Controlled Dissolution of Organosilica Sol−Gels as a Means for Water-Regulated Release/Delivery of Actives in Fabric Care Applications

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Organosilica sol−gels functionalized with amino groups are used for the controlled release of encapsulated molecules including biomolecules. These gels prepared by hydrolysis of aminosilane precursors are characterized by water-induced changes in network formation and dissolution and as a result can be used as controlled release matrixes on the basis of the water content of the medium. The gels were initially evaluated for release of simple organic dyes such as Congo red as a result of the water-induced dissolution of gels. These gels can also be used as a means for controlled release of encapsulated entities including enzymes such as proteases that are used in fabric care applications. The rapid dissolution of these gels when placed in water-rich media provides an effective strategy for release and/or delivery of actives with potential utility in fabric care applications. The encapsulated enzymes are able to retain their activity and remain stable for extended periods. The gels were characterized by FTIR spectroscopy, which indicates the formation of a loosely held network of hydrogen-bonded particles in these gels. The results indicate potential utility of these materials as versatile delivery matrixes where the release of actives can be initiated by facile water-induced dissolution of these organosilica sol−gels.

Introduction

Controlled release strategies rely on efficient trigger mechanisms that can be regulated precisely at the molecular level. Efficient regulation of physicochemical properties of the host matrix that can be coupled with release of actives is the key to controlled release strategies.[1,2] In this direction, there exist several strategies for controlled release ranging from degradation or dissolution of the host matrix to porosity changes and/or altered physicochemical interactions between the matrix and encapsulated molecules.[3−8]

Advances in biotechnology have spurred the use of enzymes in personal care and fabric care applications, and a large number of laundry detergent formulations employ enzymes for effective cleaning mechanisms.[9] Alkaline proteases are among the most widespread enzymes used in fabric care applications. However, long-term stabilization of enzymes in detergents presents unique challenges. The hostile environment of detergents usually leads to aggregation, precipitation, denaturation, and loss of activity of enzymes.[10] An additional consideration is the activity of proteases, which leads to autolytic degradation of isolated alkaline proteases in the basic environment of detergents.[11] Finally, long-term stability of the enzymes in an environment that goes through constant changes in temperature remains an issue in the practical application of these enzymes in detergents.

At present, enzymes are widely used in the detergent industry.[12] Proteases added to detergents bring about hydrolysis of the peptide linkages in protein-based stains and thereby provide useful benefits to cleaning of fabrics.[13] Preventing denaturation of the protein molecule in the multicomponent detergent environment consisting of linear alkyl benzenesulfonates and other ionic and anionic surfactants presents considerable challenges.[14,15] The problem is further heightened when mixtures of enzymes are used in detergents to serve different purposes. A multienzyme composition containing proteases poses additional stability issues since the enzyme hydrolyzes peptide linkages of all the other proteins including itself. As a result, there is a current need for matrixes that can be used for immobilization,

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stabilization, and subsequently fast release of the proteases when placed in a water-rich environment to elicit the desired function during normal fabric care applications.[16]

The stringent criteria posed by the specifications of the enzymes used in detergents necessitate use of specialized materials that can provide not only the required stability of the enzymes but also the physical isolation of the proteases as well as fast release when placed in an aqueous medium. While organic polymers can provide the stability for the enzymes, their dissolution in water is limited. Additionally, depending upon the nature of the polymeric matrix, the process of immobilization may not necessarily provide the spatial isolation of protease to prevent autolytic and heterolytic degradation of proteins in the presence of proteases. Similarly, degradable matrixes exhibit slow rates of release due to inherent limitations of the chemical pathways that accompany the process of chemical degradation of the polymeric structure. As a result, such materials are widely used for slow release applications.[4] On the other hand, the criteria for the enzymes in detergents dictate that the proteins are released during the normal fabric care process, which requires a fast release of actives that can be accomplished within minutes. Consequently, materials that exhibit fast dissolution kinetics are ideal matrixes for immobilization of enzymes that are typically used in fabric care applications.

The ability to entrap the (bio)molecules through physical encapsulation in a suitable matrix provides a strategy to overcome these issues. Immobilization also helps in conserving the conformation of the protein backbone, which is essential for their activity. Immobilization of the biomolecules has been shown to be successful with organic polymeric matrixes[17,18] or inorganic silicate matrixes.[19−30] The release of the trapped molecules from the carrier could be by diffusion,[31,32] through changes in the structure of the matrix which take place as a response to external stimuli such as pH[33] and temperature,[34] or, alternatively, by degradation or dissolution of the host matrix.[35−40] The degradable host matrix can be designed to disintegrate as a response to certain environmental variables,[41] and this approach has been widely used in drug delivery applications.

In this context, sol−gel-derived materials provide an efficient matrix that can be used for encapsulation and release of enzymes. While encapsulation of molecules including proteins and enzymes is well established, the design of efficient strategies for controlled release of encapsulated molecules from a sol−gel remains a focus of current research. Sol−gel-derived materials furnish a chemically inert and mechanically robust matrix for encapsulation. Room-temperature experimental protocols make the sol−gel processes amenable for encapsulating the biomolecules. Silicate matrixes can be tailored to create a microenvironment suitable for a particular protein. For example, lipases are found to be stabilized in a hydrophobic environment.[20] Silicate matrixes formed with precursors containing functional groups (e.g., amino, hydroxyl, and carboxyl groups) are known to be hydrophilic in nature, while those formed with silane precursors containing alkyl pendant groups are known to possess a hydrophobic environment.[42] Recently, selective intake and release of molecules including proteins has been reported from organosilica sol−gels that contain both hydrophilic and hydrophobic groups as part of the network.[43]

Herein, we report a strategy for encapsulation, stabilization, and controlled release of molecules from sol−gel matrixes made from different amino-functionalized alkoxysilane precursors which have been shown to be useful for immobilization of biomolecules.[44−46] These gels are water-soluble, and release of encapsulated molecules including the protease enzyme subtilisin can be effected when the gels are placed in water. Initially, the sol−gel compositions were optimized using Congo red dye as the dopant because of its optical properties, which facilitate monitoring of the release process by optical spectroscopy. Next, the gels were evaluated for their stabilization and release of subtilisin. These sol−gel matrixes bring about controlled release of the encapsulated enzyme molecules as a response to a change in the water content of the medium. One of the objectives of the study involving stabilization and controlled release of subtilisin in

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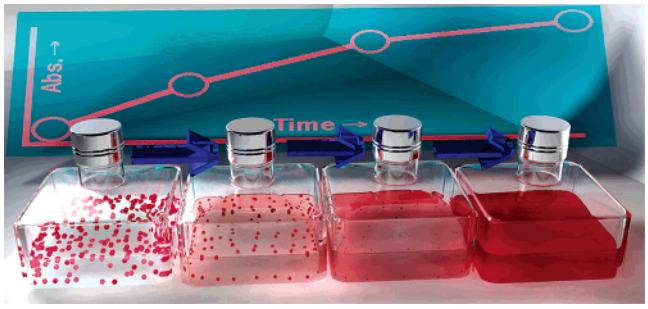

Figure 1. Dissolution-controlled release from organosilica sol−gels: a graphical representation of the process of dissolution of sol−gels that accompanies release of the encapsulated molecules along with a schematic representation of changes in absorbance of the solution as a function of time that can be used to monitor the release process.

sol−gel matrixes was to develop sol−gel-derived matrixes, which can release protease by fast dissolution in water. The sol−gel matrixes are readily soluble in water and release encapsulated molecules through matrix dissolution (Figure 1) such that the release time can be controlled by a judicious choice of precursors to regulate the dissolution.

