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Ascorbic Acid Encapsulation in Hydrophobic Silica Xerogel

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Summary

Self-assembled hybrid organo-silica sol-gel materials are rapidly expanding for new and novel applications. The microporous solid silica matrix was used as a carrier for the controlled release of ascorbic acid (AA), selected as cargo molecule. One-step synthesis procedure was optimized for the preparation of silica–molecule composites by using tetraethoxysilane and methyltrimethoxysilane as precursors. The hydrophobic silica xerogel matrices were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction. Specific surface area and porosity parameters were determined by Brunauer-Emmett-Teller (BET) technique and the matrix surface morphology by scanning electron microscopy (SEM). The observed release pattern could be interesting for the development of AA-fortified food and for use in food packaging.

Key words: hydrophobic silica xerogel, ascorbic acid, packaging, fortified food

Introduction

A promising and simple one-step technique to make matrices for time-dependent delivery of bioactive agents is the sol-gel method. The procedure involves the gelation of colloidal solution (sol). This technique has the advantage of allowing the incorporation of highly sensitive molecules, like proteins, peptides or antioxidants into the gel (*1,2*).

Silica matrices show high biocompatibility, biodegradability (*3–5*) and resistance to microbial attack. They also exhibit higher mechanical strength, enhanced thermal stability and negligible swelling in organic solvents, compared to most organic polymers. Consequently, soluble silicates cause no adverse tissue reactions and degrade in the body as $Si(OH)_4$, which can be eliminated through the kidneys (*6*). A major advantage of the use of sol-gel is that the matrix can be generated in the presence of the cargo molecule in a one-step process. The molecule can be incorporated into the sol and distributed within the porous silica xerogel network.

The release of molecules from silica xerogel can be affected to some extent by modifying the structure of the silica matrix by changing the sol-gel synthesis procedure. Sol composition, type and course of reaction, drying temperature, sol pH, catalyst type and concentration are typical experimental parameters that have an influence on the structure and surface properties of silica xerogels prepared by hydrolysis and polycondensation of tetraethoxysilane (TEOS). Adjusting these parameters allows the change of the profile of molecule release (*7*). However, the release of a compound is usually diffusion-controlled and quite fast. Organic groups linked to the oxide network by stable chemical bonds give structural

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flexibility by reducing the degree of cross-linking and providing modified chemical reactivity, resulting in a decreased drug dissolution rate (*4,6–8*).

There are many different approaches to the sol-gel synthesis of hybrid materials where combinations of two or more chemical species precursors are used. Generally, organic modifiers such as methyltriethoxysilane (MTES), bis-1,2-(triethoxysilyl)ethane (BTSE), 3-aminopropyltriethoxysilane (APTES) and tetramethoxysilane (TMOS) are widely used to alter the morphology, surface characteristics and properties of silica xerogels made with TEOS precursor. The addition of methyltrimethoxysilane (MTMS) involves the decrease of surface silanol groups, which results in more hydrophobic surface (*8*). The hydrophobization of silica xerogels with MTMS can be summarized by the reaction scheme shown in Fig. 1.

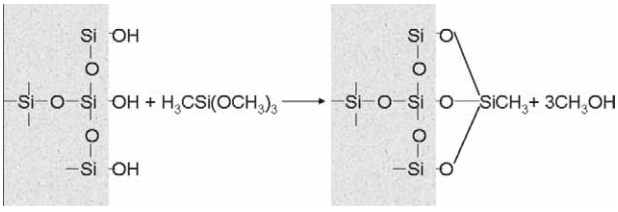

Fig. 1. Reaction scheme of hydrophobization of silica xerogels with MTMS

Ascorbic acid (AA) is an essential nutrient in human diet with well known antioxidant properties. The stability of free AA is very low at room conditions, especially due to its sensitivity to environmental surroundings such as temperature, light, oxygen, pH and water activity, which reduce its biological activity. Therefore, AA encapsulation and/or entrapment in an inert matrix is a viable alternative to be explored for the development of AA-fortified food or pharmaceuticals, which keeps AA activity protected with a longer shelf-life and better biodisponibility.

