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Nanostructural control of the release of macromolecules from silica sol–gels

Shula Radin, Sanjib Bhattacharyya, and Paul Ducheyne*

Department of Bioengineering, Center for Bioactive Materials and Tissue Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

The therapeutic use of biological molecules such as growth factors and monoclonal antibodies is challenging in view of their limited half-life in vivo. This has elicited the interest in delivery materials that can protect these molecules until released over extended periods of time. Although previous studies have shown controlled release of biologically functional BMP-2 and TGF- β from silica sol–gels, more versatile release conditions are desirable. This study focuses on the relationship between room temperature processed silica sol–gel synthesis conditions and the nanopore size and size distribution of the sol–gels. Furthermore, the effect on release of large molecules with a size up to 70 kDa is determined. Dextran, a hydrophilic polysaccharide, was selected as a large model molecule at molecular sizes of 10, 40 and 70 kDa, as it enabled us to determine a size effect uniquely without possible confounding chemical effects arising from the various molecules used. Previously, acid catalysis was performed at a pH value of 1.8 below the isoelectric point of silica. Herein the silica synthesis was pursued using acid catalysis at either pH 1.8 or 3.05 first, followed by catalysis at higher values by adding base. This results in a mesoporous structure with an abundance of pores around 3.5 nm. The data show that all molecular sizes can be released in a controlled manner. The data also reveal a unique in vivo approach to enable release of large biological molecules: the use more labile sol–gel structures by acid catalyzing above the pH value of the isoelectric point of silica; upon immersion in a physiological fluid the pores expand to reach an average size of 3.5 nm, thereby facilitating molecular out-diffusion.

Keywords

Sol-gel; Silica; Controlled release; Drug delivery; Nanomaterials

1. Introduction

Large biological molecules have short half-lives in vivo. As a result, there is significant interest in administering these molecules with delivery vehicles that protect them and that can deliver them over extended periods of time. Previously, room temperature processed silica sol–gel controlled release materials were studied for the delivery of a variety of biological molecules, including macromolecules such as the transforming growth factor TGF- β 1 and the bone morphogenetic protein BMP-2 [1–10]. These studies showed that these large molecules were released in a controlled fashion from nanoporous sol–gel materials. In addition, both TGF- β 1 and BMP-2 were released as biologically functional molecules. Separately, it was also shown that silica sol–gel to which calcium and

phosphorous oxides were added stimulated osteogenic activity of rat stromal marrow cells with and without the addition of BMP-2 [3]. The osteogenic activity resulted from BMP-2, which was present in the medium or released from the sol-gel carrier, and which acted synergistically with the Ca-P layer formed on the sol-gel substrate [3]. In another study, it was also shown that trypsin inhibitor used as a model protein with a size similar to those of the osteogenic growth factors was released in a time-and load-dependent manner. Release durations of 9 weeks and more were measured [2].

The studies briefly mentioned here demonstrate that large molecules with molecular weight (MW) in the range of 20–22 kDa can be incorporated into sol-gels and can be released in a functional form. These studies, however, also revealed the limitations of using nanoporous sol-gels with a pore size below 2 nm (i.e. microporous gels) for the release of these large molecules. In fact, the release of TGF- β 1 was slow, with only 0.5% of the initial load of 500 ng being released by 1 week of elution [1]. In addition, the release of trypsin inhibitor from these porous sol-gels also progressed slowly, albeit at a continuous zero-order release rate [2].

As sol-gel technology has been pursued with the intent to entrap large molecules [11,12], it was thought that large biomolecules could only be released by rendering sol-gel materials bioresorbable [8]. Herein, we pursue controlled release by enlarging the pore size at the nanoscale. We hypothesize that the previously observed limited release of the various macromolecules from the sol-gels was caused by a pore size equal to or below 2 nm. This pore size is typical for sol-gels made using acid catalysis under low-pH conditions (at a pH below 2, which is the isoelectric point of silica [13]). Thus, we theorize that increasing the pore size of sol-gels will facilitate the release of large molecules.