Experimental Section

Materials. The sol−gel precursors [*N*-(2-aminoethyl)-3-aminopropyl]trimethoxysilane, (3-aminopropyl)trimethoxysilane, ([3-(trimethoxysilyl)propyl]diethylenetriamine, [*N*,*N*-bis(2-hydroxyethyl)-3-aminopropyl]triethoxysilane, [*N*-(hydroxyethyl)-*N*-methylaminopropyl]trimethoxysilane, (*N*,*N*-diethyl-3-aminopropyl)trimethoxysilane, and tetramethoxysilane were procured from Gelest Inc. (Tullytown, PA). Congo red dye was purchased from Acros, and subtilisin protease (Multifect P3000, assay 34 mg/mL), which is used in commercial liquid detergent formulations, was supplied by Genencor International Inc. (Palo Alto, CA). Trizma preset crystals (pH 8.6) (tris(hydroxymethyl)aminomethane (Tris) and Tris hydrochloride) and 2-morpholinoethanesulfonic acid (MES) buffers were obtained from Sigma.

Preparation of Pristine Sol−Gels. The different sol−gel samples used in this study are labeled K1−K5. The precursors used to make these samples along with the mole fractions of the different precursors are summarized schematically in Figure 2. K1 was prepared with 0.5 mL of (3-aminopropyl)trimethoxysilane (2.86 mmol), 0.5 mL of [*N*-(2-aminoethyl)-3-aminopropyl]trimethoxysilane (2.23 mmol), and 0.4 mL (22.2 mmol) of H_2O. K2 was prepared with 1 mL of [3-(trimethoxysilyl)propyl]diethylenetriamine (3.67 mmol) and 0.4 mL of H_2O. K3 was prepared with 1 mL of (3-aminopropyl)trimethoxysilane (5.73 mmol) and 0.4 mL of H_2O. K4 was prepared with 0.5 mL of (3-aminopropyl)trimethoxysilane (2.86 mmol), 0.5 mL of [*N*,*N*-bis(2-hydroxyethyl)-3-aminopropyl]-triethoxysilane (1.41 mmol) and 0.4 mL (22.2 mmol) of H_2O. K5 was prepared with 0.5 mL of [*N*-(hydroxyethyl)-*N*-methylaminopropyl]trimethoxysilane (1.58 mmol), 0.5 mL of (3-aminopropyl)-trimethoxysilane (2.86 mmol), and 0.4 mL (22.2 mmol) of H_2O. A reference sol−gel was prepared with (*N*,*N*-diethyl-3-aminopropyl)-trimethoxysilane and tetramethoxysilane (TMOS) for the IR studies. Initially, TMOS sol was prepared by sonicating a mixture of 1.5 mL of TMOS, 0.4 mL (22.2 mmol) of H_2O, and 0.022 mL (0.04 M) of HCl for 30 min. The reference sol−gel was obtained immediately upon addition of 0.25 mL of (*N*,*N*-diethyl-3-aminopropyl)trimethoxysilane to 0.5 mL of TMOS sol.

Preparation of Dye-Containing Sol−Gels. Typically, the dye-containing sol−gels were prepared in polypropylene beakers by adding 0.4 mL of aqueous Congo red dye solution (0.032 M) to 1 mL of the silane precursor (K2 and K3) or 1 mL of a silane precursor mixture (K1, K4, and K5). The gelation period of the prepared dye-containing gels varied between 1 min and 3.5 days (Table 1). Gels were aged for 2 days under ambient conditions in uncovered polypropylene beakers before dye release experiments were carried out.

Preparation of Enzyme-Containing Sol−Gels. The various sol−gel compositions (K1−K5) prepared contained the silane precursor(s) as mentioned in the above section. Two sets of enzyme-containing sol−gels were prepared. In one set (prepared in duplicate), to 1 mL of silane precursor(s) was added 0.25 mL (13.88 mmol) of H_2O. This was immediately followed by the addition of 0.25 mL of subtilisin. The sol was stirred with a stir bar as H_2O and formulated enzyme (enzyme with water, propylene glycol, and sodium formate) were added. All the components form a clear and homogeneous sol readily. The final pH of the mixture was found to be about 8.5. The gelation period varied between 2 min and 10 days. Gels were aged for 2 days under ambient conditions in uncovered polypropylene beakers before being tested for enzyme activity. These gels did not exhibit any visible shrinkage during the aging period. Another set of gels (K1−K5) after being aged for 2 days were covered with Parafilm and stored under ambient conditions for long-term release studies.

In another set of enzyme-containing sol−gels, the sols were prehydrolyzed by adding 0.25 mL (22.2 mmol) of H_2O to 1 mL of the organosilane precursors. The prehydrolyzed sols were "aged" for 30−90 min (Table 1) depending on the composition. After the aging period of the prehydrolyzed sol, 0.25 mL of formulated enzyme was added with stirring. The viscous, aged sol was readily soluble in the subtilisin concentrate (with about 50% water), giving a uniform sol once again. The sols turn viscous once again in 30−90 min and form a soft gel in about 24 h to 10 days. K2 could not be prepared following this protocol because of its short gelation period (2 min). Gels were aged for 2 days before being tested for enzyme activity. Another set of gels after being aged for 2 days were covered with Parafilm and stored under ambient conditions for 1 month to monitor release.

