

Biodegradability of sol–gel silica microparticles for drug delivery

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Abstract The biodegradability of porous sol–gel silica microparticles in physiological buffers has been investigated using a USP4 flow-through dissolution tester. In the open configuration, which most closely models in-vivo conditions, the particles dissolved rapidly at pH 7.4, with a rate dependent on the surface area and media flow rate. In the closed configuration, the fastest dissolving 4 mg silica sample was almost completely dissolved in 100 mL of buffer after 36 h. The initial dissolution rates appeared relatively linear but dropped off as dissolved SiO₂ concentrations approached 20–25 ppm. Addition of serum proteins acted to slow dissolution by 20–30%, suggesting a slower degradation in vivo. Silica microparticles administered for controlled release drug delivery would therefore be expected to be eliminated relatively rapidly from the body, depending on the sample size and local fluid flow conditions.

Keywords Biodegradability · Encapsulation · Drug delivery · Dissolution · Microparticles

1 Introduction

Considerable research effort is being applied to improving drug efficacy by developing drug/delivery device combinations which address limitations encountered during

administration of the drug alone. Silica particles, both nano and micron-sized, present a novel drug delivery option, in comparison with the better known organic systems such as liposomes, dendrimers, polymers, etc. [1, 2]. One important concern which must be addressed, however, is the fate of silica particles in the body, following delivery of the drug payload. In particular, nanoparticles taken up into cells must degrade effectively to avoid the toxicity risk associated with accumulation of non-degradable fine particles. More generally, biodegradability is a key parameter for the acceptance of new drug delivery systems by regulatory authorities.

Despite a number of studies (both in vitro and in vivo) demonstrating that silica implants are degradable materials [3–8] there remains a widespread misconception that silica is insoluble in aqueous conditions. This is perhaps not surprising given the refractory nature of ceramic materials. Nevertheless, the dissolution of silica in aqueous solution has been widely discussed in older literature, and indeed may be essential to growth in mammals, particularly in bone and connective tissue development [9, 10].

Silica undergoes hydrolysis to form silicic acid, Si(OH)₄. In vivo, silicic acid from the degradation of silica gel granules, was found to diffuse through the blood stream or lymph and was excreted in the urine at a rate of 1.8 mg silicon per day [11]. The reaction to form silicic acid is catalysed by OH⁻, hence the rapid increase in hydrolysis rate with increasing pH. Comparison of the hydrolysis rates at pH = 9 and 2 shows an increase in excess of three orders of magnitude. However, the equilibrium solubility, which depends on the nature of the silica, remains relatively constant from pH 2 to 9, but increases sharply with increasing pH above 9 [10]. For *nonporous*, amorphous silica, the equilibrium concentration of silicic acid is ~70 ppm silica at 25 °C. In comparison, crystalline silica is ten times less soluble. The CeramiSphere silica nano- and microparticles

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are produced using sol–gel chemistry, and are highly porous, high surface area materials. The solubility is therefore greater than nonporous amorphous silica, and is approximately 120 ppm [10].

Here we report the results of an investigation into the biodegradability of CeramiSphere silica microparticles in physiological conditions, using the standard USP4 flow-through dissolution method. In contrast to the static methods usually reported in the literature [3–8], in the USP4 apparatus, the fluid flows through the powder entrapped in a dissolution chamber, which gradually dissolves. This allows for a much more accurate modelling of the situation in vivo. The design of the sample cell is such that nanoparticles cannot be confined without further modification, and thus the kinetics of nanoparticle dissolution will be reported separately. An ‘open system’ configuration (see Fig. 1a) was used to simulate the in vivo degradation and to compare the relative rates of dissolution of several types of CeramiSphere microparticles, and a commercial sample of silica microparticles with similar surface area. The CeramiSphere silica particles were synthesised using the same procedure, but with different silica precursor materials to try to ascertain the relative influence of both the particle internal structure (i.e. porosity and surface area) and chemical structure (degree of condensation) on the dissolution rate. A ‘closed system’ configuration (see Fig. 1b) was used to compare the

dissolution rate with addition of serum proteins, to better correlate the in vitro data with the in vivo situation.