It is known that the pore size and porosity of sol-gels can be varied by modifying such sol-gel processing parameters as the pH of the hydrolysis reaction, the water and alcohol content, and the catalyst selected [13]. Thus, in this study we set out to increase nanopores by selecting catalysis pH values with the potential to enlarge this nanopore size, specifically intermediate-pH values (pH 3–7 [13]).

A unique aspect of the study is the selection of the molecule to determine the effect of its size variation on the release from the present nanostructured sol-gel controlled release materials. With the objective of focusing uniquely on the effect of size without any extraneous influence arising from variations in molecule chemistry, we selected a model molecule the size of which can vary over a wide range. Model macromolecules that have been used before include trypsin inhibitor [2], bovine serum albumin [8] and lysozymes [14]. We selected dextran conjugates, a polymer of biological origin which is available in sizes ranging from 3 to 500 kDa. In this study, we used three sizes, 10, 40 and 70 kDa, and we determined the relationship between this increasingly large molecular weight of the incorporated molecule, on the one hand, and the processing parameters, the nanostructural properties prior to and subsequent to simulated in vivo conditions, and the release kinetics, on the other hand.

2. Methods and materials

2.1. Experimental design

In a first series of experiments we determined the effect of varying sol-gel processing conditions on the characteristics of the porous nanostructures, including the pore size distribution of the sol-gels prior to and after immersion in phosphate buffer solution (PBS). We used either acid catalysis at pH 1.8 or 3.05, or two-step acid-base processing, with the pH of the second step ranging from 5.35 to 7.25. In these first experiments we did not

incorporate any molecules in the sol–gels. In the second series of experiments, however, we did, and we restricted the selection of processing conditions to those that yielded a wide variation in pore size as followed from the first series of data. We have extensively described typical sol–gel processing conditions before [1–4]. Herein, we describe those parameters and parametric values that are of significance to this study.

2.2. First series of experiments – catalysis conditions

The processing parameters used for the synthesis of sol–gels in the intermediate-pH range are listed in Table 1.

For the one-step acid-catalyzed process at pH 1.8 or 3.05, 0.1 N HCl and 0.1 N glacial acetic acid were used to catalyze the hydrolysis of the silica precursor tetraethoxysilane (TEOS; Sigma–Aldrich, St Louis, MO) [13]. The sols were synthesized by mixing and stirring TEOS, deionized (DI) water and HCl or glacial acetic acid. A 0.7 ml volume of the acid solution was used for 10 ml of TEOS until a single-phase, hydrolyzed sol–gel solution was formed. The molar ratio of water to TEOS (the ratio R) was 8. When hydrolyzed, the pH of this sol–gel was 1.8 or 3.05 (Table 1) and the time to formation of the hydrolyzed sol varied from 70 to 90 min. The time to gelation of this sol at room temperature varied from 24 to 30 h.

The liquid sols were cast into 22 mm diameter polyethylene vials (1 ml per vial) and allowed to gel in sealed vials. Subsequent to gelation and aging for 2 days, the vials were unsealed and the wet gels were allowed to dry at room temperature until the weight was constant. In addition to drying at room temperature, the gels were dried at 37 °C for 24 h. The dried sol–gels (xerogels) were ground and sieved to obtain granules in the size range 150–350 μm .

With respect to the two-step acid–base processing, we first catalyzed the hydrolysis reaction at a pH 1.8 by mixing TEOS, 0.1 N HCl and DI water ($R = 8$). When the hydrolysis was complete, the acid-catalyzed sol was cooled down to 4° °C in an ice bath. Subsequently, various amounts of 0.1 N NH_4OH solution were added to the sol to increase the pH to a value above 5, and the pH and the time to gelation of these acid–base catalyzed sols (ABC sols) were monitored. A Solvotrode combined electrode designed for measuring pH in non-aqueous solutions (Metrohm, Riverview, FL) was used. Processing parameters of the acid–base catalysis (pH of the sol) and the time to gelation are summarized in Table 1. In a similar way as for the xerogels prepared in one step, the ABC sols were cast into plastic vials and allowed to gel and dry at room temperature. This was followed by grinding and sieving to produce granules in the size range 150–350 μm .