Release of Encapsulated Molecules from Sol−Gels. The dye-containing sol−gels were powdered with a mortar and pestle, added to 100 mL of water in a 250 mL beaker, and stirred with a 1 in. × 0.25 in. magnetic stir bar at 200 rpm. The release of the dye molecules was followed spectrophotometrically by monitoring the absorbance at wavelengths of 340 and 500 nm at regular time intervals until no further change in absorbance was observed. Enzyme-containing sol−gels were powdered by gentle crushing with a pestle in a mortar. The powdered sol−gels (amounts given in Table 1) were then added to 10 mL of water and the resulting mixtures stirred with a magnetic stir bar. The release of protease was evaluated through an assay of the external aqueous medium. For typical assay measurements, stirring was momentarily stopped to allow the particles to settle down and an aliquot of 10 μL of the clear supernatant solution was pipetted out. The aliquot was diluted 500 times with 10 mM MES buffer (pH 5.5) containing 10 mM $CaCl_2$ and 0.005% Tween before the assay was carried out. The release was studied until a constant enzyme assay value was obtained.

Enzyme Activity Assay. The released enzyme from the sol−gels was estimated spectrophotometrically by carrying out an assay at 25 °C in Tris buffer (pH 8.6). *N*-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide (suc-AAPF-pNA) (100 mg/mL of DMSO) was used as the substrate molecule. The enzyme released from the sol−gels hydrolyzes the amide linkage of the substrate molecule, thereby generating the yellow-colored chromophore *p*-nitroanilide, which can be monitored spectroscopically. The rate of release of *p*-

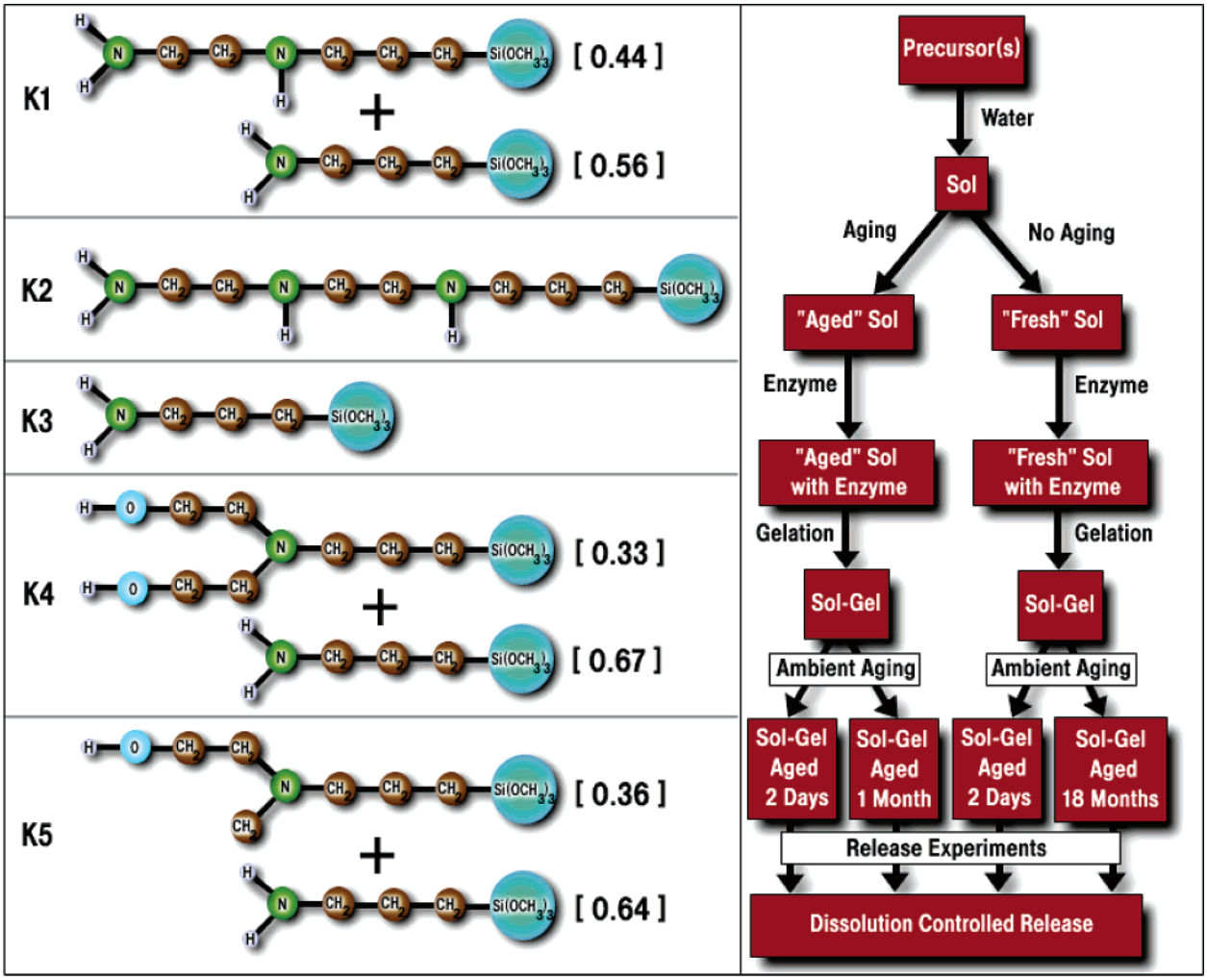

Figure 2. Schematic depiction of different precursors used in the preparation of sol−gels along with their mole fractions (left panel) and the overall experimental strategy for investigating the dissolution-controlled release (right panel).

Table 1. Aging Period of the Sols, Gelation Period, Dissolution Period, and Melting Points of the Sol−Gels

sol−gel	aging time[a] (min)	amt of gel with enzyme (g)	gel dissolution time[b] (min)	gelation time			gel dissolution time[c] (min)			melting point[d] (°C)
				pristine gels	with dye	with enzyme	pristine gels	with dye	with enzyme	
K1	30	0.573	10	23 h	23 h	10 d	4.79	5.32	3.44	72.4
K2	0	0.6102	20	4 min	1 min	2 min	7.63	6.63	4.44	133
K3	30	0.5	15	15 h	14 h	24 h	4.65	6.61	4.84	46.9
K4	90	0.4589	13	3.5 d	3.5 d	10 d	4.71	8.18	4.47	59.8
K5	90	0.4876	14	3 d	3 d	10 d	4.13	7.23	4.59	64.9

[a] The time period between the preparation of sol and addition of the formulated enzyme to the sol. [b] Time taken to completely dissolve the amounts of enzyme containing gel mentioned in column 3 in 10 mL of water with stirring. [c] Dissolution of 0.1 g of powdered gel in 10 mL of water with stirring with a stir bar. The time represents the average of three samples. [d] Obtained from DSC data.

nitroanilide (measured from the absorbance at 410 nm) forms the basis of the assay. Typically, to 1 mL of Tris buffer (10 mM Trizma, pH 8.6, 10 mM $CaCl_2$, 0.005% Tween) in a cuvette was added 10 μL of the enzyme-containing sample solution followed by 10 μL of the substrate, and the mixture was stirred for 1 min. After 1 min of stirring, the rate of change of absorbance at 410 nm was recorded for 10 s. The percentage of enzyme released from the sol−gels is calculated by comparing the assay of enzyme released with respect to the assay of total enzyme added to the sol−gels.