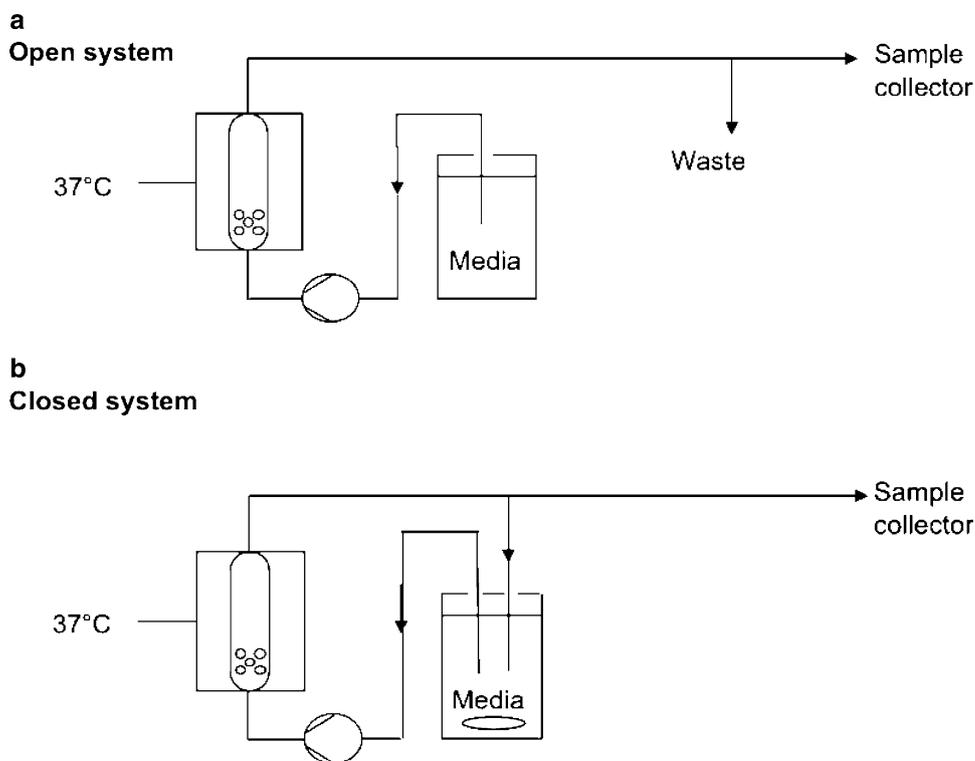
2 Experimental

2.1 Particle synthesis

CeramiSphere microparticles are formed by addition of a sol–gel silica precursor solution to a vigorously stirring surfactant solution of sorbitan monooleate or sorbitan monolaurate (Span 80 or Span 20 respectively) in non-polar solvent (typically cyclohexane or kerosene), with a mass ratio of 1:10–2:10 surfactant:solvent. While the surfactant/solvent combination primarily determines the particle size, the particle microstructure is entirely determined by the nature of the sol–gel silica precursor solution. Three different types of particles were investigated.

- (1) ‘polymeric silica’. The sol–gel solution consisted of a mixture of tetramethylorthosilicate (TMOS), water and methanol, in a molar ratio of 1:4:4 Si:H₂O:methanol. The water was adjusted to pH = 2 by addition of acid, to catalyse the hydrolysis reaction of TMOS. The sol–gel solution was stirred for 16 h to complete hydrolysis of the TMOS before addition to the surfactant solution [12].

Fig. 1 Schematic description of flow-through dissolution system. **a** ‘open’ system, in which the media is passed once only through the material and sampled continually. **b** ‘closed’ system, in which the media is continuously cycled past the material, and sampled periodically



- (2) ‘colloidal silica’. The sol–gel solution consisted of 30 wt% colloidal silica (Bindzil 30/360, Eka Chemicals). The pH was reduced from 10 to 6 by addition of an appropriate amount of acid after addition of the sol–gel solution to the surfactant solution [13].
- (3) ‘waterglass silica’. The sol–gel solution consisted of aqueous sodium silicate solution (27% SiO₂, Sigma-Aldrich) which had been diluted to 5 wt% SiO₂. The pH was reduced to 10 by treatment of the silicate solution with cationic ion-exchange resin, before addition to the surfactant solution. The pH was reduced from 10 to 6 by addition of acid, as above.