2.3. Characterization of the porous structure

Gas (nitrogen) sorption analysis (BET analysis; Autosorb 1, Quantachrome, Boynton Beach, FL) was used for the characterization of the properties of the porous structure (specific surface area (SSA), pore volume (PV) and pore diameter (PD)). Sol–gel granules without dextran were outgassed at 50 °C for 24 h. Absorption–desorption isotherms and multipoint BET methodology were used [15]. The pore size distribution was determined using desorption isotherm data and the program based on the Barrett, Joyner and Halenda (BJH) numerical method [15].

In addition to the analysis of as-synthesized materials, we also determined the changes in pore properties caused by in vitro immersion in conditions akin to controlled release conditions. Using similar experimental principles as in previous studies [16], sol–gel granules were immersed in PBS (pH 7.4) at a weight-to-solution ratio of 5 mg ml^{-1} for durations ranging from 3 to 72 h. The immersion experiments were conducted in an

incubator at 37 °C and the specimens were placed on a shaker table at 150 rpm. After immersion, the granules were rinsed with ethanol, dried in an oven at 37 °C and outgassed at 50 °C for 24 h.

2.4. Synthesis of sol–gels with incorporated dextran

Dextran–Texas Red-conjugated molecules with MWs of 10, 40 and 70 kDa (Molecular Probes, Eugene, OR) were added to the sol–gels under conditions that took into consideration the sensitivity of this molecule to low pH, temperature and light. These precautions were also continuously applied during storage, handling and experimentation, including the immersion tests. Dextran is not stable at pH values below 3, thus the pH of hydrolyzed sols was always above 3 when any dextran was added.

The processing conditions selected for two dextran loads (10 and 20 mg per g of silica in the sol–gel) are summarized in Table 2. Synthesis conditions followed from the first series of experiments (synthesis conditions given in Table 1 and some results in Table 3). Acetic acid catalyzed sol–gels (AC sol–gels) were processed at pH 3.05 and ABC sol–gels were subsequent to a first step at pH 1.8 brought to a pH between 5.5 and 5.7, such that the time to gelation would be between 15 and 25 min at 4 °C. Dextran of various MWs was added to the sols in DI water solutions (25 mg ml⁻¹).

As shown in Table 1, a further increase in the pH would have resulted in a sharp reduction of the time to gelation, which renders the incorporation and uniform mixing of the dextran molecules tenuous. Nevertheless, considering the beneficial increase in pore size at pH values above 7 (see Table 3), a three-step process, with the final step at pH above 7, was also used for the dextran incorporation. The three-step process included the previously described two-step process, with dextran incorporation at the second step at pH ranging from 5.5 to 5.7. The dextran-containing sols were cast into plastic 22 mm diameter vials and predetermined amounts of the base were added to the sols to achieve a pH above 7. The base addition resulted in a short duration of gelation (1–2 min).

2.5. In vitro release measurements

In vitro release of dextran was determined using PBS (pH 7.4, 37 °C), with vials placed on a shaker table at 150 rpm. The solution was exchanged at predetermined time periods (between 1 and 35 days). Dextran-containing granules with a size range as before (150–350 µm) were immersed in the PBS solution at a weight-to-solution volume ratio as before (5 mg ml⁻¹). Amber vials were used for the immersion experiments and for storage of solutions containing dextran. The concentration of dextran–Texas Red conjugates in solution was determined using an ultraviolet/visible light spectrophotometer (Ultraspec Plus, Pharmacia LKB, Piscataway, NJ) at 595 nm.

