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In-vitro controlled release of doxorubicin from silica xerogels

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Abstract

This study aimed at the development of a novel silica xerogel matrix as a delivery tool for an anti-cancer drug. Doxorubicin was incorporated as a hydrochloride salt during hydrolysis and polycondensation of tetraethylorthosilicate (TEOS) in the sol-gel process. The effect of sol-gel synthesis parameters (drug concentration, size of the device and lyophilizing process) on the release rate of the drug were investigated. In addition, dissolution rate, as well as weight loss of silica xerogel, was evaluated. In general, both the lyophilizing process of xerogels and the increase in size of non-lyophilizing device significantly decrease both the rate of drug release and the rate of dissolution of matrix. The overall release process was found to be governed by diffusion control and simultaneous zero-order dissolution of the xerogel.

Introduction

Doxorubicin is one of the most potent anti-tumour agents used generally in the treatment of bone cancer (Itokazu et al 1996; Minko et al 2000; Fan & Dash 2001). At present, the treatment is systematic and, due to the narrow therapeutic index of doxorubicin, a substantial increase in systematic dose to achieve high concentration of the drug at the bone sarcomas is not possible. Additionally, the bone is a moderately perfused organ and the chance of achieving an effective therapeutic efficacy of doxorubicin is likely to be low (Fan & Dash 2001). Another problem associated with systematic treatment is also the systemic toxicity, especially cardiotoxicity and immunosuppression (Minko et al 2000). To reduce the toxicity of doxorubicin and improve delivery to the tumour site, various targeted drug delivery systems, such as liposomes (Li et al 1998), nanoparticles (Yoo et al 2000), microspheres (Stolnik et al 1995), conjugates and polymeric micelles (Fan & Dash 2001, Greish et al 2004), have been used. However, these delivery systems are usually administered intravenously and are not adequate for the treatment of bone cancer.

The addition of anti-cancer drugs to implantable delivery matrix is a promising strategy for modifying their biodistribution, reducing drug toxicity and thus improving the therapeutic efficacy of anti-tumour agents. Recently, hydroxyapatite implants (Itokazu et al 1996) and gelatin cross-linked with glutaraldehyde (Fan & Dash 2001) containing doxorubicin have been investigated as potential, implantable delivery systems for the treatment of bone cancer. Actually, it seems to be a very attractive idea to look for materials that could release an anti-tumour agent, such as doxorubicin, in a local and controlled way while showing bioactive properties (e.g. to avoid bone resorption). The sol-gel derived silica materials may be interesting as multifunctional biomaterials. They are biodegradable, biocompatible and also bioactive (Kortesuo et al 2000; Radin et al 2002, 2005). The manufacture of amorphous silica materials occurs by hydrolysis and condensation reactions of precursors, such as tetraethylorthosilicate, at low temperature (Livage 1997). Silica xerogel prepared by the sol-gel method is a porous material that contains interconnected bottle-neck-like pores formed by a three-dimensional SiO_2 network. The bioactivity of silica materials (e.g. silica functions like the tissue in which they are implanted) has been studied extensively by Kortescuo et al (2000) and Radin et al (2002, 2005). These authors have shown that the biodegradation of silica occurs through hydrolysis of the siloxane bonds and dissolved non-toxic silica acid affects osteoblast and fibroblast formation, resulting in increased collagen formation that in turn bonds implantable silica material to bone. In addition, the silica material causes no

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adverse tissue reactions and dissolved silica acid can be easily eliminated through the kidneys. The sol-gel method has also received much attention in implantable materials research due to its unique advantages, such as low-temperature processing, ultrahomogeneity, chemical and thermal stability of final products and their good compatibility with other materials (Livage 1997; Dunn et al 1998). There is a growing interest in the use of porous sol-gel derived silica materials in drug release applications both for conventional drug molecules (Ahola et al 2000; Kortesuo et al 2000) and for sensitive enzymes (Dunn et al 1998). The drug can be added to the mixture at some time during the formation of the colloidal silica particles (sol) or gel. After an aging and drying process, the drug-loaded silica gels are obtained.

Doxorubicin-loaded silica xerogels were obtained in the author's previous study (Prokopowicz et al 2005). The goal of these methods was a complete loading of doxorubicin without losses or leaching out and uniform distribution of the drug within silica xerogel networks.

Drug release is generally known to be diffusion-controlled (Ahola et al 2000). The main factors influencing the drug release behaviour are the size of pores, the size of material and physicochemical properties of the drug and the degree of loading. An important factor influencing the structure of silica xerogels is the pH of the solution. In the case of an acidic catalyst, rapidly proceeding hydrolysis causes the formation of linear polymers, resulting in a solid material with less porosity. In contrast, basic catalysis favours the formation of agglomerate consisting of branched colloidal particles, which yields a porous material with a high adsorption capacity (Livage 1997; Curran & Stiegman 1999).

In this paper, we report the preparation of a novel doxorubicin-loaded silica xerogel by the modified version of the two-step (acidic and basic catalysis) procedure of Ahola et al (2001). Freeze-drying, as well as vacuum-drying at 4°C, was selected as the drying technique of obtained matrices, because the encapsulated doxorubicin is thermosensitive and traditional drying at an accelerated temperature is impossible. Freeze-drying is a well-established method for the preservation of unstable molecules over long periods of time, as well as being a means of storage under sterile conditions, and this technique has not yet been applied in the drying of these systems. Therefore, this paper discusses the effect of freeze-drying, as well as the size of matrices and the amount of doxorubicin, on the rate of release of drug in-vitro. The materials containing an anti-cancer drug can find use (e.g. as implantable drug delivery devices that can provide targeted disease control) locally or as supplements in biodegradable materials, such as polylactide and polyglycolide, that are used as temporary implants in certain bone surgery (Kursawe et al 1998).