In addition to the CeramiSphere microparticles, a commercial sample of silica microparticles (Davisil silica gel, Sigma-Aldrich) was tested for comparison.

3 Particle characterisation

²⁹Si magic-angle-spinning (MAS) NMR spectra were measured to determine the relative degree of condensation of [SiO_x] units in the silica particles. High-resolution solid-state ²⁹Si MAS NMR spectra were acquired at ambient temperature using a Bruker MSL-400 NMR spectrometer ($B_0 = 9.4$ T) operating at the ²⁹Si frequency of 79.48 MHz. All measurements were performed using a Bruker 7 mm double-air-bearing probe with single pulse (Bloch decay) methods utilising high-power ¹H decoupling during data acquisition. The MAS frequencies implemented for these measurements were ~5 kHz. To ensure that a quantitative speciation analysis was achieved from these single pulse ²⁹Si MAS experiments, a single ²⁹Si $\pi/4$ pulse width of 2.5 μ s and a pre-acquisition delay of 10 μ s were used in conjunction with recycle delays of 30–60 s. All ²⁹Si chemical shifts were externally referenced to tetramethylsilane (TMS) via a high purity sample of kaolinite, and all data were simulated and deconvoluted with the Omnic spectral analysis package (Thermo Electron Corporation) to establish the relative proportion of Q₄ ([SiO₄], –111 ppm), Q₃ ([SiO₃(OH)], –102 ppm) and Q₂ ([SiO₂(OH)₂], –92 ppm) species [14] present in these samples.

Particle sizes were determined by light scattering (Malvern Mastersizer 2000) of samples suspended in water and ultrasonicated to disperse aggregates. The surface area and porosity of samples dried at 60 °C was determined by N₂ sorption analysis, using an ASAP 2001 (Micromeritics). Data was modelled using the classical BET and BJH approach using Micromeritics software. In the case of the waterglass silica, drying at 60 °C resulted in complete collapse of the micropores, forming a low surface area, dense solid. The measurement was repeated

after freeze-drying the sample to preserve the original microstructure.

The silica content of the samples was determined by thermal analysis in air, using a Setaram Tag24 Simultaneous Thermoanalyser. The silica content was calculated as the ratio of mass remaining at 800 °C to that of the initial sample mass, expressed as a percentage.

3.1 Dissolution tests

Dissolution tests were conducted using a Sotax CE7Smart USP 4 ‘flow-through’ dissolution tester. The tester is composed of seven cells, one of which serves as a control. The cells are held at 37 °C, and fitted with glass microfibre filters to prevent loss of sample. Open system tests were conducted using 200 mg samples, subjected to a flow of 2 mL min⁻¹ PBS (0.01 M). 10 mL samples were collected over a period of 1 h, for a total of 6 h. Each sample was run in duplicate, and the results averaged. Additional tests were run with specific samples to assess the influence of several experimental parameters. The effect of increasing the media flow rate was determined by comparing the dissolution rate of 250 mg samples of polymeric silica samples in a flow of 2 and 4 mL min⁻¹ PBS respectively over a period of 6 h. The effect of increasing the sample size was investigated by comparing dissolution rates of samples of colloidal silica (100, 200 and 400 mg silica respectively) subjected to a flow of 2 mL min⁻¹ PBS. Finally, the dissolution rate in simulated gastric fluid (150 mM NaCl, 5 mM PO₄³⁻, 100 μ g mL⁻¹ pepsin, adjusted to pH = 2.0 with HCl) was determined for 200 mg samples of the polymeric, waterglass and davisil silica.