3. Results

3.1. The effect of processing conditions on the porous structure of as-synthesized sol–gels

The sorption analysis showed that the absorption-desorption isotherms of the two types of sol–gels (the AC and the ABC sol–gels) were very similar. The AC sol–gel catalyzed at pH 1.8 or 3.05 and the ABC sol–gel processed at a final pH 5–6 were characterized by type I isotherms typical of “microporous” materials (materials with a pore size not exceeding 2 nm). With increasing pH value of the ABC processing above pH 6, there was a gradual transition from the type I isotherm to the type IV isotherm typical of “mesoporous” materials (materials with a pore size ranging from 2 to 50 nm). The isotherms of the ABC sol–gels processed at a pH above 7 showed a full transition to type IV isotherms.

The BET characteristics of the porous structures derived from the isotherms are shown in Table 3. These characteristics include the SSA, total PV, and average and peak PD. Parameters studied also included the pore size distribution (PD vs. PV) and Figs. 1 and 2 show the effect on pore size distribution of increasing the pH from a value of 3.05 (Fig. 1) to a value above 7, namely 7.05 or 7.25 (Fig. 2).

As shown in Fig. 1 and Table 3, AC sol–gels catalyzed at pH 1.8 and 3.05 and ABC sol–gels processed in the pH range from 5 to 6.7 are micro–mesoporous materials with an average pore size of 2.2 nm. The pore size distribution in both materials (Fig. 1) shows a large volume of micropores with a pore size ranging from 1.6 to 2 nm, along with a minor presence of mesopores with pores ranging from 2 to 5 nm. Although an increase in pH from 1.8 to 6.7 did not affect the pore size, it did affect the PV and the SSA, and led to a noticeable increase in these parameters (Table 3).

As shown in Fig. 2 and Table 3, a major shift towards a mesoporous structure occurs when acid–base catalyzed sol–gels are processed at a pH value above 7. The pore size distribution in the acid–base catalyzed sol–gels processed at pH 7.05 and 7.25 shows a major presence of pores in the range from 2 to 6 nm and the appearance of a peak pore diameter (PPD) centered at 3.5 nm (Fig. 2). The data reveal that a large portion of the pores now has a size in the 3–4 nm range. The increase in the pore size is also accompanied by a significant increase in the SSA and PV (Table 3).

3.2. The effect of immersion on the porous structure

Immersion in PBS for 24 h did not affect the isotherms of the acid catalyzed sol–gels: type I isotherms typical for microporous materials were observed before and after immersion. In contrast, there was a major effect of immersion on the isotherms of the ABC sol–gel: there was a major shift from type I isotherms before immersion to type IV isotherms typical for mesoporous materials after immersion. Fig. 3 shows the time-dependent effect of immersion on the pore size distribution of the ABC-1 sol–gel and the gradual change from a microporous to a mesoporous material arising from immersion in PBS. The effect of immersion on the BET characteristics of the AC and ABC sol–gels are also summarized in Table 4.

The mesoporous character is clear, given that the pore range is 2–8 nm after immersion. Very quickly (this is, already by 3 h of immersion) a peak starts to develop at 3.5 nm, implying that a large number of pores form with a size centered around this dimension. The further increase in the peak height with immersion time indicates a continuous increase in the volume of pores with a size in the 3–4 nm range. By 72 h of immersion, there was a total shift to the mesoporous range and a major increase in the volume of the 3–4 nm pores. Additional BET data specifically related to the PV are included in Table 4.

The findings of the sorption analysis also suggest two possible principles for achieving the goal of higher release kinetics of larger molecules by pursuing the release from larger pores in sol–gels. First, use acid–base two-step processing with a basic step at a pH above 7; or, second, use acid–base processing with a basic step at a pH between 5 and 6 and achieve larger pores upon subsequent immersion in biological fluids. Both methods result in the formation of mesoporous structures with a large portion of pores in a small size range of 3–4 nm. The advantage of acid–base processing at lower pH values, below pH 6, is the longer time to gelation.