Materials and Methods

Materials

All reagents and the drug were obtained from Sigma Chemical Company and used without further purification: doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}HCl$, $M = 580.0\,g\ mol^{-1}$), tetraethylorthosilicate (TEOS, $M = 208.33\,g\ mol^{-1}$); ethanol 98%(v/v); hydrochloric acid 0.01 M and ammonia 0.01 M. The release medium was simulated body fluid solution (SBF, pH 7.4). SBF had the following composition (in mM): NaCl 136.8, $NaHCO_3$ 4.2, KCl 3.0, $K_2HPO_4{\cdot}H_2O$ 1.0, $MgCl_2{\cdot}6H_2O$ 1.5, $CaCl_2{\cdot}2H_2O$ 2.5 and Na_2SO_4 0.5. It was buffered at pH 7.4 with a tris[hydroxymethyl]aminomethane solution (50 mM) and concentrated hydrochloric acid.

Preparation of standard solutions of doxorubicin by a static (volumetric) method

One millilitre containing 2 ± 0.1 mg doxorubicin hydrochloride was dissolved in 25 mL of de-ionized water (pH 6.4) in a silanized polypropylene volumetric flask in the absence of light. Various standard solutions of doxorubicin (3.2–32 $\mu g\ mL^{-1}$) were then prepared from this stock solution after appropriate dilution. The solutions were prepared quickly, to limit the exposure to light, and carefully, due to the high toxicity of doxorubicin. The pH of standard solutions ranged from 6.4 to 6.6. The pH values of the doxorubicin solutions were determined with a pH meter (Teleko, pH-METR N5170E).

Spectrophotometric measurements

Quantitative determinations of doxorubicin were performed using a Hewlett Packard 8452A Diode-Array UV/VIS spectrophotometer connected to an IBM Pentium 100 computer. The analytical wavelength $\lambda = 480$ nm was selected for the determination of the drug.

Synthesis of doxorubicin-loaded SiO_2 xerogels

The overall reaction of synthesis of SiO_2 xerogels is as follows:

$$Si(OC_2H_5)_4 + 2H_2O \Rightarrow SiO_2 + 4C_2H_5OH \quad (1)$$

according to which, 1 mol of TEOS ($208.33\,g\ mol^{-1}$) yields 1 mol of pure silica ($60\,g\ mol^{-1}$). The doxorubicin-loaded SiO_2 xerogels were synthesized by a two-step sol-gel process at room temperature and under atmospheric pressure in a polypropylene flask and kept out of the light. TEOS (3.48 g) and ethanol (6.14 g) were slowly magnetically stirred for 15 min. Next, de-ionized water (1.8 g) with a catalyst—HCl (120 μL, 0.01 M, pH 3)—was added. The solutions were stirred for 6 h to obtain uniform sol. The sols were hydrolysed in a covered flask for one day before a base catalyst—ammonia (80 μL, 0.01 M, pH 10.5)—was added. The pH of homogeneous sol was raised to 5–5.5. The total molar ratio of TEOS–H_2O–C_2H_5OH–HCl–NH_4OH was held constant at $1{:}6{:}8{:}8\times10^{-5}{:}5\times10^{-5}$. After 4 h of the sol stirring, doxorubicin hydrochloride solution containing various amount of doxorubicin was added. The obtained sols with doxorubicin were cast into disc-shaped polypropylene moulds and allowed to stand undisturbed in a refrigerator at +4C° for polycondensation. After 48 h, the resulting gels were subjected to aging and drying in the vacuum chamber (100 Pa) under silica gel at +4°C for 10 days to obtain solids. The loading degree of doxorubicin was varied between 1.2 and 4 mg g^{-1}.

Some of the resulting disc-shaped solids were crushed in a ball mill to obtain granules and then sieved. Before studies, the granular forms of xerogels and discs were weighed and the dimensions were measured with a micrometer screw gauge.

The final xerogels were divided into two groups. The first batch (S) was used immediately in subsequent experiments. The second batch (LS) was placed in the drying chamber of an Alpha 1–2 LD Freeze-Dryer (Christ, Germany), and cooled to −55 °C. Lyophilization was performed at a pressure of 2 Pa for 48 h.

To evaluate whether the selected parameters of lyophilization were sufficient for drying, random samples (n = 10) with masses varying between 0.2 and 1 g were taken for analysis. The correlation between percent of water loss (g) per unit mass of xerogels and the mass taken for analysis was evaluated statistically using linear correlation method at $\alpha = 0.05$.

Textural characterization of SiO_2 xerogels

The bulk density of xerogels was determined using the buoyancy method with the Mohr balance (Poland, Mohr balance, Labart). A randomly selected piece of xerogel was weighed on the Mohr balance in air and after that in ethanol. The bulk density of the xerogel was calculated from the following formula:

$$\rho_1 = m_1 \times \rho_o/(m_1 - m_2) \quad (2)$$

where: ρ_1 is the bulk density of the xerogel, m_1 is the mass of the xerogel, m_2 is the mass of the xerogel immersed in ethanol and ρ_o is the density of ethanol (0.81 g mL^{-1} at 20°C).