Closed system tests were conducted using 5 mg samples in 100 mL of either PBS or PBS with 10% bovine serum, flowing at 10 mL min⁻¹. Each 100 mL bottle was continually stirred for the duration of the measurement. It was necessary to dilute and prefilter the serum to remove protein aggregates, and to add 200 mg L⁻¹ of sodium azide to prevent bacterial growth in the serum and resulting blockages in the system. 1 mL samples were withdrawn at nominated intervals (10 min, 1, 2, 3, 7, 13, 19, 24, 30 and 36 h) over a period of 36 h, and diluted with water to 10 mL for analysis. Each sample was run in at least quadruplicate, and the controls in duplicate, and the results averaged.

The concentration of Si in the dissolution samples was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and converted to [SiO₂]. The concentration present in the control sample run under identical conditions was subtracted from the sample result at each time point. However, this value was generally very low, typically <0.5 ppm SiO₂, and never exceeded 1.5 ppm SiO₂.

4 Results and discussion

4.1 Open system

The dissolution with time of 200 mg samples of the three CeramiSphere microparticles and one commercial (davisil) silica sample treated using the open system configuration (Fig. 1a) is shown in Fig. 2a. The particles were held in a minimum flow (2 mL min⁻¹) of 0.01 M PBS over a period of 6 h. The dissolution rate appeared relatively constant with time, with the exception of the waterglass silica particles. In this case the rate slowed considerably after 40% had been dissolved, to ~24% of the initial rate. This is most likely to be due to a considerable proportion of smaller particles (38 wt% < 1 μm) in the original sample which are expected to dissolve more rapidly than the larger particles. The colloidal silica particles were slowest to dissolve (8% dissolution in 6 h). The davisil sample dissolved slightly faster, with 11% dissolved after 6 h. The polymeric silica particles showed a median dissolution rate (26% dissolution in 6 h).

The initial dissolution rates, expressed as milli gram of silica dissolved per mg of silica in the sample, per hour, are

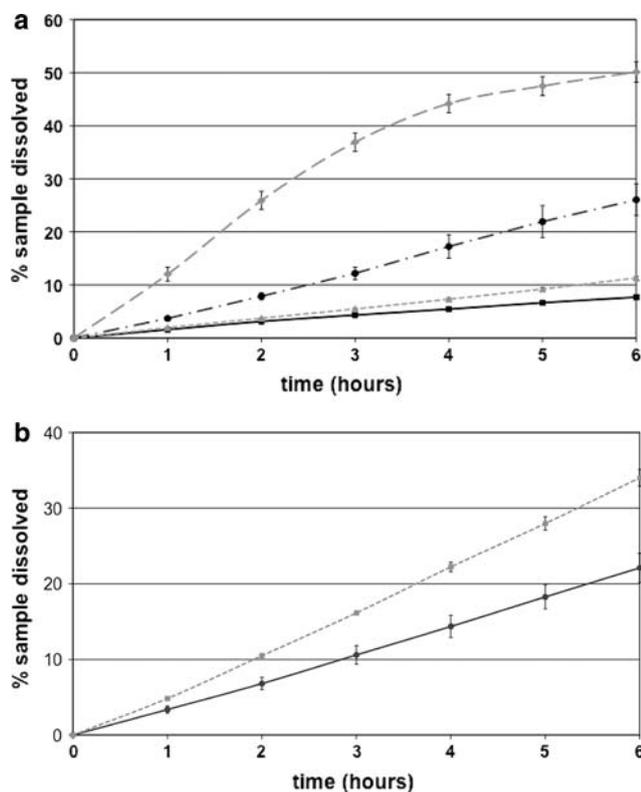


Fig. 2 Open system dissolution of **a** 200 mg microparticle samples in 2 mL min⁻¹ flow of 0.01 M PBS. (—■—) colloidal silica, (- - ▲ - -) Davisil silica, (- - ● - -) polymeric silica and (- - ◆ - -) waterglass silica, **b** 250 mg polymeric silica in (—●—) 2 mL min⁻¹ and (- - ■ - -) 4 mL min⁻¹ flow of 0.01 M PBS