Instrumentation. A Hewlett-Packard model 8453 UV−vis spectrophotometer was used to monitor the release of dye molecules and for enzyme activity assays. ATR-FTIR analysis was carried out on a Thermo Nicolet Nexus 670 FTIR ESP unit fitted with an Avatar multibounce HATR smart accessory. The spectra were recorded in the range 4000−500 cm^{-1} with 200 scans and a resolution of 2 cm^{-1}. Attempts were made to obtain TEM micrographs of the sol−gels using a Hitachi 7100 TEM instrument. However, only a few of the prepared sol−gels (K2 and K3) could give meaningful data. The other gels were found to be unstable under the conditions. Differential scanning calorimetry (DSC) of the weighed amounts of dried sol−gels (3.4 mg) was carried out on a Mettler-670e DSC unit. Samples were heated from 0 to 200 °C at the rate of 10 deg/min.

Results and Discussion

Release of Congo Red Dye from Sol−Gels. The sol−gel compositions were initially optimized for encapsulation and controlled release of Congo red dye molecules. The dye molecules were used in the initial optimization experiments

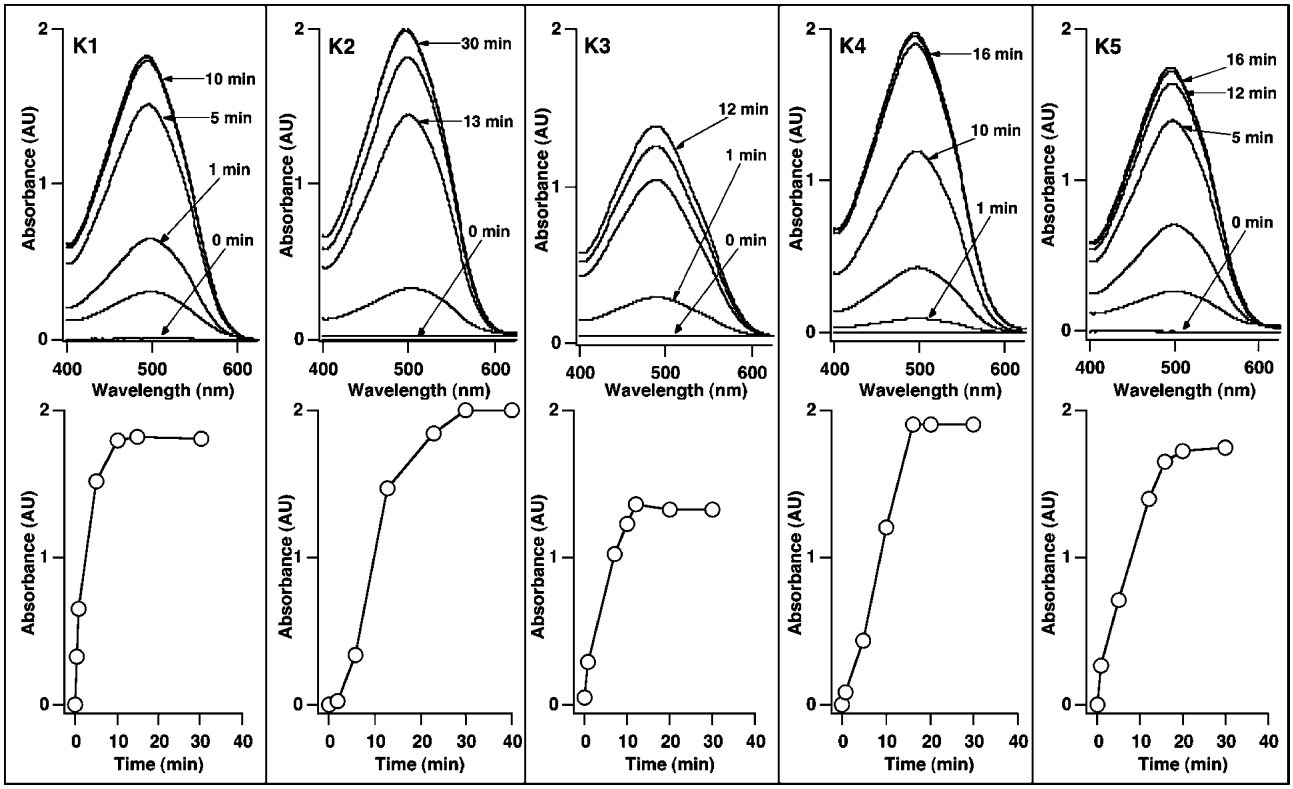

Figure 3. Release of Congo red dye monitored at 500 nm. The top panel shows the changes in UV−vis spectra with respect to time, and the bottom panel shows the absorbance profiles as a function of time for each gel system.

because of the ease of monitoring the release of the colored dye molecule. The UV−vis spectra of the aqueous medium in which the controlled release of the dye-containing gels was carried out are given in Figure 3. Congo red gives a peak at 500 nm. As the dye is released from the sol−gels, the absorbance at 500 nm is found to increase with time and reach a maximum (Figure 3). At this point it was observed that the sol−gel matrix dissolved completely, giving a clear red-colored solution. Release of the dye molecules by dissolution of the sol−gel matrix clearly indicates that the encapsulated dye is released from these matrixes by degradation of the host matrix. The process of complete dissolution of the aged sol−gels took about 10−15 min for the sol−gels labeled K1, K3, K4, and K5. On the other hand, K2 samples took ca. 30 min to release the encapsulated dye when placed in 10 mL of water. The time taken for dissolution of a constant measured amount (0.1 g) of dye-containing gels in 10 mL of water is shown in Table 1.