The specific surface area, the pore diameter and the pore volume were measured using the BET method based on nitrogen gas adsorption (Micromeritics ASAP 2000). Before the measurement, samples were vacuum dried for 24 h at 25°C.

An FTIR spectrometer (Jasco model 410, 4 cm^{-1} resolution) equipped with the Horizontal Attenuated Total Reflectance Accessory (45° incident angle, zinc selenide ATR prism) was used to record HATR/FTIR spectra of non-lyophilized and lyophilized xerogel surface in the range of 1300–750 cm^{-1}. The thickness of the gel layer was about 1 mm (i.e. much greater than the effective penetration depth of the IR light passing the ATR crystal and attenuating in the bulk of the sample). For a better comparison, the spectra were normalized to maximum absorption of dominant peak at ~ 1070 cm^{-1} attributed to the asymmetric stretching of siloxane bands.

The FTIR spectra of lyophilized and non-lyophilized SiO_2 xerogels were taken between 650 and 4000 cm^{-1} using a Jasco model 410 FTIR in transmission mode and KBr as a background material.

Three independent textural measurements were carried out for each type of xerogel.

Study of in-vitro release

The stability test of solutions of doxorubicin

To evaluate the stability of free doxorubicin under conditions of release test (see paragraph below), the doxorubicin was dissolved in SBF of pH 7.4 and stored in the absence of light in a covered polypropylene flask for 20 days at 37°C. The degree of degradation of free drug in the solution was measured spectrophotometrically.

Release test of doxorubicin-loaded SiO_2 xerogels

The samples of doxorubicin-loaded SiO_2 xerogels used in the release tests are listed in Table 1. The release study was performed twice for an initial time up to 8 h and for an extended time up to 50 h. In the first case, drug-loaded xerogels were subjected to release testing at 37 ± 0.5°C in 10 mL of SBF pH 7.4 under sink condition (the theoretical highest concentration of drug in the medium was much below the 10% drug aqueous solubility, which is a requirement for fulfillment of sink conditions). The release medium in a polypropylene flask was shaken in a shaking water bath (75 shakes per min) and kept out of the light. At various time intervals (identical for all gel samples), 2-mL volumes of the solution were filtered through a membrane filter (cellulose acetate, 0.22 μm) and the spectrophotometric assay of doxorubicin was performed. The release medium was replaced by a fresh 2 mL of SBF buffer to maintain constant volume. For the extended-time study, the release medium was regularly replaced, following the

Table 1 Loading degree and textural properties of doxorubicin-loaded SiO_2 xerogels

Sample	Loading degree (mg g^{-1})	Lyophilization	Mass of matrices[a] (mg)	Formulation and size of matrices (± 0.1 mm)	S_{BET} (m^2 g^{-1}); pore size (nm); porosity (%); bulk density (g cm^{-3})[c]
LS1	1.4	+	230 ± 0.5	Granules 1 ÷ 2	480; 2.8; 37; 1.4
LS2	1.2	+	232 ± 0.4	Granules 1 ÷ 2	
LS3	2.0	+	230 ± 0.5	Granules 1 ÷ 2	
LS4	4.0	+	231 ± 0.3	Granules 1 ÷ 2	
S1	1.4	−	250 ± 0.2	Granules 1 ÷ 2	390; 2.5; 50; 1.1
S2	1.4	−	248 ± 0.3	Granules 0.5 ÷ 0.8	
S3	1.4	−	249 ± 0.5	Disc[b]: $\phi \times D = 2 \times 4$	
S4	1.4	−	252 ± 0.4	Disc[b]: $\phi \times D = 2 \times 10$	

[a]Mean of 5 measurements. [b]Disc with thickness ϕ and dimension D. [c]Median of 3 measurements; percentage of porosity was calculated using the formula: $P\% = [1 - (\rho_b/\rho_d)] \times 100$, $\rho_s = 2.2$ g cm^{-3} (ρ_b, ρ_s are bulk and skeletal density, respectively) (Iler 1979).

measurement of drug release, every 8 h during the time of release. The volume of release medium was 10 mL for all doxorubicin-loaded SiO_2 xerogels. The rest of the experimental conditions were the same as for the initial release. Quantitative determinations of the amount of doxorubicin released were based on pre-calibration of the spectrometer using standard solutions of the drug.

Experimental data were fitted using a semi-empirical power law equation:

$$M_t/M_\infty = M_b/M_\infty + kt_n \quad (3)$$

where M_t and M_∞ are the amounts of drug released at time t and the total amount of loaded drug, respectively, and M_b is the amount of burst-released drug, defined as a an initial large bolus of drug released before the release rate reaches a stable profile (Huang & Brazel 2001). The value of n approaching 1 corresponds to zero-order release kinetics, $0.5 < n < 1$ means a non-Fickian release model and n approaching 0.5 indicates Fickian diffusion for non-swelling polymers (Ritger & Peppas 1987). From the plot of log M_t/M_∞ versus log t, the release constant (k) and the release exponent (n), characteristic of the release mechanism, were calculated.

Dissolution test of SiO_2 xerogels

Some of the samples of weighed doxorubicin-loaded SiO_2 xerogels were subjected to determination of the hydrolysis rate under the same conditions as the release test. Dissolution of the SiO_2 xerogels was determined by measuring dissolved silicic acid spectrophotometrically as a molybdenum blue complex at 820 nm (Koch & Koch-Dedic 1974). At the end of dissolution test, samples were removed from the medium, re-dried under vacuum at room temperature for 8 h and weighed to determine the weight loss.