listed in Table 1 alongside the most relevant physical parameters derived from the particle characterisation studies described above. The silicon connectivity is calculated as:

$$C = \sum n * Q_n$$

where n is the number of links to a neighbouring silicon and Q_n represents the proportion of silicon centres with n links to other silicon atoms according to the traditional ²⁹Si MAS NMR methodology (e.g. Q_1 denotes a silicon with one Si–O–Si bond only, whereas Q_4 denotes a silicon with 4 Si–O–Si linkages). The degree of condensation, to facilitate the comparison, is defined by Viitala et al. [8],

$$D = Q_4 / (Q_2 + Q_3)$$

where Q_n are defined as above.

It is apparent that neither particle size nor pore diameter has a significant effect on dissolution rates, at least over the micron size range compared here. Although in general, the dissolution rate trends down with increasing extent of condensation if monitored using Q_4 , this is not entirely consistent. While the polymeric sample has Q_4 , Q_3 and Q_2 proportions very similar to the waterglass sample, the dissolution rate is considerably slower. This is further exemplified by the only slight difference in connectivity and condensation observed between the water glass and polymeric sample. In contrast to what Viitala et al. [8] have postulated, the decrease in dissolution rate is not caused by an increase in the condensation (or connectivity) of the silica network. Rather, there is a clear trend linking the BET surface area with dissolution rate. This appears reasonable, given that dissolution is essentially a hydrolysis reaction, and increased surface area implies enhanced exposure of the surface to the reactant (i.e. water).

Dissolution experiments of 200 mg samples of the polymeric, waterglass and davisil silica in simulated gastric fluid at pH = 2 were also conducted. In each case, there was negligible dissolution (<0.5% after 6 h exposure), as expected given the dramatic difference in hydrolysis rates between pH 2 and 7.4 [10]. This corresponds with the observations of Begu et al. [15] who showed that impermeable silica shells deposited on liposomes were rapidly (40–60 min) dissolved at pH = 7.4 but remained intact at pH = 1.2.

The effect of flow rate was examined by comparing dissolution rates of polymeric samples (250 mg) subjected to a flow of 2 and 4 mL min⁻¹ 0.01 M PBS respectively. The resulting dissolution curves are shown in Fig. 2b. Comparison of the linear dissolution rates observed indicate that doubling the PBS flow rate resulted in a 54% increase in dissolution rate.

The effect of increasing the sample size was examined by comparing dissolution rates of colloidal silica (100, 200

Table 1 Physical parameters for the various silica samples used in the dissolution study

Analysis	Thermal	Scattering	N ₂ sorption		NMR				Degree of condensation	Dissolution
			Parameter	% silica	Mean particle size (μm)	Average BJH pore diameter (nm)	BET Surface area (m ² /g)	% Q ₄		
Waterglass	53.0	1.8	<2	>600	56.1	39.0	4.9	3.5	1.28	0.125
Polymeric	77.2	100	2.9	373	55.4	38.7	5.8	3.5	1.25	0.044
Davisil	92.9	54.8	12.7	264	73.3	24.2	2.6	3.7	2.74	0.019
Colloidal	77.3	1.3	3.1	135	78.7	19.3	2.0	3.8	3.70	0.013

and 400 mg silica respectively), subjected to a flow of 2 mL min⁻¹ 0.01 M PBS. The dissolution curves are shown in Fig. 3a. Increasing the sample size from 100 to 200 mg silica resulted in an increase of 55% in the dissolution rate (in absolute value), compared with a 66% increase from 200 to 400 mg silica. For the largest sample (400 mg), the typical dissolved silica concentrations observed were ~30 ppm. From the closed system results discussed below, we note that this is approximately the concentration above which saturation effects start to become apparent, and thus somewhat lower increases in dissolution rate would be expected for larger samples at this flow rate. Furthermore, it is interesting to note that although the mass of silica dissolved increased with increasing sample mass, the *proportion* of the sample dissolved decreased with increasing sample mass (see Fig. 3b). Both this result and the dependence of the dissolution rate on the flow rate, suggest that the dissolution of the silica particles will vary depending on their location in the body and biological parameters such as the degree of blood irrigation and the quantity injected (or ingested).

