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Abstract
Controlled release silica sol gels are room temperature processed, porous, resorbable materials with generally good compatibility. Many molecules including drugs, proteins and growth factors can be released from sol gels and the quantity and duration of the release can vary widely. Processing parameters render these release properties exquisitely versatile. The synthesis of controlled release sol gels typically includes acid catalyzed hydrolysis to form a sol with the molecules included. This is then followed by casting, aging and drying. Additional steps such as grinding and sieving are required to produce sol gel granules of a desirable size. In this study, we focus on the synthesis of sol gel microspheres by using a novel process with only two steps. The novelty is related to acid–base catalysis of the sol prior to emulsification. Sol gel microspheres containing either vancomycin (antibiotic) or bupivacaine (analgesic) were successfully synthesized using this method. Both drugs showed controlled, load dependent and time dependent release from the microspheres. The in vitro release properties of sol gel microspheres were remarkably different from those of sol gel granules produced by grinding and sieving. In contrast to a fast, short-term release from granules, the release from microspheres was slower and of longer duration. In addition, the degradation rate of microspheres was significantly slower than that of the granules. Using various mathematical models, the data reveal that the release from sol gel powder is governed by two distinct phases of release. In addition, the release from emulsified microspheres is delayed, a finding that can be attributed to differences in surface properties of the particles produced by emulsification and those produced by casting and grinding. The presented results represent an excellent data set for designing and implementing preclinical studies.

Keywords
controlled release, sol gels, microspheres, emulsification

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ABSTRACT

Controlled release silica sol gels are room temperature processed, porous, resorbable materials with generally good compatibility. Many molecules including drugs, proteins and growth factors can be released from sol gels and the quantity and duration of the release can vary widely. Processing parameters render these release properties exquisitely versatile. The synthesis of controlled release sol gels typically includes acid catalyzed hydrolysis to form a sol with the molecules included. This is then followed by casting, aging and drying. Additional steps such as grinding and sieving are required to produce sol gel granules of a desirable size. In this study, we focus on the synthesis of sol gel microspheres by using a novel process with only two steps. The novelty is related to acid–base catalysis of the sol prior to emulsification. Sol gel microspheres containing either vancomycin (antibiotic) or bupivacaine (analgesic) were successfully synthesized using this method. Both drugs showed controlled, load dependent and time dependent release from the microspheres. The *in vitro* release properties of sol gel microspheres were remarkably different from those of sol gel granules produced by grinding and sieving. In contrast to a fast, short-term release from granules, the release from microspheres was slower and of longer duration. In addition, the degradation rate of microspheres was significantly slower than that of the granules. Using various mathematical models, the data reveal that the release from sol gel powder is governed by two distinct phases of release. In addition, the release from emulsified microspheres is delayed, a finding that can be attributed to differences in surface properties of the particles produced by emulsification and those produced by casting and grinding. The presented results represent an excellent data set for designing and implementing preclinical studies.

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1. Introduction

Q3 Controlled release focuses on delivering biologically active agents locally over extended periods of time [1–3]. The site specificity of the delivery reduces the potential side effects that can be associated with general administration of drugs through oral or parenteral therapy [1]. Prevalent mechanisms for the delivery of biological agents by controlled release devices are resorption of the drug carrier material and diffusion. The resorption of these devices may, however, cause an inflammatory tissue response, which interferes with the treatment sought for with the molecules [4,5]. Thus, excellent controlled release materials are ideally biodegradable materials with generally good biocompatibility.

Room temperature processed, silica based sol gels are resorbable materials with a favorable tissue response [2,9]. They have been studied for biomedical applications that include tissue, cell and enzyme encapsulation and controlled release of drugs [2,3,6–13]. Derived from a metal alkoxide precursor, the sol is produced through a hydrolysis and polycondensation reaction [14]. Due to the mild processing conditions, high concentrations of many types of biologically active agents can be incorporated in the liquid sol. The agents are embedded in the matrix of the gel, which after condensation and drying becomes a porous, glassy solid [2,3,9–13]. Data show that controlled release of antibiotics, proteins, and growth factors is possible from this porous material [2,9–13]. These studies also demonstrate that the release is dependent on synthesis parameters such as the molar ratio of silica precursor to water, type of precursor and the concentration of bioactive drugs [2,10,11].

Controlled release sol gels are usually manufactured through an acid catalyzed process followed by casting, aging and drying. This leads to the synthesis of pellets, which can then be ground and sieved to arrive at granules or powders [2,3,10–12].

Sol gel granules made by grinding down cast discs possess an angular geometry. The sharp edges of this geometry may elicit more of an inflammatory response than that expected from microspheres. So far microspheres have mostly been made with

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biodegradable polymers such as polylactic acid, polyglycolic acids and polylactic-co-glycolic acid [15,16]. These microspheres, however, are not ideal as their degradation products have been observed to cause an inflammatory response [4,5]. This probably would not be the case for sol gel microspheres, since it has been shown previously that silica sol gel granules demonstrate a favorable tissue response and enhanced bone healing [2,9].

Sol gel microparticles have been synthesized using spray drying [17] or emulsification [6,18]. The spray dried microspheres have been used in controlled release studies, however, these particles do not have excellent release properties as the spray drying caused a major reduction of the surface area and resulted in transforming highly porous sol gels into a dense material [17].

In this study, we focus on obtaining porous, controlled release sol gel microspheres using emulsification as the synthesis route. These microspheres were made both with and without biological agents incorporated. Specifically, we incorporated the antibiotic vancomycin and the analgesic bupivacaine. The selection of these molecules was related to our parallel programs that focus on osteomyelitis treatment [19] and surgical pain control [20]. Herein, we report on the synthesis parameters of emulsified acid – base catalyzed microspheres that affect optimal controlled release, with optimization being related to achieving release kinetics of vancomycin and bupivacaine with a desirable therapeutic profile. A novel acid–base catalysis was selected in order to shorten the time to gelation of the sol. A shorter time to gelation is essential to produce sol gel microspheres by emulsification. Synthesis parameters of interest were: pH and time to gelation of the acid–base catalyzed sol, water to alkoxide ratio in the sol, drug concentration added to the sol and rotational speed of emulsification.