Statistical analysis

The release and dissolution study were repeated 5 times and values are given as mean ± standard deviation (s.d.). The textural experiments were repeated 3 times and values are presented as medians. The Kruskal–Wallis nonparametric analysis of variance was used with Dunn's post-hoc procedure to analyse differences between more than 2 groups. The non-parametric Mann–Whitney test was evaluated to determine whether differences between two groups existed. The differences were considered significant if $P < 0.05$. Statistica 6.0 (program Stat Soft Inc.) was employed for statistical evolution.

Results

Doxorubicin-loaded SiO_2 xerogels

Study of the influence of lyophilizing process on the average loss of weight of doxorubicin-loaded SiO_2 xerogels showed that the average water loss per unit mass of xerogel (1 g) was 6.8 ± 0.33%. Based on the statistical evaluation, the obtained value was found to be independent of the mass taken for lyophilization (in the analytical range of masses 0.2–1 g). This result also showed that the selected parameters of lyophilization were appropriate for the repeatability of freeze-drying of SiO_2 xerogels.

Figure 1 shows the HATR spectra of non-lyophilized and lyophilized xerogels in the characteristic silica region (1300–750 cm^{-1}). There are two dominant features at about 1070 and 1170 cm^{-1} (broad shoulder) that are attributed to asymmetric stretching vibrations of Si-O-Si in TO (transverse optical) and LO (longitudinal optical) modes, respectively (Lenza & Vasconcelos 2001). The band around 790 cm^{-1} is associated with the symmetric stretching of Si-O-Si mode. The band centered about 950 cm^{-1} is associated with the stretching mode of non-bridging oxide bands: Si-OH and $Si\text{-}O^-$. The spectra indicated that the SiO_2 network at ~1060 cm^{-1} up shifts to about 1070 cm^{-1} following freeze-drying. This also correlated with a small decrease of stretching mode of the Si-OH and SiO^- groups. A detailed inspection of the spectra of these SiO_2 xerogels (results not shown) also revealed significant changes in the bands between 3500 and 3200 cm^{-1}, corresponding to stretching and bending vibrations of the -OH group coming from free or adsorbed water, for lyophilized SiO_2 xerogels.

The loading degree and the textural properties of doxorubicin-loaded non-lyophilized and lyophilized SiO_2 xerogels are shown in Table 1. The differences between bulk densities was found highly significant ($P = 0.01$) and also significant difference was found between specific surface values ($P < 0.05$). No significant differences between pore size and matrices size were found ($P > 0.05$).

Study of in-vitro release

Stability test of solutions of doxorubicin

According to literature, doxorubicin hydrochloride is stable in acidic medium over the pH range 4.5–6.5, but rapid decomposition occurs at a higher pH (6.5–12) (Li et al 1998). The temperature also has an influence on the degradation of doxorubicin and with its increase decomposition is accelerated.

Doxorubicin has also been found to adsorb onto various materials, such as glass, polyethylene and polytetrafluoroethylene, but not to polypropylene (Fan & Dash 2001). Therefore, a polypropylene flask was used in this release study.

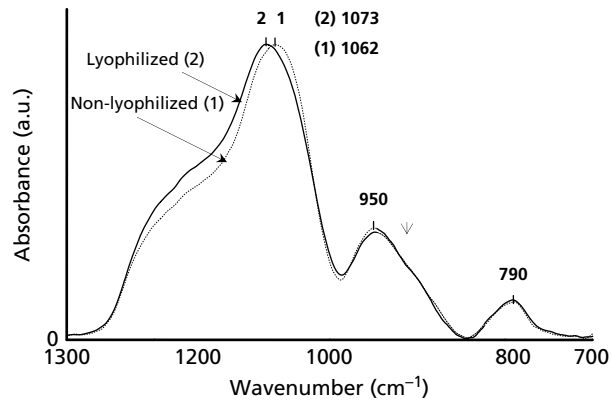

Figure 1 HATR/FTIR spectra of non-lyophilized (1) and lyophilized xerogel (2).

The degradation profile of free doxorubicin under the same conditions as the release test followed a zero-order kinetics (r close to 0.99) and the rate of degradation was found to be 6 wt%/day (results not shown). Therefore, the calculated degree of degradation of doxorubicin during 8 h in the release study was about 2 wt% and this loss was not reflected in the release study.

The effect of size of device

The effect of size of device on the release of drug was studied for granules (S2) and two disc-shaped xerogels with different diameters: 4 mm (S3) and 12 mm (S4) (Table 1) containing 1.4 mg g^{-1} of doxorubicin as a model formulation (Figure 1). The kinetic data are listed in Table 2.

The obtained drug release was diffusion controlled from the granular formulation of xerogel (n = 0.40) whereas for the disc-shaped xerogels the release exponent significantly deviated from the diffusional release mechanism (n close to 0.8 for S3 and S4). As seen in Figure 2A and Table 2, the initial burst release of doxorubicin corresponding to 15 wt% in half an hour was only seen for the granular formulation of the device. In the case of disc-shaped xerogels with different diameters, the obtained difference between initial release of doxorubicin was statistically insignificant ($P > 0.05$) and about 2.0 wt% was released from S3 and S4, respectively.

