993). Drug release mechanism from a microcrystalline cellulose pellet system. *Pharmaceutical Research*, *10*, 356–361.

Peppas, N. A. (1985). Analysis of Fickian and non-Fickian drug release from polymers. *Pharmaceutica Acta Helvetiae*, *60*, 110–111.

Perri, F., Qasim, A., Marras, L., & O'Morain, C. (2003). Treatment of *Helicobacter pylori* infection. *Helicobacter*, *8*(Suppl. 1), 53–60.

Petersson, K., Pedersen, B. T., Staerk, D., Krogfelt, K. A., & Larsen, C. (2004). N^4-alkyloxycarbonyl derivatives of cytosine: physicochemical characterisation, and cytosine regeneration rates and release from alginic acid gels. *European Journal of Pharmaceutical Sciences*, *23*, 337–345.

Rubino, J. T., & Yalkowsky, S. H. (1987). Cosolvency and cosolvent polarity. *Pharmaceutical Research*, *4*, 220–230.

Sloan, K. B., & Wasdo, S. (2003). Designing for topical delivery: prodrugs can make the difference. *Medicinal Research Reviews*, *23*, 763–793.

Steffansen, B., Ashton, P., & Buur, A. (1996). Intraocular drug delivery. In vitro release studies of 5-fluorouracil from N^1-alkoxycarbonyl prodrugs in silicone oil. *International Journal of Pharmaceutics*, *132*, 243–250.

Whitehead, L., Fell, J. T., Collett, J. H., Sharma, H. L., & Smith, A. (1998). Floating dosage forms: an in vivo study demonstrating prolonged gastric retention. *Journal of Controlled Release*, *55*, 3–12.

Zatz, J. L., & Woodford, D. W. (1987). Prolonged release of theophylline from aqueous suspensions. *Drug Development and Industrial Pharmacy*, *13*, 2159–2178.