2. Materials and methods

Sol gel derived silica microspheres were synthesized using an acid–base catalyzed hydrolysis of tetraethoxysilane (TEOS, Strem Chemicals, Newburyport, MA) followed by emulsification.

2.1. Typical sol synthesis

TEOS (10 ml) and 0.1 M HCl (2.4 ml), with and without the addition of de-ionized water (DI), were mixed and stirred to form an acid catalyzed sol. The molar ratio, *R*, of total water (including water in 0.1 M HCl) to TEOS varied from 2.5 to 10. Pharmaceutical agents were then added to the sol. Sols with 20 mg/g and 30 mg/g of vancomycin (drug to SiO_2 ratio), and sols with 50 mg/g and 80 mg/g of bupivacaine were made by adding corresponding amounts of the drugs. Prior to base addition, the acid catalyzed sol was cooled down to 4 °C in an ice bath. Subsequently, 0.08 M NH_4OH was added dropwise to the sol, which was thoroughly stirred. This changed the process to an acid–base catalyzed process. Depending on the amount of base added (2.2–2.4 ml) the pH of the sol was between 4.5 and 6; under these conditions, the time to gelation varied from immediate gelation to 1 h. To produce microspheres with and without the drugs, the pH was ideally set to 5.5, which led to a time to gelation between 15 and 40 min. Typically, 5 ml of the sol was applied dropwise onto 100 ml of vegetable oil stirred at speeds between 220 and 880 rpm by using a 2 inch × 3/8 inch magnetic stirrer. The stirring continued until microspheres precipitated to the bottom of the beaker. The microspheres were filtered through a 40 μm nylon filter, rinsed with DI water and left to dry overnight in a laminar flow hood.

2.2. Addition of biological molecules – variation of the ratio R

Vancomycin (vancomycin–HCl; Abbott Labs, Chicago, IL), as a first molecule, was dissolved in DI water at 100 mg/ml for incorporation into the sols. Bupivacaine (Spectrum, New Brunswick, NJ), the other molecule, was dissolved in methanol at 70 mg/ml for incorporation into the sols. The vancomycin and bupivacaine solutions were added to the acid catalyzed liquid sol to achieve calculated drug concentrations (mg of drug per gram of dried silica) of 20 and 30 mg/g of vancomycin and 50 mg/g of bupivacaine.

The water content of the sol was found to be critical in obtaining a clear sol without the precipitation of the molecules. The effect of water content on the incorporation of vancomycin into the sol was studied by using acid catalyzed sols without any DI water added to the sol (taking into account the presence of H_20 in the acid, *R* is equal to 2.75) or sols with total water/TEOS molar ratios (*R*) of 5, 6, 8, and 10. As shown in Table 1, acid catalyzed sols without extra water added to the sol became cloudy upon drug addition, indicating precipitation of the drug. When water was added to reach $R = 4$, low doses of vancomycin (16.7 mg/g) could be added to the acid catalyzed sol. However, precipitation of vancomycin was still seen when base was added. At *R* equal to 5, low doses (doses up to 20 mg/g) were successfully incorporated: no precipitation was observed after the addition of the drug and base. It must be pointed out, though, that after incorporation of the base, vancomycin precipitation was observed at higher doses such as 28 mg/g. Only at total water/TEOS ratios of 8 and above was the higher load successfully incorporated. This suggests that, in contrast to the sol gel synthesis with low water content as described by others [6], incorporation of these drugs requires the presence of a sufficient amount of water with *R* values greater than 5.

The addition of pharmaceutical agents and the variation in *R* also altered the pH and time to gelation of the sol. The volume of base was modified to maintain the time to gelation within the optimal range of 15–40 min.

2.3. Materials characterization

Morphology and size distribution of the microspheres were determined microscopically using an image analysis system consisting of a high resolution video camera and Image-Pro Plus 4.0 analysis software (Media Cybernetics, Silver Spring, MD). Sieving was also used to determine the size distribution. Nylon microporous filters of 70, 105, 210, 350, 500, and 710 μm were used. In addition, scanning electron microscopy (SEM, JEOL-6400) was used for imaging the morphology of microspheres in the size range below 100 μm.

Porosity of acid–base catalyzed ground granules and emulsified microspheres was measured using gas (nitrogen) sorption analysis (Autosorb 1, Quantachrome). Granules and microspheres of the same R8-30 V composition were used for the analysis (they contained 30% vancomycin by weight and were synthesized with water/TEOS ratio of 8). Prior to the analysis, the samples were outgassed at 50 °C for 24 h. Adsorption–desorption isotherms and multipoint BET [28] were used to determine porosity characteristics such as the specific surface area, pore volume, pore size distribution and the average pore size.

2.4. In vitro release and degradation study

In vitro release and degradation properties of microspheres were studied in phosphate buffered saline (PBS, Gibco, pH = 7.4) at 37 °C in comparison to those of granules. The size of particles was between 210 and 500 μm. As was the case for the microspheres, the ground granules were also produced from acid–base catalyzed sols. 1 ml of the sols was cast into vials, aged for 3 days and dried at room temperature until there was no further weight loss. The resulting sol gel discs were ground and then sieved to produce granules of the right size range.

For the release studies, 25 mg of particles were immersed in 5 ml of solution (5 mg/ml) and the solutions were exchanged daily. The dissolution experiments were conducted differently. In fact, in order to prevent solution saturation with silicon, 5 mg of particles were immersed in 5 ml of solution (1 mg/ml) and the solutions were exchanged at 6, 10, 24 and 48 h.

The concentration of drug released into solution was measured every 24 h. A time zero measurement was not included, as desorption phenomena are not typically observed with sol gel particles.

Table 1
The effects of water/TEOS molar ratios (*R*) and vancomycin load (drug to SiO2 ratio in weight %) on the incorporation of vancomycin into acid catalyzed (AC) and acid–base catalyzed (ABC) sols.