The compared release rates after burst release for granules (S2) and disc-shaped xerogels with smallest diameters (S3) was found to be statistically different ($P < 0.05$). In addition the difference was highly significant ($P < 0.01$) for comparison of the largest disc-shaped xerogel (S4) with others. By examining these release profiles in detail it can be seen that the rates were relatively fast during the first 8 h (Figure 2A) and between 43 wt% and 35 wt% of doxorubicin was released from the granules (S2) and smallest disc-shaped xerogels (S3), whereas 20 wt% was released from the largest disc-shaped xerogel (S4). After that, the release rate slowed down for all formulations of xerogels, and about 70 wt% and 60 wt% was released from the granules and smallest disc-shaped xerogels (S3), respectively, and 38 wt% from the largest disc-shaped xerogel (S4) in 50 h (Figure 2B). The difference

Table 2 Release kinetics parameters for extended time of release

Sample	Release exponent n	Kinetic constant k (wt%/h^n)	Correlation coefficient r	Initial release at 0.5 h (wt%)
LS1	0.88 ± 0.05	1.80 ± 0.02	0.968	–
LS2	1.10 ± 0.06	1.23 ± 0.06	0.925	–
LS3	0.94 ± 0.05	1.72 ± 0.08	0.936	–
LS4	0.80 ± 0.04	3.28 ± 0.06	0.979	0.8 ± 0.04
S1	0.42 ± 0.09	15.0 ± 0.08	0.985	10 ± 2.5[a]
S2	0.40 ± 0.03	18.5 ± 0.04	0.994	14 ± 1.3[a]
S3	0.80 ± 0.04	4.20 ± 0.05	0.956	2.1 ± 0.5
S4	0.80 ± 0.06	2.00 ± 0.01	0.974	2.4 ± 0.8

–, No determination. [a]Burst release.

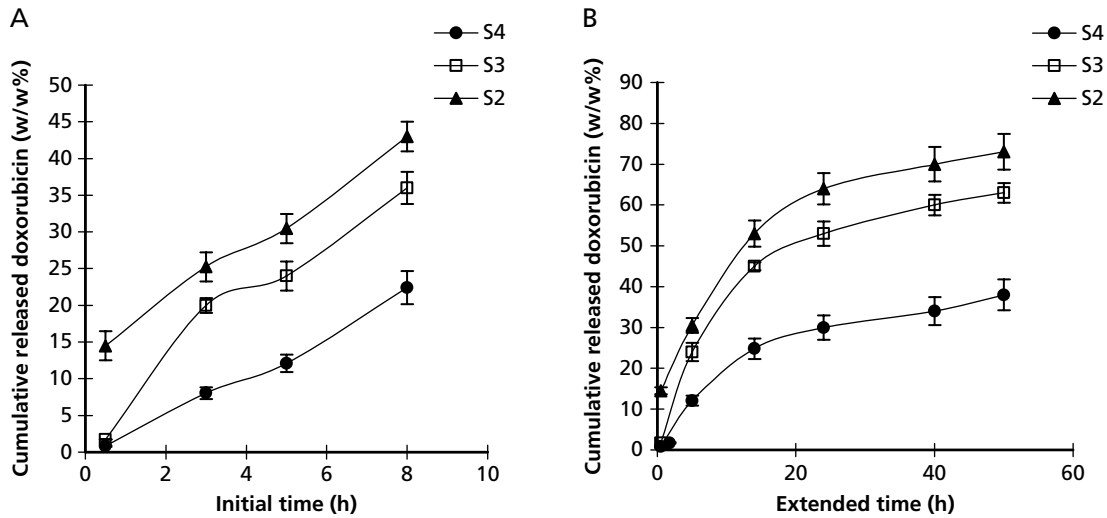

Figure 2 Doxorubicin release from non-lyophilized silica xerogel devices containing 1.4 mg g^{-1} of doxorubicin hydrochloride as a function of initial time (A) and extended time (B) of release test. S2, granules with size 0.5–0.8 mm; S3, discs with a diameter of 4 mm and thickness of 2 mm; and S4, disc with a diameter of 10 mm and thickness of 2 mm.

between means of total release of doxorubicin was significant ($P<0.05$). In general these results indicate that an increase in the device size in the examined range significantly decreased the total release of doxorubicin. Additionally, a significant decrease in burst release was found for all disk-shaped formulations independently of the size of diameter.

The dissolution rates of the SiO_2 xerogels are in good agreement with the doxorubicin release data, as seen in Figure 3. Dissolution rate decreased as the size of device increased and the difference was highly significant ($P=0.01$) for comparison of S4 with S3 and S4 with S2. Significant difference was also found between S3 and S2 ($P<0.05$). By examining the dissolution profiles in detail (Figure 3), it can be seen that the dissolution rate was relatively slow for disc-shaped xerogels (S3 and S4) compared with granules (S2) during the initial time. After the lag time, all the profiles were acceptably linear ($0.992< r <0.996$) according to zero-order release over a 50-h period and the weight loss was 8–20 wt% for different formulations.