3.3. Release kinetics of dextran with varying size

The cumulative release of dextran molecules with MWs of 10, 40 and 70 kDa from the AC sol–gels is shown in Fig. 4. The release kinetics of dextran molecules from both the AC and

ABC sol-gels is plotted comparatively in Fig. 5a–c. The effect of MW and dextran load (10 vs. 20 mg g⁻¹) on the dextran release from the acid-base catalyzed sol-gels is shown in Fig. 6.

All dextran molecule sizes showed a time dependent release from the AC sol-gels (Fig. 4). The data also reveal a decrease in the rate of release with increasing MW: 40% of the 10 kDa molecules was released by day 25, but only 25 and 16%, respectively, of the 40 and 70 kDa molecules. In addition, the larger molecules showed a stage of delayed release: no release could be detected for several days.

Fig. 5a–c shows the difference in release kinetics of dextran molecules from the AC sol-gels (processed at pH 3.05) and the ABC sol-gels (processed at pH 5.5 and 5.7). The data reveal enhanced release of all dextran molecules for the ABC sol-gels in comparison to the AC sol-gels. In fact, whereas 44% of the 10 kDa dextran was released from the AC sol-gel by day 30, the release from the ABC sol-gel was 64% (fig. 5a). The enhanced release from the ABC sol-gels was also observed for the larger dextran molecules (40 and 70 kDa). The initially delayed release of these large molecules from the AC sol-gel was not, however, observed for the release from the ABC sol-gels (Fig. 5b and c). The amount released was also significantly enhanced in the case of both large molecules. Whereas the release of 40 and 70 kDa molecules from the AC sol-gels by day 23 was 25 and 16%, respectively, the release of these molecules from the ABC sol-gels was increased to 36 and 24%, respectively.

Fig. 6 shows the effect of MW and the dextran load (10 vs. 20 mg g⁻¹) on the release from the ABC sol-gels processed at pH 5.5–5.7. Similar to the release of dextran of various MW from the AC sol-gels, the release rate of dextran molecules from the ABC materials also decreased with increasing MW. It also follows from Fig. 6 that the release of 10 and 40 kDa molecules from the ABC sol-gels was load dependent and that the amount released increased with the load. In contrast, the release of the large 70 kDa molecules was load independent. There is also a major difference in the release profiles of the dextran molecules with different MWs. Whereas both the 10 and 40 kDa molecules showed release kinetics approximating first order, the 70 kDa molecules showed release approximating zero order.

When the release data of the 10 and 40 kDa molecules shown in Fig. 6 were analyzed as a function of the square root of time, the resulting graphs (not shown) included an initial 1 day delayed release, followed by a linear relationship. The linearity of the released concentration versus square root of time might suggest that the release of the 10 and 40 kDa dextran molecules from the ABC sol-gels can be described by the Higuchi model for the diffusion controlled release from porous matrices [17]. The initial 24 h delay in release might be related to the time needed for the transformation of the ABC sol-gels into mesoporous materials during immersion. When the mesoporous structures with an enlarged pore size are formed, the initial delayed release is followed by a stage of faster, diffusion controlled release.

Regarding the ABC sol-gels with a final, third synthesis step at pH exceeding 7, the release of the large 40 and 70 kDa dextran molecules is further enhanced (data are included in Table 5 without graphical representation). A comparison between the ABC sol-gels processed at a pH between 5 and 6 and the ABC sol-gels synthesized first at the same pH between 5 and 6, followed by a final third step above pH 7, showed faster initial release kinetics and a greater cumulative release over 3 weeks for the latter. The data in Table 5 show an additional release of both the 40 and 70 kDa molecules by 21 days of immersion of about 10%. The increased release is presumably associated with the formation of a mesoporous structure with enlarged pore size and greater porosity resulting from the catalysis at pH 7.05.