Vancomycin loading	Water to TEOS molar ratio (*R*)								
	DI water-free	4		5		8		10	
	AC sol	AC	ABC	AC	ABC	AC	ABC	AC	ABC
16.7 mg/g	Cloudy	Clear	Cloudy	Clear	Clear	–	–	–	–
22.2 mg/g	–	–	–	Clear	Clear	Clear	Clear	Clear	Clear
28 mg/g	–	–	–	Clear	Cloudy	–	–	–	–
33 mg/g	–	–	–	–	–	Clear	Clear	–	–

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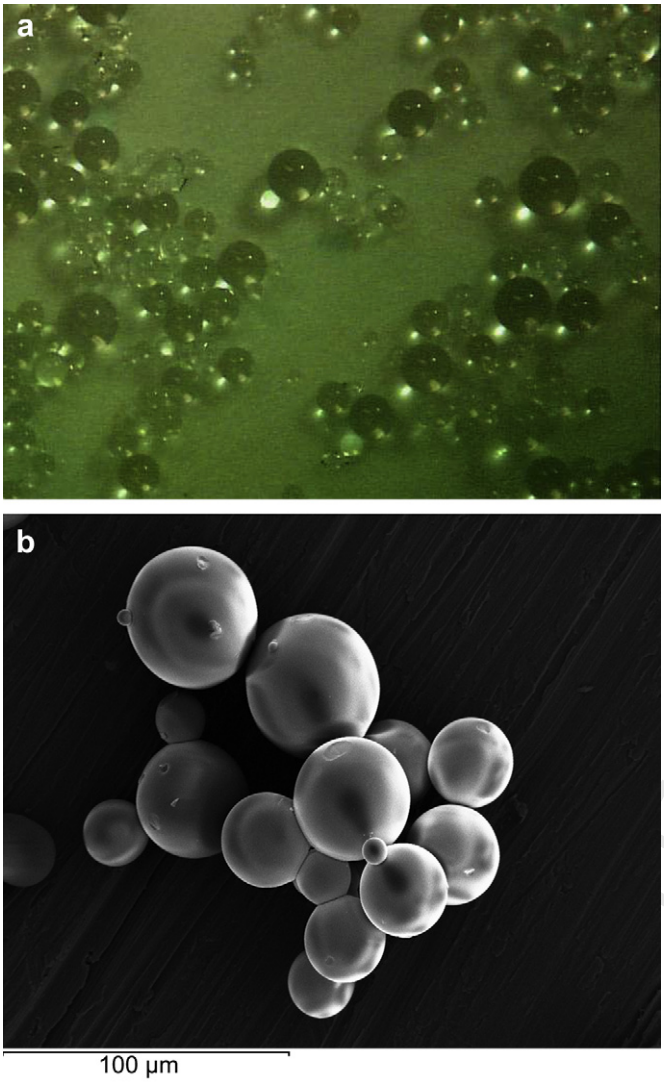

Fig. 1. a and b. Optical and SEM micrographs of emulsified acid–base catalyzed silica microspheres. The optical (original magnification: 60×) and SEM (original magnification 600×) images show the appearance of microspheres in the size range of 100–300 and 10–40 μm in diameter, respectively.

Vancomycin and bupivacaine standards were prepared by dissolving appropriate amounts of the drug in PBS. Bupivacaine was dissolved in PBS through gradual heating in a water bath to 55 °C. The release of vancomycin and bupivacaine was measured spectrophotometrically (Ultraspec Plus, Pharmacia LKB, Piscataway, NJ) at 280 and 265 nm respectively.

The silicon concentration was measured using Atomic Absorption Spectrophotometry (AAS, 5100, Perkin Elmer, Norwalk, CT).

2.5. Modeling release and dissolution kinetics

Various models as described in the modeling section were applied to analyze the release and dissolution profiles obtained using microspheres and granules made from sols with *R* equal to 8 and 30 mg/g vancomycin load, or sols with *R* equal to 6 and a 50 mg/g bupivacaine load.

3. Results

3.1. Microsphere characterization

3.1.1. Morphology and size distribution

Using the acid–base catalyzed synthesis and emulsification process, silica sol gel microspheres with and without drugs were successfully produced. As illustrated in Fig. 1a and b, the particles appear as smooth microspheres. The appearance of vancomycin containing microspheres is illustrated in Fig. 1a and b. Images of microspheres in the range from 200 to 500 μm and of particles below 100 μm are shown in the optical (Fig. 1a) and SEM (Fig. 1b) micrographs, respectively.

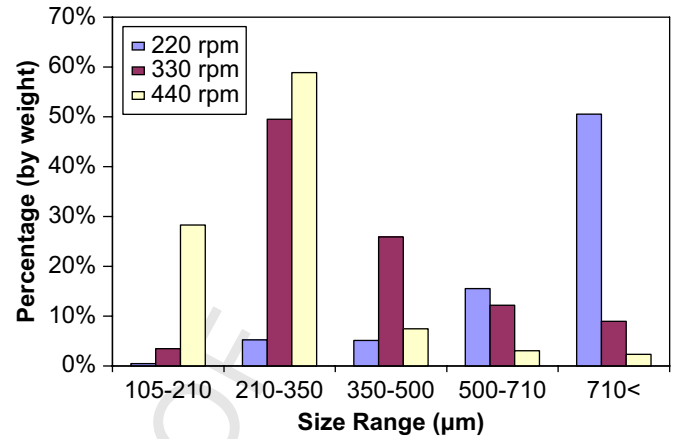

Fig. 2. Size distribution of drug-free microspheres produced at various stirring speeds, as measured by sieving. The fractions dimensions are μm. With increase of stirring speed, the size of microspheres decreased. At 440 rpm, about 30% of the microspheres was in the size range 100–210 μm.

The size distribution as a function of speed of stirring during emulsification is shown in Fig. 2. These results reveal that the size of the microspheres is mainly dependent on this speed. At lower speeds (around 220 rpm) about 50% of spheres formed were greater than 710 μm. Non-spherical particulates precipitated along with the microspheres. When the speed of stirring was increased, the size of the microspheres decreased and non-spherical particulates were not observed. At 330 rpm, about 50% of the microspheres were in the size range of 210–350 μm. At 440 rpm, the percentage of the microspheres in this size range increased to almost 60%. The percentage of the microspheres in the size range of 105–210 μm also increased substantially. This percentage reached 28%, in contrast to less than 4% at the emulsification speed of 330 rpm. With further increases of stirring speed (to 660 rpm and beyond), most of microspheres were below 100 μm. At 880 rpm, most of microspheres were in the size range of 10–40 μm.