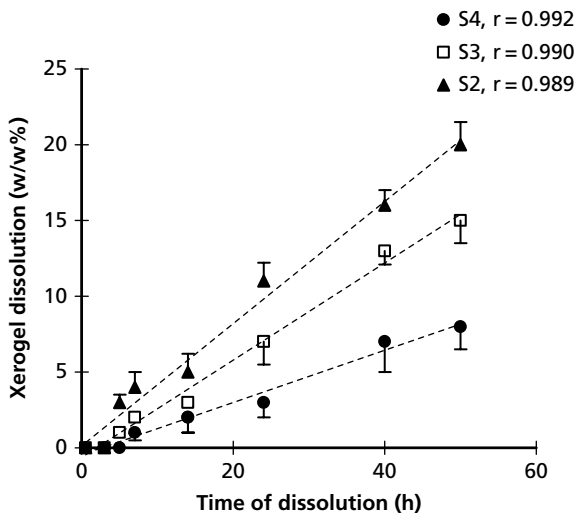

Figure 3 Dissolution profiles of non-lyophilized silica xerogel devices containing 1.4 mg g^{-1} of doxorubicin hydrochloride as a function of time of dissolution test. S2, granules with size 0.5–0.8 mm; S3, discs with a diameter of 4 mm and thickness of 2 mm; and S4, disc with a diameter of 10 mm and thickness of 2 mm. Dashed lines are linear fits of the calculated values.

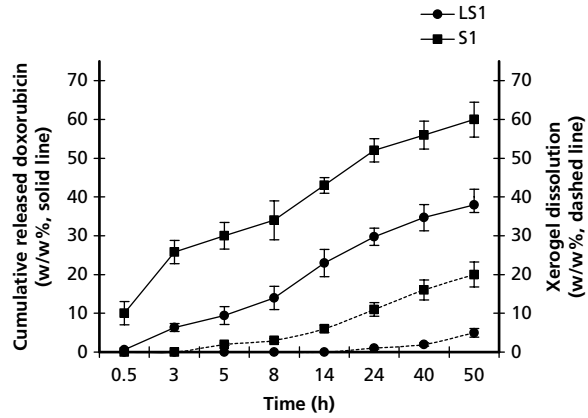

Figure 4 Doxorubicin release (solid line) and dissolution profiles (dashed line) from silica xerogel granules containing 1.4 mg g^{-1} of doxorubicin hydrochloride as a function of time. S1, non-lyophilized; LS1, lyophilized xerogel.

The effect of lyophilization

Comparison of the release profiles of LS1 and S1 with a model drug load of 1.4 mg g^{-1} is depicted in Figure 4. The differences between LS1 and S1 formulations were highly significant ($P<0.01$). The drug release from non-lyophilized xerogel (S1, n = 0.42, Table 2) was found to be diffusion controlled whereas for the lyophilized xerogel, the release exponent significantly deviated from the diffusional release mechanism (LS1, n = 0.88, Table 2). As seen in Figure 4 and Table 2, the burst release amounting to about 10 wt% in half an hour was seen only for the non-lyophilized xerogel, whereas a lag time before the release was observed for lyophilized devices. The release rate was relatively faster for the first 8 h for non-lyophilized formulations compared with lyophilized, and about 32 wt% and 15 wt% of doxorubicin was released from S1 and LS1, respectively. By examining the influence of lyophilization on drug behaviour among lyophilized formulations, Figure 5A presents in detail the release of doxorubicin over the initial time of release up to 8 h from lyophilized xerogels (LS2–LS4) containing different loads of drug (see Table 1). When fitted to the zero-order release ($0.990<r<0.998$), the drug release was linear and the obtained release exponent in the Peppas equation, n, was close to 1 for all the profiles, suggesting that the release rate of doxorubicin was independent of the time. During this time, the difference between initial release was not significant ($P>0.05$) and also the dissolution of these xerogels was not significant ($P>0.05$). After the initial time of 8 h, the rate of release slowed down for both the lyophilized and non-lyophilized xerogels (Figure 4B, 5B). However, the release constant, k, of lyophilized xerogel (LS1) was significant smaller than that of non-lyophilized xerogel (S1) and about 38 wt% of the drug was released from lyophilized device compared with 64 wt% for non-lyophilized xerogel at the end of study (Figure 4). These results corresponded well to dissolution rates of those xerogels (Figure 4). The difference was significant ($P<0.05$) during this time and the rate of dissolution of lyophilized xerogels was two-fold smaller compared with non-lyophilized materials. After 50 h of release study, about 5 wt% decrease of mass for lyophilized formulations was observed (Figure 4).

Generally, the obtained data suggest that lyophilizing of xerogels significantly decreases both burst and total release of doxorubicin and also dissolution of xerogel.

The effect of loading degree of doxorubicin

The effect of loading degree of doxorubicin on the release of drug was studied for lyophilized granules (Table 1, Figure 5) with different drug loads of 1.2, 2.0 and 4.0 mg g^{-1} for LS2, LS3 and LS4, respectively. The differences were not significant