Release of Subtilisin from Sol−Gels. The enzyme-containing sol−gels were prepared using two different protocols. In the first method, the enzyme-containing gels were prepared by addition of formulated enzyme, immediately after addition of water to the siloxane precursor mixture. However, the assay of the enzyme released from these sol−gels indicates significant loss of enzyme activity possibly due to heat released during the hydrolysis and condensation reactions of the siloxane precursors with water or denaturation of the enzyme due to interaction with the silane precursor monomers. Similarly, the exothermic acid−base reactions of aminosilane precursors likely generate a substantial amount of heat, and the temperature of the mixture after addition of water to silanes was found to increase by about 10 °C. To confirm that this heating of the mixture was the cause of denaturation, experiments were carried out by mixing the enzyme and precursors along with water kept in an ice bath to form the K3 gel samples. These gels made at low temperature were found to retain 100% of the enzyme activity. Therefore, to prevent such denaturation of enzymes, a modified protocol was used. In the modified protocol, the siloxane/water mixture was allowed to "age" for 30−90 min prior to addition of the enzyme. On addition of water, trialkoxyaminosilanes readily undergo hydrolysis;[47,48] however, letting the sol stand under ambient conditions for some time helps the system to achieve equilibrium conditions and also allows the sol to return to room temperature after the initial exothermic reactions. The activity of the enzymes was found to increase dramatically when the prehydrolyzed sol was used, indicating a lesser degree of denaturation and better retention of native activity. The changes in the sol−gel protocol do not affect the gelation or dissolution time of the sol−gels but enhance the stability of the enzyme when the prehydrolyzed sol is utilized for encapsulation of the enzyme. Gels prepared by both the methods were evaluated for their release properties.

The enzyme-containing sol−gels dissolve completely in water in about 10−20 min (Table 1). To measure relative differences in their dissolution time, a measured amount (0.1 g) of sol−gel with enzyme was dissolved in 10 mL of water, and the results are reported in Table 1. The mechanisms of release of the dye molecules and the enzyme are similar in

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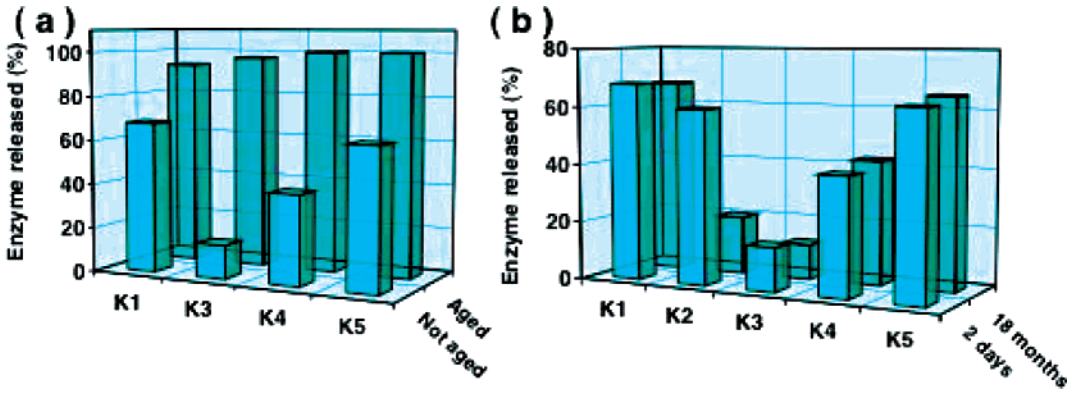

Figure 4. (a) Percentage of subtilisin released by dissolution of gels made from sol which was prehydrolyzed or "aged" under ambient conditions and sol which was not prehydrolyzed or "not aged" prior to addition of subtilisin. (b) Percentage of subtilisin released by dissolution of gels aged for 2 days and 18 months under ambient conditions. The gels were prepared by immediate addition of the formulated enzyme to the sol.

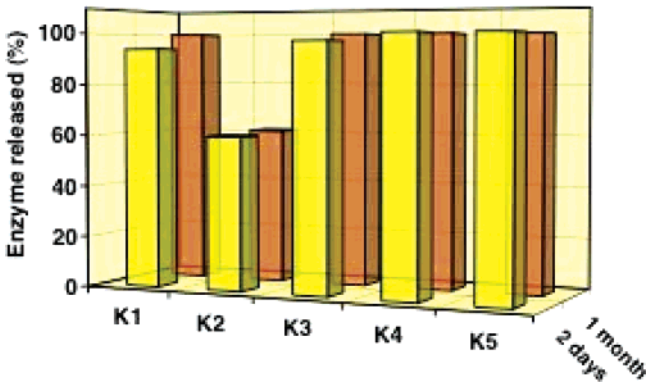

Figure 5. Percentage of subtilisin released upon dissolution of sol−gels aged for 2 days and for 1 month. All the sol−gels except K2 were prepared by adding enzyme to a prehydrolyzed aged sol.

these systems, namely, through dissolution of the host matrix. Since the encapsulated subtilisin is released by dissolution of the sol−gels, it is prudent to assume that all the enzyme molecules are released and there is no unreleased subtilisin trapped in the matrix. As such the percentage of enzyme released from these dissolving sol−gels reflects the active enzyme retained during the encapsulation process. Figure 4 shows release of enzyme from gels. Encapsulation in sol−gels K1, K2, K4, and K5 (without aging of sols) retained about 60−70% of the enzyme activity, while K3 prepared with (3-aminopropyl)trimethoxysilane retained very low enzyme activity (13%). All the gels (except K2), which were prepared without aging of the sol, retain the same activity even after 18 months (Figure 4b). On the other hand, samples prepared from aged sol retain almost all of the activity (Figure 4a). The results suggest the absence of denaturation of subtilisin within the sol−gel matrixes over a period of time. Similarly, with samples prepared from a prehydrolyzed and aged sol, the activity is substantially enhanced (Figure 5) as compared to that of samples prepared with fresh sol (Figure 4). Furthermore, as shown in Figures 4b and 5, the initial activity is retained for an extended period of time. Almost the same percentage enzyme activity was obtained from the sol−gels that were aged for 2 days and 1 month (Figure 5) and for 18 months (Figure 4b). Using prehydrolyzed sol prior to addition of the enzyme retained the enzyme activity upon encapsulation remarkably. Figure 4a shows the comparative enzyme release profiles of the sol−gels prepared with sols that were aged and not aged. It was found that 92−98% of the enzyme activity was retained in the sol−gels prepared with aged sols. The difference is especially prominent in the case of K3 where the amount of enzyme released increased from 13% to 98%.

Overall, it appears that the use of a modified sol−gel protocol wherein the sols were aged for a period of 30−90 min prior to the addition of the enzyme leads to substantial enhancement in retention of native activity (Figures 4a and 5). Furthermore, there is minimal loss of activity even after storage of the sol−gels with enzyme under ambient conditions for an extended period of time (Figures 4b and 5). The results also indicate that the low enzyme activity of the released subtilisin, especially from the K2 and K3 systems, could be because of possible denaturation during the process of encapsulation itself, rather than due to changes within the microenvironment of the sol−gels after the encapsulation. Aging of the sol−gels is a possible pathway to bring about denaturation of the proteins. Over a period of time, as the silicate network gets more extensive and rigid, the local environment turns more conducive to protein−silica interactions.[49,50] Loss of stability of proteins in silica matrixes is generally attributed to unfolding which occurs due to electrostatic interaction of the active sites of the enzyme with the silicate matrix.[51,52] Our results indicate that such denaturizing interactions are predominantly absent in the present systems. The data on the long-term stability of the enzymes in the gels that were stored under ambient conditions for an extended period rule out denaturation of enzymes after encapsulation and suggest long-term storage stability of the enzyme in the sol−gel matrixes. Furthermore, the results provide an initial feasibility evaluation of the potential of the sol−gels for utilization of the enzymes in a fabric care system wherein long-term storage stability is a critical requirement.