3.1.2. Porosity of acid–base catalyzed ground granules and emulsified microspheres

The results of the absorption–desorption analysis including the specific surface area (SA), total pore volume (PV), average pore diameter (PD) and the PD distribution are shown in Table 2. The data suggest that, although some micropores with a size below 2 nm (typical for microporous materials) were present in both granules and emulsified microspheres, the average pore size was characteristic for mesoporous materials. Most pores were in the mesoporous range above 2 nm. The average pore diameter for granules and spheres was 2.38 and 2.55 nm, respectively, and the pore size distribution was 1.5–5 nm and 1.5–7 nm, respectively.

Table 2
Characteristics of porosity of acid–base catalyzed sol gel granules and emulsified microspheres including surface area (SA), pore volume (PV), average pore diameter (PD) and pore diameter distribution. Composition of both granules and microspheres was similar (water/TEOS ratio of 8 and 30% vancomycin concentration).

Material	SA, m^2/g	PV, cc/g	PD ave, nm	PD range, nm
R8-30VG	517	0.31	2.38	1.5–5
R8-30V MS	283	0.18	2.55	1.5–7

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Although the average pore size and the pore size distribution of granules and microspheres were not significantly different, the surface area (SA) and pore volume (PV) were different. In contrast to SA and PV values for granules of 517 m^2/g and 0.31 cc/g, those of microspheres were 283 m^2/g and 0.18 cc/g. Thus, the microspheres exhibited a reduction in surface area and pore volume by a factor slightly less than 2.

3.1.3. *Release of vancomycin and bupivacaine from microspheres and granules*

The cumulative release of vancomycin as a function of immersion time, drug load and water/TEOS molar ratio (*R*) is shown in Fig. 3. It was found that microspheres with vancomycin concentrations of 20 mg/g, which were synthesized from sols with *R* equal to 5, released only 6% of the original load after 4 days. Increasing *R* to 8, the rate and amount of release increased considerably: 36% of the original load was released after 12 days. Using sols with *R* equal to 8, but with a further increase of the load up to 30 mg/g, the rate and amount of release are obviously larger; however, the percentage release remains the same.

The data in Fig. 4 demonstrates a major difference in the release profiles from emulsified microspheres and ground granules even though both were produced from the same sols (*R* equal to 8 and 30 mg/g vancomycin). In contrast to a fast, short-term release from granules, vancomycin release from microspheres was slower and of longer duration. Obviously then, a higher percentage of the original vancomycin load was released from sol gel granules at same immersion times. Within the confines of the experiment, the granules released 90% of the load over 7 days, whereas the microspheres released only 36% of the load over 14 days.

As shown in Fig. 5, microspheres with bupivacaine also showed a time dependent release. In addition, similarly to vancomycin release, the release profiles of bupivacaine from microspheres and granules produced from same sols (*R* equal to 6 and 50 mg/g bupivacaine) were also largely different. The granules showed a burst release of 50% of the bupivacaine load over 1 day. There was a total release of 74% over 7 days. In contrast, microspheres showed a more gradual release: only 41% of the original load was released over 12 days.

3.1.4. In vitro *dissolution properties of microspheres and granules*

Dissolution behavior of the silica based particles was evaluated by measuring changes in the silicon concentration as a function of immersion time. Fig. 6a and b show the cumulative silicon release from (a): vancomycin containing, and (b): bupivacaine containing microspheres or ground granules. Although the granules and microspheres were made using sols of the same composition (*R* equal to 8 and 30 mg/g vancomycin, or *R* equal to 6 and 50 mg/g bupivacaine), the data in Fig. 6a and b show a major difference in dissolution profiles. Dissolution profiles of granules loaded with either drug were typical for dissolution of silica materials. The initial silicon dissolution rates were 3.4 or 5.1 μg/mg/h for granules with vancomycin or bupivacaine, respectively. In contrast, the dissolution of microspheres with either drug was initially delayed (up to 6 h). This initial stage was followed by dissolution at a rate of 0.81 or 1.5 μg/mg/h for microspheres with vancomycin or bupivacaine, respectively. Thus, the data reveal a major reduction of the dissolution rate of the microspheres in comparison to that of granules.

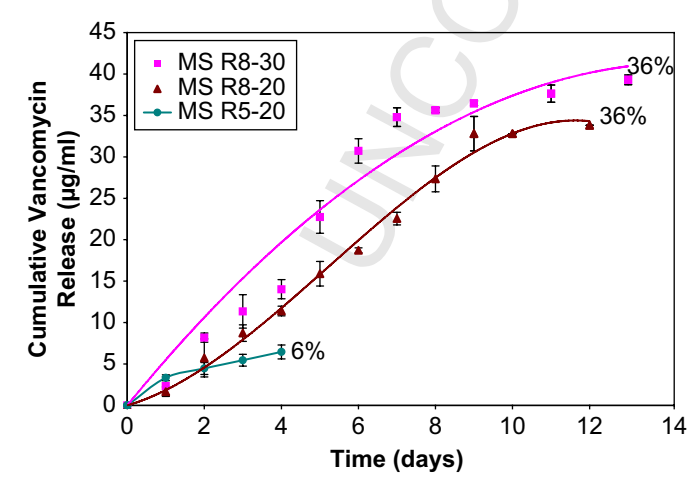

Fig. 3. Cumulative vancomycin release (μg/ml) from microspheres (MS) as a function of immersion time in PBS, load (20 or 30 mg/g), and water/TEOS molar ratio (*R*). At $R = 5$, only a limited release of 6% was observed. At $R = 8$, load dependent release continued up to 2 weeks. The error bars represent the standard deviation ($n = 3$).