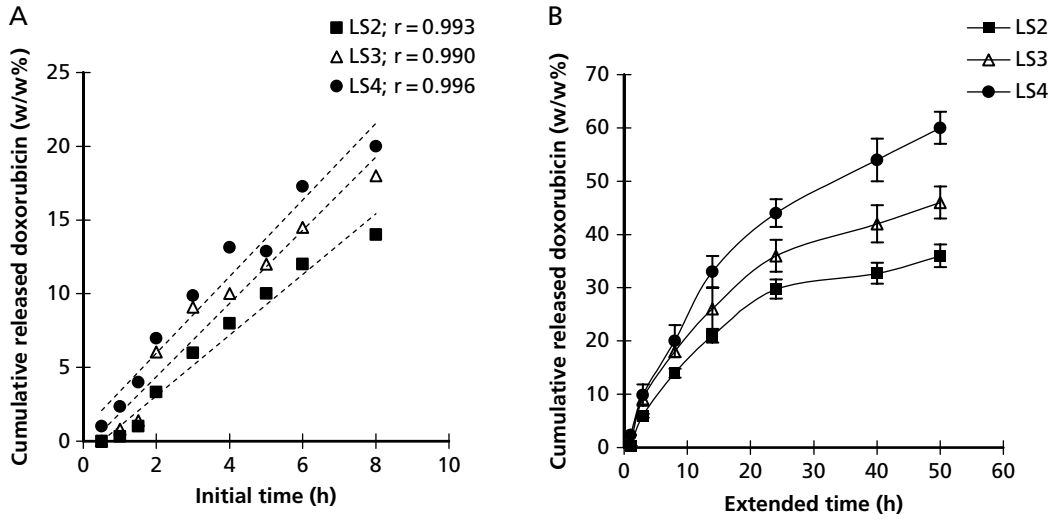

Figure 5 Doxorubicin release from lyophilized silica xerogel granules containing 1.2 mg g^{-1} (LS2), 2.0 mg g^{-1} (LS3) and 4.0 mg g^{-1} (LS4) of doxorubicin hydrochloride as a function of initial time (A)and extended time (B) of release test.

for the initial time (8 h) (Figure 5A). Significant differences were observed in the extend time study of doxorubicin release ($P < 0.05$) (Figure 5B). Among these xerogels, the formulation with drug close to 4.0 mg g^{-1} had the highest k value, indicating that the drug was released fastest from this formulation (Table 2). The differences between the largest drug loading (4.0 mg g^{-1}) and smallest tested (2.0 and 1.2 mg g^{-1}, respectively) were highly significant ($P = 0.01$). In addition, the differences between formulations with 1.2 and 2.0 mg g^{-1} of loading degree were significant ($P < 0.05$). These results also indicate that the drug release was proportional to the loading degree of doxorubicin.

The drug loading was found to not significantly influence the dissolution rate of xerogels ($P > 0.05$).

Discussion

This study evaluated the effect of the size of delivery device, the drying through lyophilization of drug-loaded SiO_2 xerogels and loading degree on the behaviour of drug release. The obtained results indicate that the release of doxorubicin from all types of SiO_2 xerogel was governed by diffusion and simultaneous zero-order dissolution of the xerogel. The rate of water-soluble doxorubicin release from the porous, non-swelling, inorganic SiO_2 xerogels may depend on: the water (simulated body fluid) concentration gradients at the xerogel–water interface; textural properties of the xerogel; the size of delivery device; the loading degree and the uniformity of drug distribution in delivery devices; the rate of dissolution (degradation and erosion) of xerogel; and the interactions between the drug and the surface of xerogel.

The rate of drug release from sol-gel materials depends on the sol-gel processing parameters, such as pH of solution and molar ratio of water to precursor (Ahola et al 2000, 2001). In this study, the pH of the homogeneous, hydrolysed sol was raised to 5–5.5 with ammonia to avoid destabilization of doxorubicin hydrochloride and the molar ratio of water to precursor was equal to 6. According to literature, the rate of condensation of gels increases with an increase in pH above the isoelectric point (2.5) of sols, resulting in a more porous structure (Curran & Stiegman 1999). Regarding the influence of the molar ratio of water to precursor on the structure of silica, most authors agree that the increase of this value above 4 increases formation of more hydrolysed species and thus yields a stronger oxide silica network with a more branched structure (Elferink et al 1996). The SiO_2 xerogel obtained in this study is characterized by mezoporosity (pore diameters above 2 nm (Curran & Stiegman 1999) with porosity above 30% and the surface area about 400 m^2 g^{-1}). All samples obtained by freeze-drying had a significant weight loss because of the sublimation process of both physically adsorbed and H-bonded water from the porous structure of the xerogel. However, the observed increase in bulk density following freeze-drying is attributed to the significant volume shrinkage of gels. Therefore, these gels were examined only in the granular form because of the significant changes in matrix size following freeze-drying.

On the other hand, the increase in specific surface, as well as shift of stretching vibration, of siloxane bonding to the highest value number corresponds to a more polymerized SiO_2 network with a stronger Si-O bond strength, and a shorter bond length (Lenza & Vasconcelos 2001). The formation of new Si-O-Si bonds and decrease in Si-O-H and $Si\text{-}O^-$ bonds by further polycondensation reactions continues during freeze-drying. According to literature (Fidalgo & Ilharco 2004), the silanol groups may undergo further condensation upon aging/drying of the gels, stiffening the silica network and yielding a more polymerized and more branched structure. The differences both in molecular structure and physical properties of the silica surface before and after lyophylizing may influence the drug release and dissolution of silica matrices.

In the sol-gel loading of doxorubicin in the molecular dispersion, the drug is uniformly dissolved in the polymer matrices (Prokopowicz et al 2005) so the release rates continuously diminish with time due to the increasing diffusional distance that hinders drug diffusion from the centre of the device. In addition, the release of drug significantly increases with an increase of loading degree between 1.2 and 4 mg g^{-1}. A study of the effect of the size of device on the release of doxorubicin showed, generally, that both the release of drug is significantly faster and the burst release significantly higher with decreasing device diameter and also surface-area-to-volume ratio of xerogels. Additionally, the high burst release of about 15 wt% in half an hour was only seen in the release profiles of non-lyophilized granules with size varying from 0.5 to 0.8 mm. This suggests that these devices are possibly too small for controlled drug delivery.