The aim of the present work is to study hydrophobic silica xerogel as a carrier for ascorbic acid (vitamin C) to be used in food and pharmaceutical packaging.

Materials and Methods

AA was incorporated into silica hybrid gels during sol-gel synthesis using tetraethylorthosilicate (TEOS), and a second precursor methyltrimethoxysilane (MTMS). Silica xerogels were made under N_2 atmosphere following one-step procedure as follows: AA (290 mg) was dissolved in a mixture of double distilled water (0.55 mL) and methanol (1.25 mL). Two precursors TEOS and MTMS were added to the mixture of AA while it was stirred. The control xerogel was synthesized without the addition of AA. The formulations of hydrophobic AA-silica composites are given in Table 1. Silica sol was cast in glass vials kept at 30 °C for polycondensation and ageing. After gelation, the resulting gel was dried at 30 °C for 10 days. All oven xerogels were treated in a vertical ball mill (Retsch, Haan, Germany) for 3 min at 200 rpm. The xerogel granules were sieved through a mesh size between 1.18 and 2.00 mm. Ascorbic acid, a white crystalline powder (Fig. 2), tetraethylorthosilicate (TEOS) and methyltrimethoxysilane (MTMS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Material characterization was performed by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

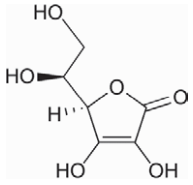

Fig. 2. Chemical structure of ascorbic acid (AA)

FTIR spectra were obtained using a Bruker IFS 66 FTIR spectrometer (Bruker, Sao Paulo, Brazil). Each sample was scanned 32 times in the 4000–400 cm^{-1} spectral range and recorded with a resolution of 4 cm^{-1}. The KBr pressed disc technique was used at two sample concentrations: 0.6 and 2 mg dispersed in 200 mg of KBr.

X-ray diffractograms of xerogels were obtained using an X'Pert PW 3050 X-ray diffractometer (PANalytical, Almelo, the Netherlands). X-ray diffraction patterns were obtained in the 2θ=2–35° region using CuKα radiation (λ=1.5405 Å) with a step length of 0.02° and a step time of 4 s.

The matrix surface morphology was examined by scanning electron microscopy (SEM). The microscopic analysis was carried out with a Philips SEM 505 instrument (Philips, Rustburg, VA, USA). Samples were dried at room temperature and covered with a gold layer.

Specific surface area and porosity parameters were determined using the Brunauer-Emmett-Teller (BET) technique based on nitrogen gas adsorption. The experiments were carried out on 0.2 g of sample in a Micromeritics ASAP 2020 (Micromeritics, Norcross, CA, USA). Before adsorption analysis, the samples were degassed for at least 24 h at 40 °C at the degasification port of the adsorption apparatus, with a residual vacuum of 0.67 Pa.

For AA release studies, tests were performed at 37 °C and 150 rpm under sink conditions. Desorption of AA was studied in aqueuous medium at pH=5.0. As a dissolution medium for degradation of the xerogel matrix, 0.1 M HCl was used. The test volume of dissolution medium was 2 mL. At each sampling interval, a 0.6-mL

Table 1. Formulation of hydrophobic AA-silica composites

Sample	*n*(TEOS)	*n*(MTMS)	*n*(MeOH)	*n*(H_2O)	*n*(AA)	pH	Gelation time
	mmol	mmol	mmol	mmol	mmol		day
Silica	27.3	14.0	61.7	49.0	–	9.0	2
Silica+AA	27.3	14.0	61.7	49.0	1.64	2.3	5

sample was withdrawn from each flask and replaced immediately with an identical volume of fresh medium. The release tests were performed using the fraction size in the range from 1.18 to 2.0 mm obtained from sieving.

The AA concentration in the samples was evaluated by isocratic HPLC method. The HPLC system comprises a Waters Symmetry C18 column (4.6×150 mm, 5 μm particle size), photodiode array detector Waters 2996 (λ=242 nm) and Waters 2465 electrochemical detector (operating mode: direct current, potential 0.15 V). The mobile phase consisted of buffer (0.02 M phosphate, pH=2.5, adjusted with phosphoric acid/methanol (99:1 by volume)) and was run at a flow rate of 1.0 mL/min, and the injection volume was 10 μL. The experiments were carried out twice, and the values in the graph represent the mean average values.