4. Discussion

In this study we explored the ability to affect and enhance the release of macromolecules from nanostructured sol–gels by varying the porous structures through modification of the sol–gel synthesis conditions. We also addressed the question whether nanoporous structures could be produced that, upon immersion in a physiological solution, would enable expedient release of macromolecules. Dextran–Texas Red conjugated molecules with molecular weights of 10, 40 and 70 kDa were used as model macromolecules. Sol–gel processing in the intermediate pH range (3–8) was used for the synthesis of dextran-containing sol–gels.

The sorption data analysis showed that the porous structures of the AC sol–gels (synthesized with a final pH at 1.8) and the ABC sol–gels (synthesized with a final pH slightly below or at about 5.7) are not significantly different. Both materials are micro–mesoporous, with an average pore diameter of 2.2 nm. Only ABC sol–gels synthesized with a second step at a pH above 7 showed a shift to mesoporosity with a major presence of pores in a narrow size range of 3–4 nm. The hydrolysis reaction occurs fast at higher water acidity than at lower water acidity [18]. In higher acidic water conditions, smaller clusters are formed due to fast hydrolysis and slower condensation. As smaller clusters aggregate, small pores form. In low acidic conditions, larger clusters are formed due to fast condensation and slower hydrolysis, and when a large amount of water is used, as in this case ($R = 8$ compared to $R = 4$ for the normal sol–gel reaction), this effect becomes more prominent when the pH is 7 or higher (alkaline) (Fig. 7). Interestingly, we also found that similar mesoporous structures with a major presence of 3–4 nm pores and a peak pore diameter at 3.5 nm develop as a result of immersion in PBS (the pH of which is 7.4). A comparison of Figs. 2 and 3 shows that the mesoporous structures produced by ABC synthesis at pH above 7 and those resulting from immersion in the PBS solution with a pH of 7.4 were similar. In the case of ABC synthesis, after 2 h of hydrolysis at the lower pH value of 1.8, the pH of the sol is increased to >5 . At this higher pH, the rate of hydrolysis is very slow and may be suppressed. As a result, the final sol–gel silica network may contain ethoxy groups. Furthermore, when these ABC gels were immersed in PBS at pH 7.4, these ethoxy (OCH_2) groups were replaced by hydroxyl (OH) groups.

Since the size of the hydroxyl group is smaller than that of the ethoxy group, the pores will be relatively enlarged after substitution.

The present observations imply that the room temperature silica sol–gel synthesis first catalyzed at an acidic pH (1.8 or 3.05) is still subject to major structural rearrangement, whether it is in a second synthesis step catalyzed at higher pH values (5–6 or above 7), or as a result of immersion in a physiological solution, which of course has about neutral pH values. These observations suggest a unique approach for releasing large macromolecules in vivo: incorporate macromolecules in labile sol–gels that will undergo a structural transformation in vivo to arrive at nanostructures of mesoporous variety.

Since physiological immersion of ABC-processed silica with the second step at a pH between 5.5 and 5.7 leads to a major enlargement of the pores, we used this ABC process for all experiments whereby dextran macromolecules were incorporated into the sol–gel. All molecular sizes were successfully incorporated and released in a long-term controlled, time-dependent manner. The release rates decreased with increasing MW.

In addition to a decrease in the release rate, the release of the larger molecules, 40 and 70 kDa, from the AC sol-gels processed at pH 3.05 was delayed for several days (Fig. 4). However, as the data show, the two-step ABC process with the second catalysis step at a pH in the range from 5 to 6 enhanced the release rate of all molecular sizes of dextran. It is worth mentioning here that nanoconfinement of a large polymer does not have any effect on its molecular weight, although it can affect the molecular dynamics and chain conformation [19,20]. Furthermore, the delay of release of the 40 and 70 kDa molecules from the AC sol-gels was significantly less when incorporated using the ABC catalysis (Figs. 5 and 6). In particular, the analysis of the cumulative release vs. square root of time showed that the delay in release was within the time period of the first measurement point (day 1). During this time, the mesoporous structures form and the pores are greatly enlarged. This observation is supported by the sorption analyses: differences in the release kinetics from the AC and ABC sol-gels were associated with major differences in the porous structures of these sol-gels after immersion in PBS (pH 7.4). Whereas the AC sol-gel remained mostly microporous, there was a remarkable change in the structure of the ABC sol-gel, from being mostly microporous before immersion to mostly mesoporous after immersion. The size of the mesopores was in the range from 2 to 10 nm, with the majority being in the narrow size range from 3 to 4 nm.