Infrared Spectroscopy. To characterize the structure of these gels, vibrational spectroscopy was used. ATR-FTIR spectra of the various sol−gels (K1, K2, and K3) investigated in this study are given in Figures 6 and 7. The K4 and K5

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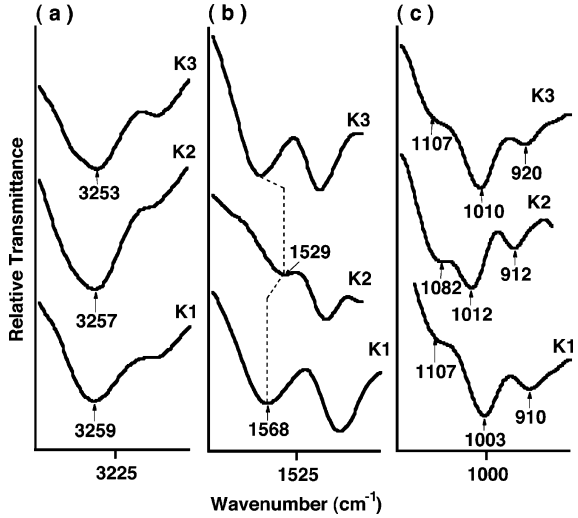

Figure 6. Comparative ATR-FTIR spectra of sol−gel samples.

gels form waxy solids that could not be powdered, and therefore, it was not possible to obtain any meaningful ATR-FTIR spectra. In primary amines, a symmetrical N−H stretching mode is observed near 3295 cm^{-1} and N−H bending vibrations are observed near 1607 cm^{-1}.[53] Both these peaks are observed in all the samples investigated in this study (Figure 6a,b). On the other hand, the reference sol−gel, which contains an *N*,*N*-dialkyl-substituted moiety, did not show any peaks around 3200 and 1600 cm^{-1}, confirming that these peaks are indeed due to N−H stretching and bending vibrational modes, respectively. The bands corresponding to N−H vibrational modes are considerably downshifted. In the K1 sample, the band corresponding to the N−H stretching mode is shifted to 3259 cm^{-1} and the band due to N−H bending vibrations is shifted to 1568 cm^{-1} (Figure 6a,b). Similarly in the case of K3, these bands are observed at 5253 and 1568 cm^{-1}, respectively. In the case of K2, the band corresponding to N−H bending vibrations is shifted to 1529 cm^{-1}. The shift to lower wavenumbers suggests participation of amine groups in hydrogen bonding.[47] This is a characteristic of strong hydrogen-bonding interactions or, alternatively, the formation of an NH_3^+ group.[54,55] However, an asymmetrical NH_3^+ deformation mode was not observed near 1600 cm^{-1}. According to Boerio et al.,[54] this band is sometimes very weak due to the orientation effect. Therefore, the observed downshift suggests a strongly hydrogen bonded amino group or possibly the formation of an ammonium group in these sol−gels.

All the sol−gels show a peak at ∼3668 cm^{-1}, suggesting the presence of hydrogen-bonded silanol groups.[56,57] Bands corresponding to isolated hydroxyl groups (∼3750 cm^{-1}) were not observed. Also, bands characteristic of $\nu_s(CH_2) \approx$ 2870 cm^{-1} and $\nu_{as}(CH_2) \approx$ 2920 cm^{-1} corresponding to the CH_2 groups of the organic moiety of the silanes are found in all the systems. The siloxane frequencies lie between 1200 and 800 cm^{-1}.[56,57] The main band in the sol−gels was found near 1010 cm^{-1} and a smaller peak near 920 cm^{-1} (Figure 6c). The peak near 920 cm^{-1} corresponds to the nonbridging, free, broken $Si-O^-$ bonds belonging to a cluster containing less than six Si atoms.[58] When the average size of the clusters increases and becomes greater than the dimension of (SiO_6) rings, the possibility of forming such SiO^- moieties decreases, and instead a peak corresponding to a stretching Si−OH bond around 950 cm^{-1} is observed.[59] The peaks observed at 1100 and 1160 cm^{-1} are usually assigned to the stretching vibrations of Si−O−Si belonging to a more linear and less cross-linked structure.[59] Sol−gels K1 and K3 show a shoulder at 1107 cm^{-1} which in the case of K2 is downshifted to 1082 cm^{-1}.

A comparison of ATR-FTIR spectra of formulated enzyme-containing sol−gels with those of the pristine sol−gels is shown in Figure 7. A noticeable upshift in N−H bending vibrations is observed for the K1 and K2 sol−gels upon addition of the formulated enzyme. The K2 sol−gel shows a peak at 1529 cm^{-1} in the pristine form, but that peak is not observed in the formulated enzyme-containing gels. Instead, the formulated enzyme-containing K2 sol−gels show a peak near 1576 cm^{-1}. Similarly, for these gels the energies of the N−H stretching vibrational modes in the 3250 cm^{-1} region also exhibit upshifts. All of these energies are still lower than those observed in primary amines, indicating the presence of hydrogen-bonding interactions. Addition of formulated enzyme appears to cause a relative upshift in energies of the N−H vibrational modes, indicating relative disruption of hydrogen-bonding interactions. These shifts in the N−H bands indicate that, even though the formulated enzyme-containing sol−gels continue to have hydrogen bonding, the extent of interaction is likely affected by the addition of the enzyme. In this context, it is interesting to note that similar upshifts in the Si−OH vibrational mode at ∼900 cm^{-1} are also observed, indicating a reduction of hydrogen-bonding interactions. Overall, it appears that addition of formulated enzymes in these sol−gels reduces the hydrogen-bonding interactions of both N−H and Si−OH groups. These reduced interactions are likely contributing factors in the faster dissolution of formulated enzyme-containing gels. Comparison of ATR-FTIR spectra of the formulated enzyme-containing and pristine sol−gels in the siloxane region (1200−800 cm^{-1}) (Figure 7c) further confirms the changes in structure resulting from incorporation of formulated enzyme in the sol−gel. On addition of formulated enzyme, a distinctive peak around 1100 cm^{-1} is noticed in K1, K2, and K3 sol−gels. These peaks are typically found in more linear and less cross-linked silicates.[59] Taken together, the FTIR data suggest interactions of the formulated enzyme during sol−gel matrix formation such that the final material has diminished hydrogen-bonding interactions and results in formation of a less cross-linked structure. Usually the gelation process occurs by polymer-