Results and Discussion

N_2 adsorption studies and SEM were carried out on unloaded xerogel for matrix characterization. The adsorption isotherm (not shown), indicates a microporous material with a mean pore size of 0.96 nm and specific surface area of 3.83 m^2/g. The estimated density of AA-xerogel was 0.3 g/cm^3. SEM image (Fig. 3) of silica xerogel appears as a dense material with a rough surface because the nanosize pores cannot be resolved using SEM.

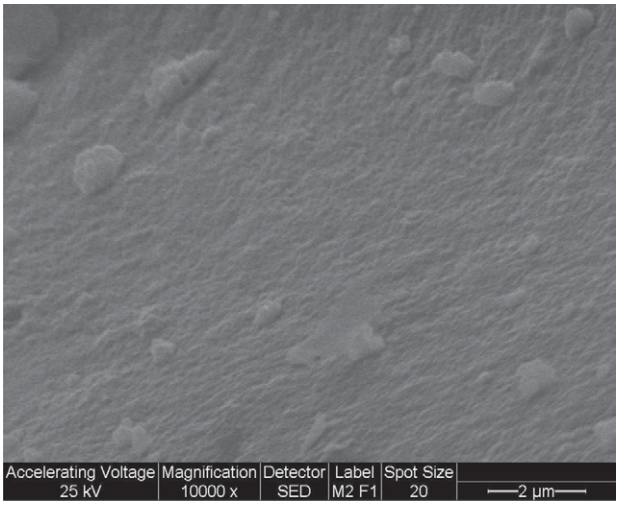

Fig. 3. SEM micrographs of silica xerogel. Highly porous silica xerogels appear as a dense material with a fine structure

The X-ray spectrum of unloaded xerogel, in Fig. 4 marked with a, presents two broad reflection lines. The reflection lines peak at 2θ=11 and 23 degrees, corresponding to d values of 0.790 and 0.386 nm, respectively. The line at 2θ=23 with a peak width of 10 degrees originates from the disordered O-Si-O continuous random network of the amorphous material and may be related to the contribution of the 1st Si neighbours (*9*), while the line at 11 degrees could be assigned to the microstructure, *i.e.* to the xerogel pore structure. The characteristic size associated with this line (0.790 nm) agrees reasonably well with the mean pore size obtained using BET (0.96 nm).

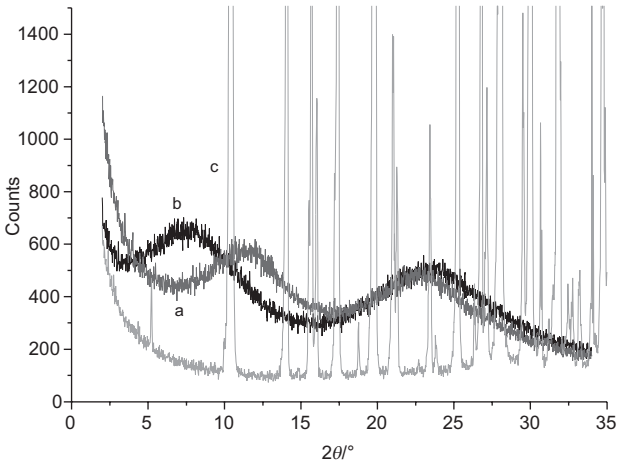

Fig. 4. XRD spectra of xerogels loaded and unloaded with AA. Spectra: a: silica, b: silica with AA, and c: free crystalline AA

The X-ray spectra obtained for AA-loaded xerogel (Fig. 4, marked with b) showed that the first amorphous halo shifts to lower angles, from 11 to 7.5 degrees (d= 1.160 nm), while the second one remained almost unshifted. This shift indicates that the microstructure was expanded when AA was incorporated. However, this expansion is not uniform, as evidenced by the small change observed in the second halo angular position. This kind of shift is usually recorded at the intercalation of ascorbic acid into the layered structures.