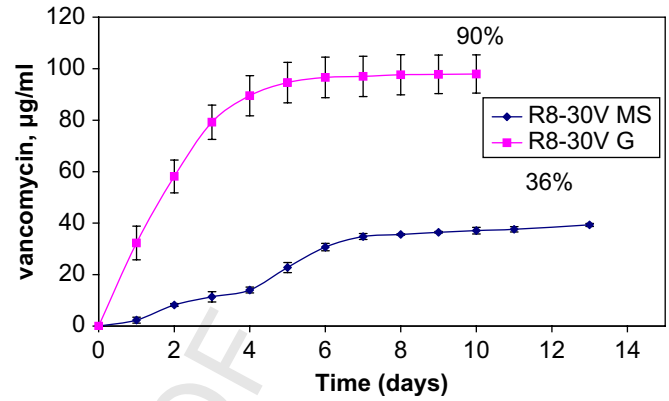

Fig. 4. Cumulative vancomycin release (μg/ml) from microspheres (MS) or ground granules (G) as a function of immersion time in PBS. Although the microspheres and granules were made from same sols (acid–base catalyzed, *R* equal to 8, 30 mg vancomycin per gram of silica), the release profiles were different. In contrast to a fast release from granules with 90% of the load released by 6 days, the release from microspheres was slower and continued up to 14 days. The error bars represent the standard deviation ($n = 3$).

4. Modeling of dissolution and release kinetics

4.1. Dissolution

When dissolution of silica in aqueous solutions is at a steady state of silica dissolution–deposition, the dissolution is given by a first order reaction (Eq. (1)) [21]. This equation indicates that the

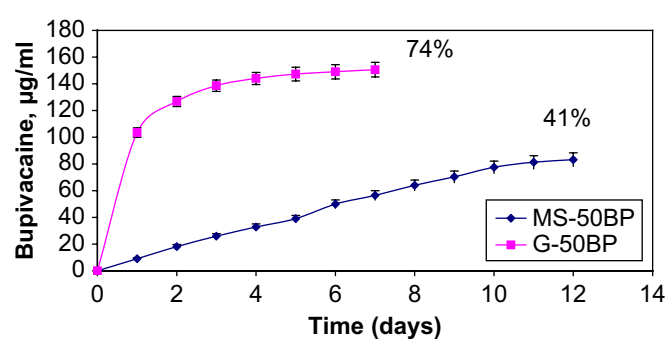

Fig. 5. Cumulative bupivacaine release (μg/ml) from microspheres (MS) or ground granules (G) made from same sols (acid–base catalyzed, *R* equal to 6, 50 mg bupivacaine per gram of silica). In contrast to granules, microspheres showed a slower release extended over a longer period of time. The error bars represent the standard deviation ($n = 3$).

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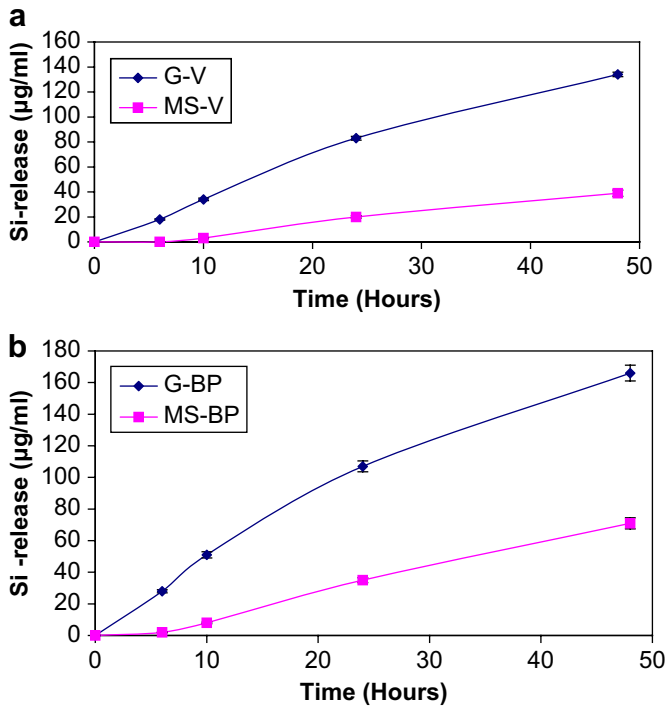

Fig. 6. a and b. Dissolution profiles (measured as the cumulative Si-release versus immersion time in PBS) of acid–base catalyzed microspheres (MS) and ground granules (G) loaded with: (a) vancomycin (V) or (b) bupivacaine (BP). The emulsified microspheres and ground granules were derived from same sols (R equal to 8, 30 mg/g vancomycin, or R equal to 6, 50 mg/g bupivacaine). The error bars represent the standard deviation ($n = 3$).

dissolution is dependent on diffusion of the solute away from the solid–liquid solution interface.

$$\frac{dC}{dt} = kS(C_e - C), \quad (1)$$

where, C: concentration of silicon in solution, C_e: equilibrum concentration or "solubility" of silicon, S: surface area of solid phase, k: rate constant, t: time.

In solutions with a neutral pH and a considerable amount of silicon, S is approximately constant and $C = 0$ at $t = 0$; the equation then takes on the form:

$$\ln\left(\frac{C_e - C}{C_e}\right) = kSt \quad (2)$$

This equation was used to determine the dissolution rate constant, k, for the dissolution of granules and microspheres. As previously determined in our laboratory, the equilibrium silicon concentration for sol gel silica particles in neutral solutions (pH 7.4) is 2.5 mM [22]. Since both granules and microspheres used for the dissolution study were in the same size range, 210–500 µm, we assumed that the difference in the purely geometrical surface area of these particles was minor. In fact, the data (see Table 2) show that the order of magnitude of the specific surface area and the pore volume is the same, with slight reduction caused by emulsification. For this comparative analysis, we also assumed that the surface area was approximately constant within the time frame of the experiment (2 days). Although our previous studies showed that the specific surface area and pore size of room temperature processed acid catalyzed silica sol gels (xerogels) can change during immersion in neutral solutions [22], we assume that the structures of acid–base catalyzed sol gels used in this study are less prone to immersion induced changes. This assumption is based on the fact that room temperature processed acid catalyzed sol gels are microporous (pore size 1 nm), weakly branched and weakly crosslinked structures [14]. In contrast, the acid–base catalyzed sol gels are mesoporous (pore size 2–5 nm), highly branched and have more globular structures [14].

The dissolution rate constants k given by Eq. (2) and the associated correlation coefficients, R_c, are shown in Table 3. The results of this regression analysis are characterized by high correlation coefficient values ($R_c > 0.96$), and therefore, the dissolution data can be well described by typical first order dissolution.