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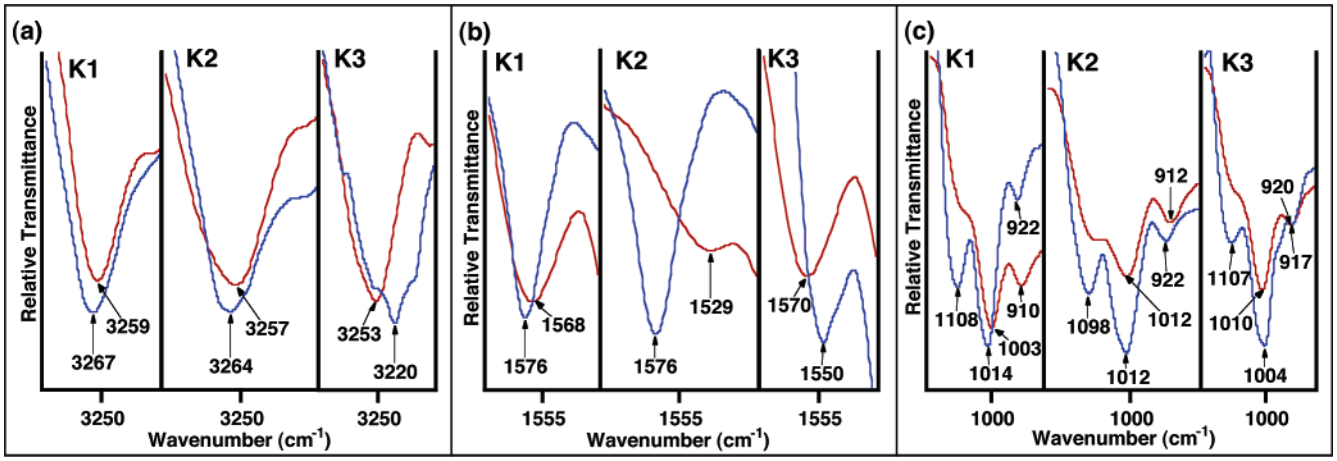

Figure 7. Comparative ATR-FTIR spectra of K1, K2, and K3 sol−gels with and without enzyme: (a) ATR-FTIR spectra in the 3250 cm^{-1} region; (b) ATR-FTIR spectra in the 1550 cm^{-1} region; (c) ATR-FTIR spectra in the 1000 cm^{-1} region; (red spectra) pristine sol−gels; (blue spectra) formulated enzyme-containing sol−gels.

ization of the monomers to form particles, which grow in size, linking to form chains followed by branched chains, and finally formation of an extensive network, which is physically seen as a gel.[60] Hydrogen bonding seems to be holding the particles together. The amino groups present on the siloxanes increase the extent of hydrogen bonding, finally forming the gels by gradual evaporation of water. Further, the formulated enzyme-containing K1 and K2 gels seem to have comparatively less hydrogen-bonding interactions, thus making the gels more easily dissolved than the pristine or dye-containing gels. These results are consistent with dissolution and gelation period data on these gels.

On the other hand, the K3 sample shows a reverse trend compared to that observed for K1 and K2 samples upon incorporation of the formulated enzyme. The ATR-FTIR spectra of these gels when formulated enzyme is incorporated exhibit a downshift in the N−H stretching and bending modes (Figure 7a,b). Similarly, a slight downshift in the Si−OH vibrational mode is also observed (Figure 7c). The results indicate that hydrogen-bonding interactions in these gels are slightly enhanced upon incorporation of the formulated enzyme. This is further validated by the experimental data on dissolution of these gels, which show a slightly longer dissolution time as compared to pristine sol−gels. Therefore, to a certain extent, it appears that enhanced hydrogen-bonding interactions lead to slower dissolution while reduction in hydrogen-bonding interactions within the gels leads to a faster dissolution of the gels.

Amino-Functionalized Organosilica Sol−Gels as Controlled Release Matrixes. The results on release of dye and enzyme provide some estimate of the potential utility of these materials in dissolution-controlled release. The quick dissolution times (ca. 10 min) are ideal for fabric care applications where a rapid release during the washing process is necessary. As shown by TEM measurements (Figure 8), these gels are characterized by a loosely held network of particles with pore sizes on the order of 0.5 nm which is suitable for dissolution-controlled release. Additionally, FTIR shows the possible formation of protonated amino groups

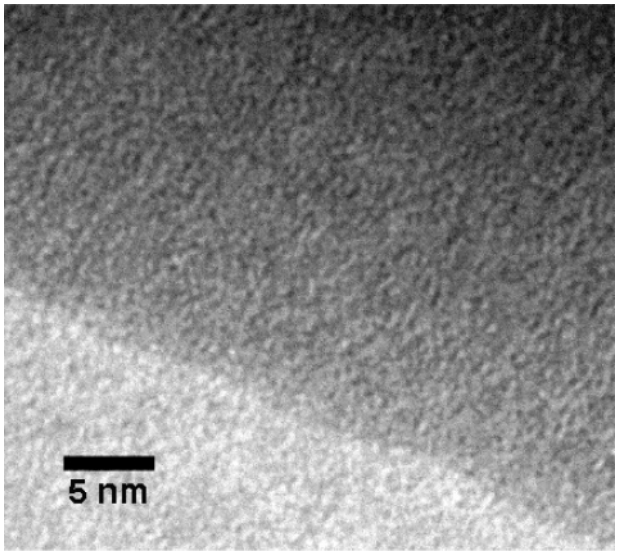

Figure 8. Transmission electron micrograph of the K3 sol−gel sample.

which due to individual electrostatic repulsions prevent formation of a network and facilitate dissolution when the gels are placed in water.