5. Conclusion

In summary, by using dextran conjugates with molecular weight between 10 and 70 kDa as model molecules, this study demonstrates that these large molecule sizes can be incorporated and then released in a controlled manner from silica sol-gels processed in the intermediate-pH range (5–6). The study also shows an MW-dependent release of the dextran molecules, and a decrease in the release rate with increasing MW. The release is extensively affected by the formation of a mesoporous nanostructure. The formation of favorable mesoporous structures having a major presence of 3–4 nm mesopores can be achieved via acid–base processing with the last catalysis step in the pH range above 7. However, even when this mesoporous structure is not present upon synthesis via acid–base processing with the last catalysis step at a pH below 7, it develops subsequently by immersion in physiological solutions (PBS, pH 7.4).

Acknowledgments

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1–7, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at <http://dx.doi.org/10.1016/j.actbio.2013.04.039>.

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Fig. 1.

Desorption volume (Dv) as a function of PPD in as synthesized sol-gel AC-1 (acetic acid catalyzed at pH 3.05). The pore size distribution indicates that the material is micro-mesoporous, with a major presence of micropores (pore diameter below 2 nm) and some mesopores in the size range from 2 to 5 nm. The pore size distribution in the ABC-1 sol-gel (acid-base catalyzed at pH 5.35) was similar.

Fig. 2.

Desorption volume as a function of PD in ABC-5 and ABC-6 sol-els (acid-base catalyzed at pH 7.05 and 7.25, respectively). The data show that both materials are mainly mesoporous, with pores ranging from 2 to 7 nm. The peak at a pore diameter of 3.5 nm indicates that the majority of the mesopores were in a narrow size range between 3 and 4 nm

Fig. 3.

Desorption volume as a function of PD in the acid–base catalyzed sol–gel ABC-1 after immersion in PBS for time periods ranging from 0 to 72 h. The immersion conditions were set at a weight-to-solution volume ratio of 5 mg ml^{-1} . The immersion produced a major change from a mostly microporous to a mostly mesoporous material having pores between 2 and 10 nm. The appearance of a major peak at 3.5 nm indicates that the majority of the pores were centered around this size.

Fig. 4. Cumulative release of dextran molecules from acetic acid catalyzed sol-gel as a function of time of immersion. When the molecule size was 10, 40 and 70 kDa, 40, 25 and 16%, respectively, was released at 23 days of immersion.

Fig. 5. Comparative cumulative release, as a function of time of immersion, of dextran with a molecule weight of 10 kDa (a), 40 kDa (b) or 70 kDa (c) from either AC or ABC sol-gels at a dextran load of 10 mg g⁻¹.

Fig. 6. Cumulative release ($\mu\text{g ml}^{-1}$) of dextran from acid–base catalyzed (pH 5.5) sol–gels as a function of MW, dextran load and immersion time in PBS. The release is expressed in $\mu\text{g ml}^{-1}$ to show the effect of various loads (two loads were used). Both 10 and 40 kDa molecules showed a load-dependent, first-order release. In contrast, the release of larger 70 kDa molecules was a slower, zero-order release which did not visibly depend on the dextran load.

Fig. 7. Variation in the size of the pore formation. At lower pH, small clusters form, which results in a small pore size; at higher pH, larger clusters give rise to larger pores.

Table 1

Processing of AC and ABC sol–gels and the effect of pH on the time to gelation of ABC sol–gels.