4.2 Closed system

Samples equivalent to ~4 mg silica, of the polymeric, colloidal, and davisil microparticles (i.e. those samples showing linear dissolution behaviour in the open configuration) were dissolved in 100 mL of both PBS and PBS + 10% bovine serum, using the closed system configuration. The primary aim was to determine if the proteins normally present in bodily fluids (but not included in the ‘physiological buffer’ employed here) influence the dissolution rate of silica. It was also of interest to compare the dissolution in PBS of particles held in a closed environment with increasing concentrations of dissolved silica in the cycling media, with the open system in which the media is continually refreshed. The closed system experiment was continued for 36 h, until almost complete dissolution (96%) of the polymeric particles was observed in PBS.

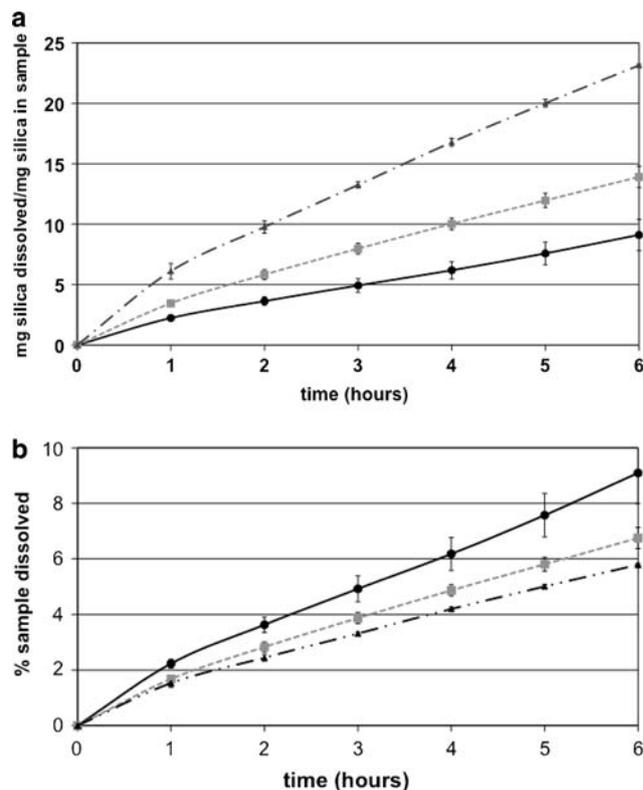


Fig. 3 Open system dissolution of (—●—) 100, (- - ■ - -) 200, and (- - ▲ - -) 400 mg colloidal silica in 2 mL min⁻¹ flow of 0.01 M PBS **a** in mg silica per mg silica in sample, **b** expressed in % sample dissolved

Figure 4a describes the dissolution behaviour of the three microparticle samples in PBS. The apparent initial lag in dissolution is due to the minimum detection limit of 2 ppm silica in this experiment, equivalent to 5% of dissolved sample. Approximately linear rates of dissolution were observed up to concentrations of ~20–25 ppm SiO₂ in solution (~60% dissolved sample), above which the dissolution rate slowed with time. As in the open system, the dissolution rate increased in the order colloidal < davisil < polymeric silica, with 67%, 88% and 96% respectively of the samples dissolved in 36 h.