Precipitation of the AA molecules in the silica xerogel was excluded on the basis of XRD. This result reveals consistently that AA molecules were incorporated into the pores of the amorphous silica xerogel. The size of AA molecule is 0.49 nm in length by 0.60 nm in width and 0.36 nm thickness.

FTIR spectra of silica xerogels are shown in Fig. 5. The characteristic vibrational peaks of SiO_2 at 780 cm^{-1} (Si-$O_{(b)}$, bending mode) and 1070 cm^{-1} (Si-$O_{(s)}$, asymmetric stretching mode) are displayed in the spectra of silica gel. The range of 1200–1080 cm^{-1} contains information about the degree of condensation (Si–O–Si bridges) and the presence of –SiOH groups on the surface. While

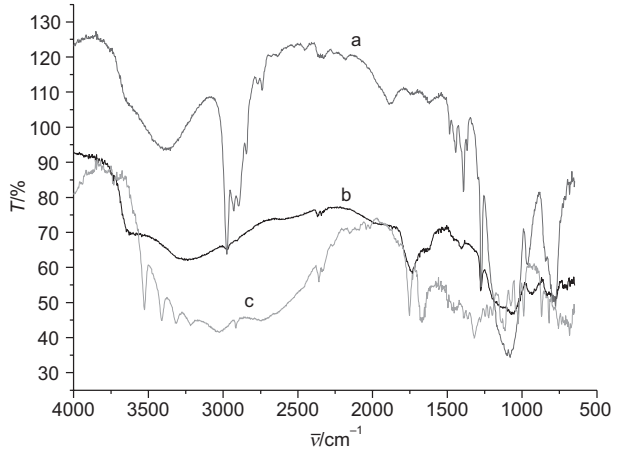

Fig. 5. FTIR spectra of AA and xerogels loaded and unloaded with AA. Spectra: a: silica, b: silica with AA, and c: free crystalline AA

the former may affect the mechanical properties of the sample, the latter determines the surface wettability of silica, which may have influence on the overall silica--drug interactions. However, when AA was entrapped in the xerogel, the carbonyl stretching ($C=O_{(s)}$) mode of AA was shifted from 1754 to 1738 cm^{-1}, indicating a strong interaction with the matrix. In addition, a displacement of at least one hydroxyl group stretching ($O\text{-}H_{(s)}$) mode was shifted from 3624 to 3628 cm^{-1}, which was possible because of the H-bridge formation with SiO_2 matrix.

AA release profile from xerogels is shown in Fig. 6. The hydrophobic silica gel was very stable in aqueous medium at pH=5.0. About 60 % of AA was released from the xerogel between 3 to 4 h. A closer analysis shows that the curves for the modified sample can be divided into two regions: the first region enclosing the period from 0 to about 10 min with a rapid drug dissolution (burst effect), followed by the second region with a considerably slower average dissolution rate. The AA release process from a solid carrier is essentially governed by both matrix dissolution and diffusion (*10*), which are strictly linked with the physicochemical properties of the matrix itself. In particular, both hydrophilic/hydrophobic character of the matrix and the nature of interaction between the incorporated molecule and the surface of the carrier play a key role. In the case of the hydrophobic silica matrix, AA can interact with a few surface silanol groups only by weak interactions (mainly by hydrogen bonding) and this could probably explain its slightly faster release kinetics.

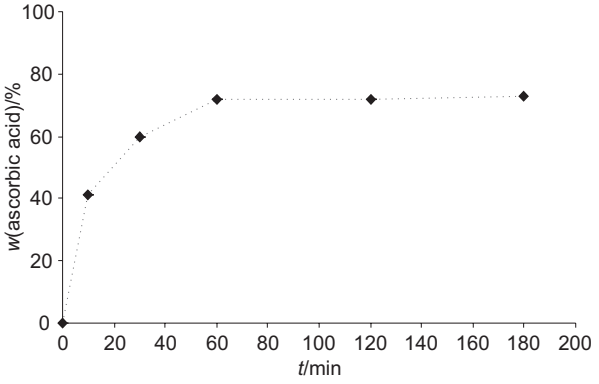

Fig. 6. Kinetic release profile of AA xerogel expressed as percentage of AA relative to the total loaded amount