Sample	Process	pH (first step)	pH (second step) ^a	Time to gelation at 4°C (min)
AC-1 ^b	One-step acid catalyzed	3.05	n/a	n/a
AC-2 ^c	One-step acid catalyzed	1.8	n/a	n/a
ABC-1	Two-step acid-base catalyzed	1.8	5.35	>30
ABC-2	Same	1.8	5.5	25
ABC-3	Same	1.8	5.7	15
ABC-4	Same	1.8	6.7	5
ABC-5	Same	1.8	7.05	3
ABC-6	Same	1.8	7.25	1.5

The same water/TEOS molar ratio of 8 was used during all acid catalyses.

^a pH for the second step of the ABC process samples varied with the amount of the 0.1 N NH₄OH basic solution added with step 2.

^b 0.1 N glacial acetic acid was used as the catalyst of the AC-1 sol–gel.

^c 0.1 N HCl was used as the catalyst for AC-2 and for the first step hydrolysis of all ABC sol–gels.

Table 2

Synthesis of AC and ABC sol-gels with dextran conjugates of various molecularweights and various loads (mg per g silica).

Sol-gel	Process	pH of the second step of two-step ABC process	MW (kDa)	Load (mg g ⁻¹)
AC	AC	/	10	10
AC	Same	/	40	10
AC	Same	/	70	10
ABC	Two-step ABC	5.7	10	10
ABC	Same	5.7	10	20
ABC	Same	5.5	40	10
ABC	Same	5.5	40	20
ABC	Same	5.6	70	10
ABC	Same	5.6	70	20

Table 3

SSA, PV, and average and peak PD of AC and ABC sol–gels.

Sample	pH (last step)	SSA (m ² g ⁻¹)	PV (cc g ⁻¹)	Average PD (nm)	Peak PD at 3.5 nm	PD range (nm)	Type of porosity
AC-1	3.05	404	0.22	2.18	/	1.5–5.0	Micro–mesoporous
AC-2	1.8	386	0.21	2.17	/	1.5–5.0	Micro–mesoporous
ABC-1	5.35	525	0.29	2.19	/	1.5–5.0	Micro–mesoporous
ABC-2	5.5	483	0.26	2.2	/	1.5–5.0	Micro–mesoporous
ABC-3	5.7	477	0.26	2.2	/	1.5–5.0	Micro–mesoporous
ABC-4	6.7	703	0.39	2.23	/	1.5–5.0	Micro–mesoporous
ABC-5	7.05	732	0.45	2.47	Yes	1.5–7.0	Mainly mesoporous
ABC-6	7.25	750	0.48	2.56	Yes	2.0–8.0	mesoporous

The peak PD and the PD range were determined using desorption isotherm data and the BJH numerical method [15]. The appearance of the peak PD centered at 3.5 nm was only observed for ABC sol–gels processed above pH 7.

Table 4

Changes in nanoporosity of acetic acid catalyzed AC-1 and ABC-1 sol-gels vs. immersion time in PBS

Sample	Time in PBS (h)	SSA (m ² g ⁻¹)	PV (cc g ⁻¹)	PD (nm)	PD range (nm)	Peak PD (nm)
AC-1	0	404	0.22	2.18	1.5–4.0	/
AC-1	24	431	0.24	2.19	1.5–5.0	/
ABC-1	0	525	0.29	2.19	1.5–4.0	/
ABC-1	3	575	0.31	2.19	1.5–5.0	/
ABC-1	10	550	0.32	2.32	1.5–5.0	3.5
ABC-1	24	540	0.35	2.55	1.5–8.0	3.5
ABC-1	72	368	0.28	3.03	2.0–10.0	3.8

Table 5

The effect of the final pH of the ABC processing on the cumulative release (%) of dextran molecules with a molecular size of 40 and 70 kDa

Incorporated dextran molecule	Number of pH steps	pH of the final step	% Released by day 21
40 kDa	2	5.5	35
40 kDa	3	7.05	45
70 kDa	2	5.5	25
70 kDa	3	7.05	34