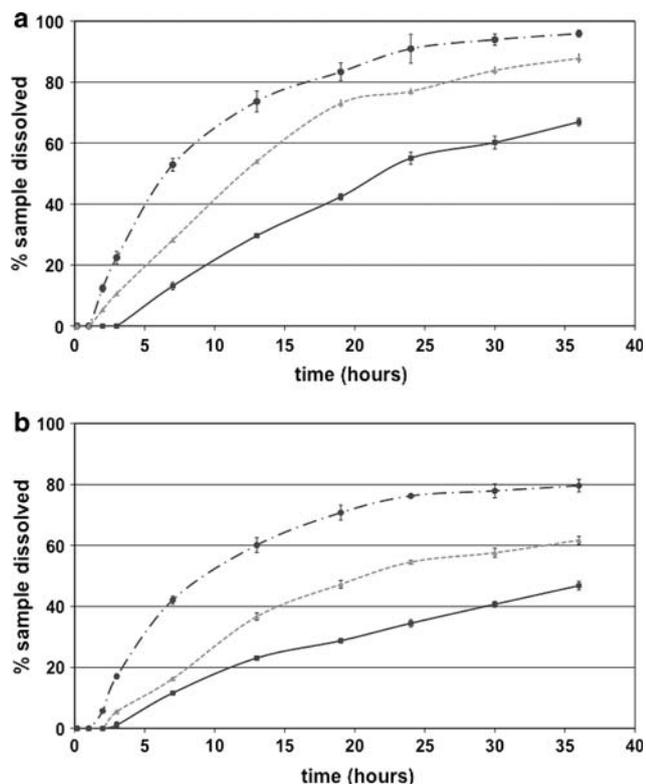


Fig. 4 Closed system dissolution of (—■—) colloidal silica, (---▲---) davisil silica, and (---●---) polymeric silica (equivalent to ~ 4 mg silica per sample) in **a** in 100 mL 0.01 M PBS, **b** in 100 mL 0.01 M PBS + 10% bovine serum

The corresponding dissolution of the three microparticle samples in 10% bovine serum is shown in Fig. 4b. A very similar pattern to that in PBS was observed, but slowed somewhat in each sample, with 47%, 62% and 80% of the colloidal, davisil and polymeric silica samples respectively dissolving in 36 h. Compared with the rate observed in PBS alone, this corresponds to a decrease of approximately 20–30%. This observation is in agreement with the findings of Falaize et al. [3], who determined rates of dissolution of sol–gel silica xerogel particles with an average size of 600 μm , in simulated body fluid (SBF) with various supplements. They found a decrease of similar magnitude (i.e. to that observed in this study) in the dissolution rate when SBF was supplemented with 10% calf serum, attributing this to the presence of serum proteins. Serum proteins such as albumin do absorb strongly on oxide particles [16, 17]. They also note little difference between dissolution rates in 10% and 100% serum, suggesting that the particle surface need only be saturated with protein to have an inhibitory effect on dissolution. This result was confirmed qualitatively both in cell transfection and in vivo experiments where a large proportion of silica particles remain intact after 24 h.

5 Conclusions

The biodegradability of a number of different sol–gel silica microparticles has been assessed using a Sotax flow-through dissolution system, using both an open and closed system configuration. Two hundred milli grams samples subjected to a minimum flow of 2 mL min^{-1} PBS showed significant dissolution after just 6 h in the open system. The initial dissolution rates observed were linear, and increased with sample surface area. For a given mass, doubling the flow rate of PBS from 2 to 4 mL min^{-1} resulted in a 54% increase in dissolution rate. Doubling the size of sample also resulted in a similar increase (55%) in dissolution rate in absolute terms (i.e. milli gram of silica) but with a smaller *proportion* of the sample dissolved as the mass increased.

Comparison of dissolution in a closed system configuration (4 mg silica in 100 mL media), showed similar dissolution patterns to those observed in the open system configuration, for the polymeric, colloidal and davisil samples. Comparing dissolution of samples dissolved in PBS, and PBS + 10% bovine serum, showed that the presence of serum proteins resulted in a ~ 20 –30% decrease in dissolution rates. The kinetics of dissolution of silica particles is also strongly dependent on the pH with virtually no dissolution taking place at pH = 2.

The implications for estimating dissolution in vivo, are that silica microparticles are expected to dissolve relatively rapidly in physiological conditions, depending on the sample size and the volume of fluid it is exposed to. Serum proteins associated with the particle surface act to reduce the dissolution rate somewhat, but do not prevent dissolution altogether. Thus the rate of elimination from the body will very much depend on the particular route of delivery, the dose of silica delivered and the local hydrodynamic conditions (e.g. blood flow).

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