AA is released from initially uniformly AA-loaded xerogel particles towards the surrounding liquid solution. Assuming xerogel spherical particles with mean radii of ~0.8 mm and taking into account the relative volumes α of solution to xerogel used in the experiments, the amount $m(t)$ of released AA at time t relative to the total amount of AA can be estimated as follows (*11*):

$$\frac{m(t)}{m_\infty} = 1 - \left(\frac{1}{1+1/\alpha}\right)\sum_{n=1}^{\infty}\frac{6\alpha(1+\alpha)e^{-D_{eff}q_n^2 t/a^2}}{9+9\alpha+q_n^2\alpha^2} \qquad /1/$$

where q_n is a function of α. The sum was performed up to the tenth term T_{10} since $T_{10}/T_1 \sim 10^{-2}e^{-3\cdot10^{-2}t}$. Comparison of $m(t)/m_\infty$ with the experimental results led to an effective diffusion coefficient of AA in xerogel at room temperature of $D_{eff} \sim 1.8\cdot10^{-7}$ cm^2/s (Fig. 7). It can be observed that the agreement is quite good up to $t^* \sim 3600$ s,

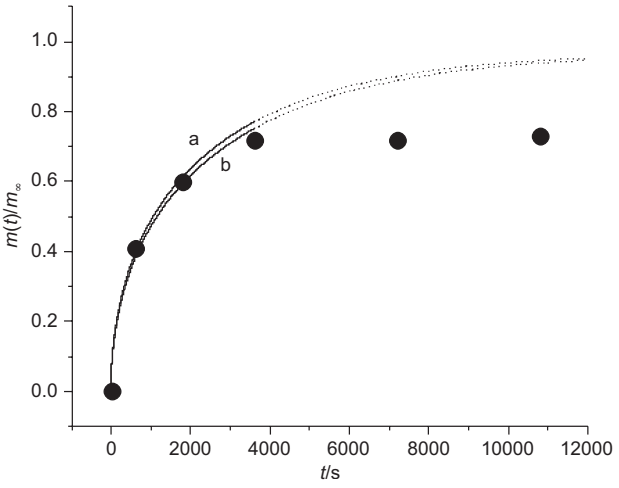

Fig. 7. AA release from xerogel. Dots: experimental results. The lines represent two release curves estimated from Robinson *et al.* (*11*) using D_{eff} values of $1.90\cdot10^{-7}$ and $1.75\cdot10^{-7}$ cm^2/s, respectively

and that then the experimental release saturates quite abruptly at $m(t)/m_\infty \sim 0.73$, indicating perhaps that some AA may be trapped in the isolated xerogel pores. The experimental results suggest that a change of the release regime may occur at times near t^*, which will be the subject of further study. The diffusion coefficient of AA in water at 25 °C is $D=(5.3 \text{ to } 6.0)\cdot10^{-6}$ cm^2/s (*11*). Taking into account the combined effect of xerogel porous radius r and of porous wall drag on the diffusion process (*12*), and using ~0.24 nm for the AA molecule mean radius and r~0.58 nm (from XRD results), another estimation of D_{eff} is performed: $D_{eff} \sim 6.2\cdot10^{-7}$ cm^2/s, which is 3.4 larger than the previous value. This can be ascribed to the additional hindering effects produced by mutual partial pore blocking of AA molecules along the pores.

Conclusions

The present study shows that ascorbic acid was incorporated in a surface-modified silica xerogel acting as a carrier system. Drug release can be controlled by partial substitution of TEOS with alkyl-substituted alkoxysilanes (MTMS) as monolithic matrix precursor. The release of AA from xerogel silica granules up to 1 hour can be described using a diffusion model, leading to an effective diffusion constant of AA in aqueous medium (inside the xerogel structure) of $D_{eff} \sim 1.8\cdot10^{-7}$ cm^2/s. This value can be understood by considering porous radius and porous wall drag effects plus additional mutual partial pore blocking effects of AA molecules along the pores. The results could present a potential for a wide range of applications in the development of fortified food packaging and drug delivery.

Acknowledgements

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