It is noteworthy that the k values for either microspheres or granules were independent of the specific drug that was incorporated. For microspheres with vancomycin or bupivacaine the k values were 0.018 and 0.019 h^{-1}, respectively, and for granules these values were 0.049 and 0.049 h^{-1}, respectively. This finding suggests that the type of drug present in the sol gel structure does not noticeably affect the dissolution rate of silica sol gel particles.

4.2. Release kinetics

Drug release from soluble matrices such as porous silica sol gels can be either diffusion controlled or dissolution controlled or both. In previous studies, the drug release from porous sol gel matrices was described as a diffusion controlled process [2,3,10–12].

Herein we first interpret the results by fitting the data to rate equations based on diffusion considerations. The Higuchi square root of time model [23] has commonly been used for modeling diffusion controlled processes of drug release. This model has been applied for diffusion controlled release from a homogenous planar matrix or from a porous matrix, from which a drug is leached by the bathing fluid that penetrates the matrix through pores and capillaries [23]:

$$Q = \sqrt{\frac{D\varepsilon}{\tau}(2A - \varepsilon C)C_t} \quad (3)$$

with Q = amount of drug released after time t, D = diffusivity of the drug in the permeating fluid, τ = the tortuosity factor of the capillary system, A = the total amount of the drug present in the matrix, C = the solubility of the drug in the permeating fluid, ε = the porosity of the matrix.

The application of the Higuchi model to the release of vancomycin and bupivacaine from microspheres and granules is shown in Fig. 7a and b. The release data are presented as the fractional release (M_t/M_∞, where M_t is the fraction of the original drug load released at time t) and plotted against the square root of time (expressed in hours). These plots revealed several stages for the release from either microspheres or granules. The stages are listed in Table 4.

The release of both drugs from granules occurs in two stages: a fast release followed by a second stage of slower release (terminal stage). The transition to the second stage occurs at 120 and 72 h for vancomycin and bupivacaine release, respectively. This transition corresponds to a fractional release of 0.9 and 0.7 (90 and 70%) for vancomycin and bupivacaine, respectively. In

Table 3
Dissolution rate constant for Si-release, k (h^{-1}), and correlation coefficient R_c for sol gel microspheres and ground granules containing vancomycin (R8-30V) or bupivacaine (R6-50BP).

Composition	Microspheres		Granules	
	k	R_c	k	R_c
R8-30V	0.018	0.97	0.049	0.99
R6-50BP	0.019	0.97	0.049	0.96

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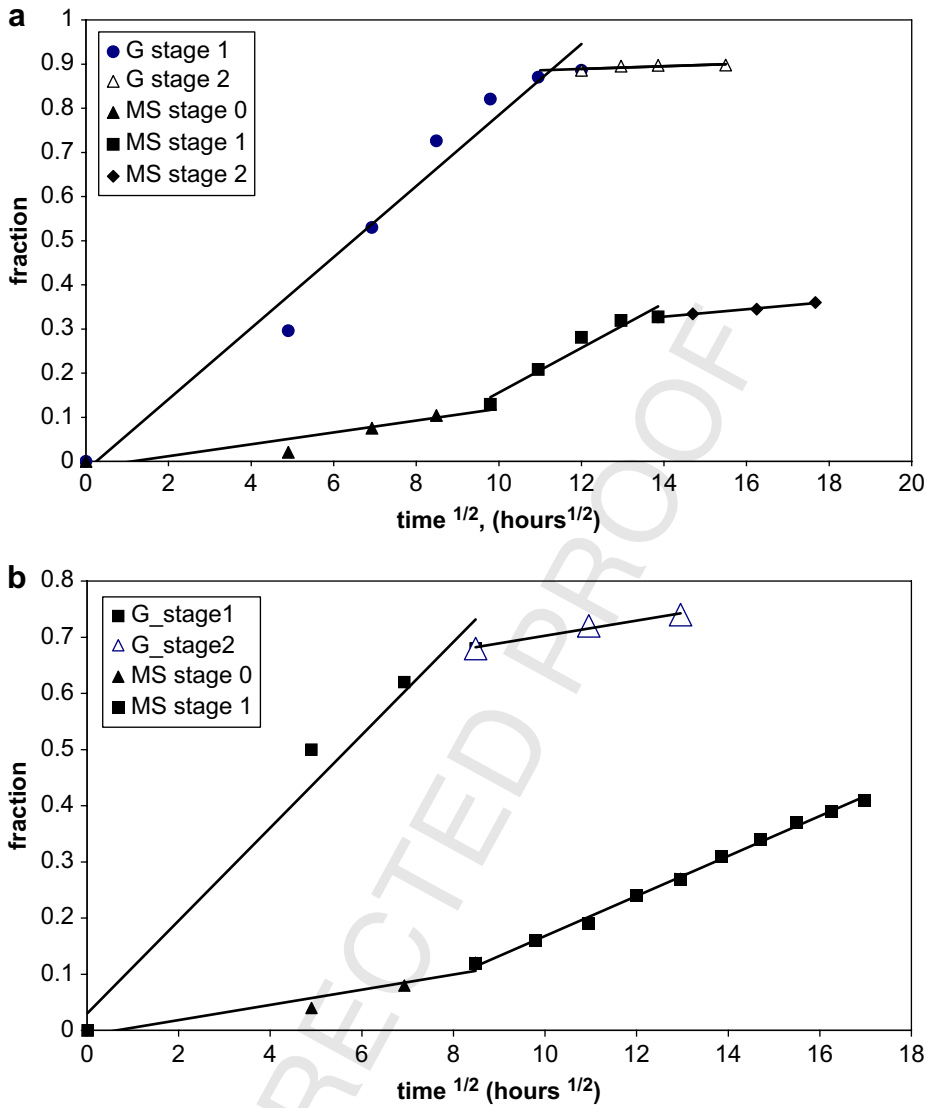

Fig. 7. a and b. Fractional release of (a): vancomycin and (b): bupivacaine from microspheres (MS) and ground granules (G) vs. the square root of time (hours $^{1/2}$). Vancomycin release was measured from particles made from sols with *R* equal to 8 loaded with 30 mg/g; bupivacaine release was measured from particles made from sols with *R* equal to 6 and 50 mg/g drug.

contrast to granules, the release from microspheres with both vancomycin and bupivacaine is preceded by a delay (stage 0). The initiation of release did not occur until 72 and 96 h for microspheres with bupivacaine and vancomycin, respectively. For microspheres with vancomycin, the first actual release phase was followed by a second stage of slower release and the transition occurred at 192 h. Microspheres with bupivacaine did not transition to this stage of slower release within the duration of the experiment.