During the time of release, the dissolution (erosion and degradation) of the devices also occurred. SiO_2 xerogels are hydrophilic polymers with silanol groups on the surface, so they take up large quantities of water and degrade by hydrolysis of the siloxane bonds through the entire network (Göpferich 1996). Finally, silica acids are released, leading to the weight loss of silica xerogels and also an increase in their pore size (Radin et al 2005). Tamada & Langer (1993) showed that the surface-area-to-volume ratio of the device defines the erosion rate and larger devices exhibit longer times of erosion than smaller ones, although the erosion rate is constant. In this study, the dissolutions study of silica xerogels showed significant differences for various sizes of xerogels and with the decrease in these parameters the rate of dissolution increases.

A study of the freeze-drying of devices on the release of doxorubicin showed, generally, that the release of drug is significantly slower and the burst release significantly smaller compared with the non-lyophilized device. The kinetic data obtained for doxorubicin release from the disc-shaped non-lyophilized and lyophilized sol-gel derived materials (rate of release close to 4 wt%/h^n and release exponent between 0.8 and 1) suggested anomalous Fickian diffusion compared with fast, diffusion-controlled release from the granular non-lyophilized xerogels (k about 18 wt%/h^n and n close to 0.4). In the case of disc-shaped non-lyophilized xerogels, the results can only be rationalized based on the dissolution process of the network of xerogels mentioned above. The dense surface layer of disc-shaped non-lyophilized xerogels dissolves significantly slower; the larger the disc diameter the slower the dissolution process. In contact with the buffer solution the doxorubicin is released only through open pores. The initial step of release of the drug (zero-order release) from these devices is likely to be based on erosion and diffusion from open pores. This conclusion is in line with the observed burst release of the drug from the granular formulations of non-lyophilized xerogels. In the case of lyophilized xerogels, the behaviour of drug release in the initial step is attributed both to the absence of physically adsorbed water within the pores of xerogels and to the more polymerized structure. The rate of release of doxorubicin depends on the rate of diffusion of water molecules within pores because these xerogels also need time to dissolve the matrix and release the drug. On the other hand, the more polymerized structure in the case of lyophilized delivery effected the decrease of dissolution of silica and thus yielded the decrease in release of drug. After the lag time, the behaviour of drug release is similar to drug release from disc-shaped non-lyophilized xerogels with the largest size.

Another important aspect affecting the rate of release of doxorubicin is the possible interaction of drug with the silica polymer. Doxorubicin has two major functional groups in its structure: a primary amine group in the sugar moiety and a primary hydroxyl group of $-C=OCH_2OH$ groups in the aliphatic side chain (Figure 6). Poupaert & Couvreur (2003) described a molecular simulation study of protonated doxorubicin interacting within a frame of n-butyl polycyanoacrylate, which showed that the cohesion in the polymer comes from a blend of dipole-charge interaction, hydrogen bonds and hydrophobic forces. The most probable stabilizing interactions of the hydrophilic silica polymers and doxorubicin are electrostatic, as well hydrogen bonding, as seen in Figure 6.

The doxorubicin molecule exists as a protonated form at the pH of simulated body fluid, whereas the silanol groups on the SiO_2 surface are negatively charged (Atkin et al 2003). A larger amount of unbound silanol groups with H-bonding water exposed on the lyophilized surface of xerogels compared with xerogels with physically adsorbed water might result in stronger bonding of the drug within the lyophilized network of SiO_2.

Conclusion

The results presented in this paper demonstrate that the sol-gel technique is a new method for loading of an anti-cancer drug within an inorganic matrix. Doxorubicin release was governed by diffusion control and simultaneous zero-order dissolution of the xerogel and it can be controlled by the size of the matrix, the loading degree and lyophilization. With an increase in the size of device and decrease in loading degree, there is a decrease in the release rate of drug. Also, lyophilizing

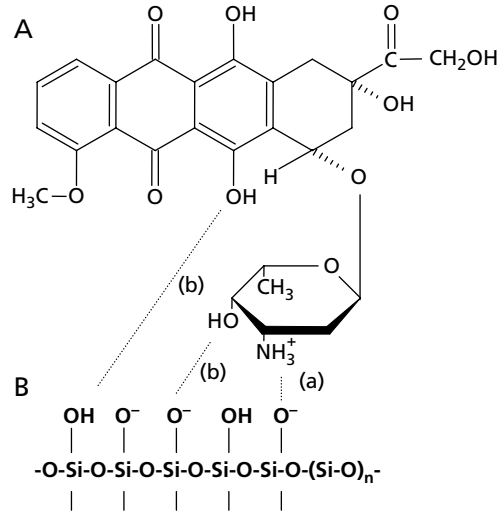

Figure 6 Schematic model of interactions of electrostatic attraction (a), hydrogen bonding (b) between silica network (B) and protonated doxorubicin (A).

of xerogels significantly influences the decrease of drug release.

The results suggested that the doxorubicin-loaded silica xerogel can be used as an implantable drug delivery device that could give targeted disease control locally. Future work will be focused on the preparation and also release study of the freeze-dried silica nanoparticles loaded with doxorubicin.

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