Table 4
Listing of the various stages of vancomycin (V) and bupivacaine (BP) release from microspheres (MS) and ground granules (G) as follows applying the Higuchi model to the data. This treatment is shown in Fig. 7a and b. Stage 0 reflects delayed release, stage 1- faster release and stage 2- slower release subsequent to stage 1.

Material	Stage 0 (h)	Stage 1 (h)	Stage 2 (h)
MS-V	0–96	96–192	192–312
G-V	n/a	0–120	120–240
MS-BP	0–72	72–288	n/a
G-BP	n/a	0–72	72–168

Using the Higuchi equation, regression analyses were performed for each stage of the release. Correlation coefficients for the vancomycin and bupivacaine release data are given in Tables 5 and 6, respectively.

In addition to the Higuchi model, other models for release have been proposed [24,25]. We analyzed the data using these models as well. They include models assuming zero order and first order release processes, as well as models that take into

Table 5
Correlation coefficient R_c for the relationship between vancomycin release from microspheres (MS) and ground granules (G) and time as expressed by each of the Eqs. (3)–(7).

Model/equation	Microspheres: stages of release			Granules: stages of release	
	Stage 0	Stage 1	Stage 2	Stage 1	Stage 2
Highuchi (3)	0.89	0.99	0.96	0.98	0.91
Zero order (4)	0.98	0.98	0.89	0.93	0.85
First order (5)	0.98	0.98	0.92	0.98	0.88
Hixson and Crowell (6)	0.94	0.98	0.94	0.93	0.86
Baker-Lonsdale (7)	0.98	0.99	0.99	0.97	0.68

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Table 6
Correlation coefficient R_c for the relationship between bupivacaine release from microspheres (MS) and ground granules (G) as expressed by each of the Eqs. (3)–(7).

Model/equation	Microspheres: stages of release		Granules: stages of release	
	Stage 0	Stage 1	Stage 1	Stage 2
Highuchi (3)	0.92	1	0.98	0.97
Zero order (4)	1	1	0.81	0.96
First order (5)	1	1	0.96	0.91
Hixson and Crowell (6)	1	1	0.87	0.97
Baker-Lonsdale (7)	0.91	0.99	0.95	0.94

account matrix dissolution and geometry of the matrices [24,25]:

$$\text{zero order release}: \frac{M_t}{M_\infty} = kt \tag{4}$$

$$\text{first order release}: \ln\left(1 - \frac{M_t}{M_\infty}\right) = -kt \tag{5}$$

cube-root Hixson-Crowell model [24,25] for drug release from systems with dissolution rate limitations:

$$\sqrt[3]{\left(1 - \frac{M_t}{M_\infty}\right)} = -kt \tag{6}$$

Baker-Lonsdale model [24,25] for drug release from diffusion rate limiting matrices of spherical shape:

$$\frac{3}{2}\left[1 - \left(1 - \frac{M_t}{M_0}\right)^{\frac{2}{3}}\right] - \frac{M_t}{M_0} = kt \tag{7}$$

When applying each of the equations to the data, correlation coefficients R_c for vancomycin and bupivacaine release that were obtained are listed in Tables 4 and 5, respectively. Although the regression analysis was performed for all data over the full release duration, the correlation coefficients of these analyses were low ($R_c < 0.91$), underscoring that the release of both drugs from granules or microspheres is very likely a process involving several steps. As we discuss below, it probably also reflects the changing Q4 pore properties as immersion extends to longer periods of time (Table 7).

Regression analyses of the data of the separate phases showed excellent correlation ($R_c > 0.91$), with stage 1 showing the best fit, a logical and expected finding ($R_c \geq 099$).

The modeling of the first stage of the release of both drugs from granules showed an excellent fit with the Higuchi model (R_c equal to 0.98 and 0.98 for vancomycin and bupivacaine data) and with the first order model (R_c equal to 0.98 and 0.96, respectively). These results are in accordance with the commonly used description of the diffusion controlled process as a first order process [23,26]. As previously mentioned, the regression analyses also revealed that the "quality of fit" is better for the first stage of release than for the second, terminal stage. The present observation is in line with knowledge that significant deviations are possible beyond 50% release [23,25]. Others have reported previously that the "quality of fit" of diffusion controlled processes is generally better for the modeling of the initial stage of release than for the modeling of the terminal phase [23,25].

Table 7
Release rate constants, k, for vancomycin and bupivacaine release from microspheres (MS) and granules (G) (determined by using the Baker-Lonsdale model for the 0.36 and 0.41 fractions of release for vancomycin (V) and bupivacaine (BP), respectively).

Composition	$k \times 10^{-3}$, h^{-1}	
	MS	G
R8-30V	0.1	2.1
R6-50BP	0.1	1.6

Focusing on the release from microspheres, all stages of the release of vancomycin fitted the Baker-Lonsdale model best (Table 5: R_c equal to 0.98, 0.99 and 0.99 for the delayed, first and second stage, respectively). These results suggest that the Baker-Lonsdale equation (Eq. (7)), which models diffusion controlled drug release from matrices of spherical shape, is more suitable for describing vancomycin release from sol gel microspheres than the more general Higuchi model. As shown in Table 4, the release data also fit the first order model well (R_c equal to 0.98, 0.98 and 0.92 for the above stages, respectively). As described before, semilogarithmic plots (Eq. (5) for the first order model) of diffusion controlled release data show a linear relationship [23,26].

Bupivacaine release data from microspheres fit all models well ($R > 0.91$), with the "quality of fit" being better for the first stage of release ($R > 0.99$). Given excellent fit between data and models across the board, mathematical data analysis was not successful to elucidate the possible rate determining mechanism for the release of bupivacaine from microspheres.

Previous studies that used various standard models for modeling drug release indicated that it is difficult to discriminate between competing models [24,25]. Herein, we did not encounter this difficulty in that the analysis of vancomycin and bupivacaine release data clearly showed the differences in the release behavior of these drugs from granules and microspheres. In addition, the release behavior of both drugs from granules can be described by a diffusion control release model such as the Higuchi model. When it comes to release from microspheres, it then appears that the Baker-Lonsdale model expressing the diffusion controlled release from matrices of spherical shape is more suitable for describing the vancomycin release from microspheres. Only when we attempted to model the release of bupivacaine from microspheres, could we not identify a differentiating mathematical description.

Turning our attention to the determination of k values, we reasoned as follows. The Baker-Lonsdale model is appropriate for describing the release behavior of the drugs from microspheres. Since this model also showed a very good fit for the release from granules, it was used for determining k values for the drug release from both microspheres and granules. With a total amount of vancomycin and bupivacaine released from microspheres being 36 and 41% (or 0.36 and 0.41 expressed in fractional release), respectively, the k values for the drug release from granules were determined for the similar fractional release. The release rate constants k are shown in Table 6.

5. Discussion

Controlled release sol gel derived silica microspheres were successfully synthesized using a new acid–base catalyzed sol gel process followed by emulsification. By using base, the gelation is short and, upon emulsification, small powder is obtained in an efficient manner. The process involves only two steps: hydrolysis and emulsification. The size of emulsified microspheres depends on the speed of stirring during emulsification and can easily be varied over a large range (from 10 to 500 μm) by changing the stirring speed.

It is established that acid–base catalyzed sol gels are highly porous, with large surface area and pore volume, and with a pore size typically in a range of 5–10 nm [14]. Another benefit of synthesizing sol gel microspheres using emulsification is that the

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"compartmentalization" of sol gel droplets during emulsification in the immiscible medium prevents changes in the sol gel structures [18]. Thus, as in Barbé et al. [18], and similarly to conventional sol gels, both the acid–base catalyzed granules and the emulsified microspheres were expected to be mesoporous. The sorption analysis data obtained for the acid–base catalyzed ground granules and emulsified microspheres (Table 2) confirmed that both particles were mostly mesoporous. In addition, the sorption analysis showed that the emulsification of an acid–base catalyzed sol did not significantly affect the average pore size and the pore size distribution of the resulting spheres.

Two drugs were used in this study, namely, the antibiotic vancomycin and the analgesic bupivacaine; both were successfully incorporated in the acid–base catalyzed emulsified microspheres. The release of both drugs from microspheres was time and load dependent. The release kinetics from emulsified microspheres was very different from those of ground granules. In contrast to a fast and short-term release from granules, the microspheres showed a slower and longer release of both vancomycin and bupivacaine. Using various release models, the analyses suggest that the release from both types of particles is mainly a diffusion controlled process. The Higuchi square root model and the first order models are suitable for describing the release profiles from granules, whereas the Baker-Lonsdale model for the diffusion rate determining release from matrices of spherical shape is more appropriate for the description of the release from microspheres.

Reduced drug release rates from microspheres were associated with reduced dissolution rates of microspheres in comparison to those of granules. Modeling of the dissolution profiles by using a first order model of diffusion rate limiting silica dissolution in neutral aqueous solutions showed that the dissolution process of both granules and microspheres can be described as a diffusion controlled process.

Others have studied the release from spray dried sol gel particles [27]. Spray drying drastically changes the properties of sol gels and results in the production of nonporous microparticles [17]. In the Czuryszkiewicz et al. study the spray-dried particles were also nonporous (SA equal to 2 m^2/g) [27]. In contrast to spray drying, an emulsification process does not affect the porous structure of sol gels due to the aforementioned "compartmentalization".

Modeling has its limitations. Few will argue that that the simple release models invoked in this analysis and in many other studies typically assume that the surface area and porosity of the substrate remain virtually constant during the release experiment. It is known that common release models, especially the models for the diffusion controlled release do not consider the surface area effect on the release kinetics, since such a release is not a surface controlled process. However, silica dissolution models do consider the surface effect on the dissolution rate. Actually, we demonstrated such an effect of immersion induced changes in the surface area on the dissolution behavior of porous xerogels [22].

In this previous study from our laboratory, microporous and mesoporous sol gels were studied and BET measurements were performed before and after different immersion durations [22]. In contrast to the nonporous spray-dried particles [27] that showed an increase of the surface area with immersion time, both microporous and mesoporous sol gels studied by us showed a significant reduction of the surface area with immersion time [22]. This result further underscores the lack of similarity between spray-dried particles and their observed behavior, and actual microporous and mesoporous particles.

The observed differences between the drug release kinetics and the dissolution rates of emulsified microspheres and ground granules are most likely related to the differences in the surface area and the pore volume (Table 2). The emulsification reduced these values by a factor slightly less than 2. This reduces solution penetration into porous sol-gel particles and causes a corresponding reduction of the diffusion rates. An additional contribution may have resulted from differences in surface morphology of the particles produced by emulsification or casting and grinding. Whereas ground granules have an irregular shape, angular geometry and multiple cracks, the emulsified sol gel microspheres are ideally smooth spheres and the surface was formed in contact with the emulsifying bath.

A study demonstrating *in vivo* biocompatibility of resorbable, acid catalyzed, controlled release sol gel ground granules was previously reported [2,9]. This previous study showed resorption of the granules. The present in *vitro* study reveals that microspheres degrade more slowly than ground granules and the data suggest improved surface properties for emulsified particles. Thus, a more gradual resorption and an even more favorable *in vivo* tissue response can be expected when emulsified powder rather than cast and ground sol gel powder is administered *in vivo*.

6. Conclusions

Controlled release sol gel microspheres were successfully synthesized by using a simple and expedient two-step process: acid–base catalyzed hydrolysis followed by emulsification. In contrast to rapid, short-term release from ground granules, the drugs incorporated in the microspheres (the antibiotic vancomycin and the analgesic bupivacaine) showed a slower, long-term release. In addition, the microspheres were characterized by longer *in vitro* dissolution durations than those of the granules.

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