The amino-functionalized sol−gels provide an environment that is conducive to enzyme stability. An initial prehydrolysis of the sol followed by some aging of the sol under ambient conditions is found to be necessary prior to addition of the enzyme. Initial reaction between water and precursors is an exothermic process and liberates heat (possibly due to acid−base reaction between amino groups and water), causing the temperature of the sol to increase by about 10 °C, which possibly leads to some denaturation of the enzyme. Similarly the presence of alkoxy precursors also may be a contributing factor in denaturation. Therefore, some aging of the sol is necessary to ensure that the precursors are fully hydrolyzed and that the temperature of the sol is conducive to enzyme stability. The gels which were made after some aging of the sol exhibit a more or less equal amount of enzyme released (94−100%) upon dissolution for all the systems (see Figures 4a and 5). After the initial prehydrolysis of the sol−gel precursor the sol contains mostly hydrolyzed siloxane moieties with terminal Si−OH groups that exhibit favorable interactions with the encapsulated

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enzyme and prevent denaturation of the enzyme. The gels are formed due to aggregation of these sol particles as the water content of the sol decreases due to drying under ambient conditions, leading to formation of a gel which is essentially an aggregation of the particles. This is confirmed by the representative TEM micrograph of the K3 sol−gel (Figure 8) system, which shows a highly porous structure formed by aggregation of particles. The dopant molecules including the enzyme are trapped in this network without significant strain on the native conformation, thereby retaining the activity of the enzyme. The enzymes are stabilized due to hydrogen-bonding and electrostatic interactions with −NH and SiOH groups. Furthermore, the gels retain a significant amount of water necessary for the stability of the encapsulated biomolecules. As a result, almost all of the enzyme (K1, 94%; K3, 98%; K4, 100%; K5, 100%) is stable and is released from the gels when placed in water (see Figures 4a and 5). It is important to point out that while the amino-functionalized sol−gels are particularly suited for stabilizing alkaline proteases, in general, the stability of enzymes is governed by a variety of factors, and further work with a broader range of enzymes is needed to identify the precise nature of specific enzyme−gel interactions.

In general, one would expect the gelation times and dissolution times of these gels to be correlated. The systems with shorter gelation times would be expected to take longer to dissolve due to an ostensibly greater extent of interactions between the sol particles. The gels in the present study are formed from precursors with a variable number of amino and hydroxy functional groups on the silane precursors. The dissolution times of these gels suggest the complex nature of interactions present in these sol−gel systems. The dissolution times of these gels are related to the interactions between sol particles, the interactions between the dopant and gel, and interactions between sol particles and water. A greater number of amino groups in the material indicates a greater extent of hydrogen-bonding interactions, leading to faster aggregations and gelation and slow dissolution. This is evident in the dissolution−gelation period data shown in Table 1. The systems with only amino groups (K1, K2, K3) have shorter gelation times as compared to systems containing hydroxy groups in addition to amino groups (K4, K5). The system with the greatest number of amino groups in the precursor (K2) shows the fastest gelation and slowest dissolution. On the other hand, systems containing precursors where both the hydrogen atoms on the terminal amino groups have been substituted with hydroxyethyl groups (K4) or with a hydroxyethyl and a methyl group (K5) show the longest gelation times. The sol−gel system K4 shows the longest gelation time, indicating the influence of substituting amino groups with hydroxyethyl groups. Furthermore, as discussed above, the incorporation of the formulated enzyme alters the hydrogen-bonding interactions, and K1, K3, K4, and K5 sol−gels exhibit longer gelation times, while the K2 samples show a faster gelation time. Overall, it appears that hydrogen-bonding interactions play a predominant role in aggregation−gelation of these gels. Likewise, the hydrogen-bonding interactions also influence the dissolution times of these gels. The gel with the highest number of amino groups (K2) shows the longest dissolution time. Reducing the number of amino groups (as in K3) or substitution of hydrogen on the amino group with a non-hydrogen-bonding methyl group lowers the dissolution times. Therefore, the results suggest gelation and dissolution of these gels is predominantly governed by hydrogen-bonding interaction within the gels. On the other hand, electrostatic interactions also contribute and regulate gelation and dissolution times as evident by the longer dissolution times of sol−gels containing the negatively charged dye molecule. The longer dissolution times with the charged dye suggest possible contribution of electrostatic interactions. The amino groups in the gels are characterized by some degree of being protonated. These positively charged groups in the matrix are likely to repel and lead to slow aggregations, leading to longer gelation times. Conversely, a greater number of protonated amino groups in the material possibly leads to a faster dissolution due to the relative ease of solubilization of charges species. Finally, the interactions between the dopant and matrix also influence the dissolution of these materials. Taken together, the experimental data suggest that the gelation and dissolution behavior of these gels is governed and regulated by a balance of interactions within the gels.

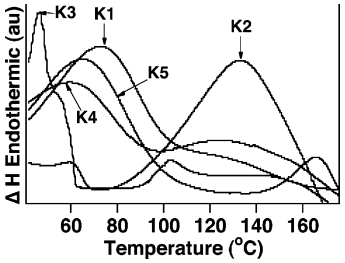

Figure 9. DSC curves of sol−gel samples showing the endothermic transitions.

A related measure of the nature of interactions in these gels is obtained from DSC measurements. The endothermic peak in the DSC data represents the melting enthalpy of the dried gels (Figure 9). On the basis of the DSC data, the melting points of these gels are listed in Table 1. The K3 gel shows the melting transition at the lowest temperature, 45 °C, while the K2 gel with a high number of amino groups shows the melting transition at 133 °C. The single-component sol−gels (K2 and K3), thus, define the two extremes of melting transitions. In other words, the system with the lowest number of amino groups (K2) shows the lowest melting point, while system with the highest number of amino groups exhibits the highest melting point. The samples with a combination of precursors exhibit melting transitions between those of these two samples. Substitution of terminal hydrogen atoms on amino groups appears to lower the melting transition temperature as shown by the low transition temperatures of the K4 and K5 systems. Thus, the K4 gel, where both the terminal hydrogens are replaced with hydroxyethyl groups, shows the melting transition at ∼60 °C, while the K5 system, where one of the hydrogens is replaced with a methyl group and the other with a hydroxyethyl group shows the transition at ∼65 °C. Similarly, increasing the number of amino groups in the system apparently increases the melting transition as shown by the high melting point of K1 (72.4 °C) as compared to K3 (46.9 °C). Overall, the

results indicate the variable nature of interactions within the gel systems as a result of the use of precursors with a variable number of amino and hydroxy groups which are also manifest in the gelation/dissolution of these gels. As such, through a precise choice of precursors, the nature of interactions and the dissolution-controlled release can be fine-tuned to achieve optimum behavior.

The dissolution and gelation times of these gels with encapsulated dopant entities also provide some insight into the nature of dopant−matrix interactions. In general, the dissolution times for the dye-containing gels are found to be slightly longer than those without the dye or with the formulated enzyme. This indicates some electrostatic interactions between the negatively charged dye and the positively charged matrix. In general, the formulated enzyme-containing gels have faster dissolution of the gels, which makes them particularly suitable for controlled release applications in detergents. The percentage of enzyme released from these gels (K1, K3, K4, and K5) remains unchanged even after 18 months, indicating long-term stability of the encapsulated enzyme. The results suggest potential utility of these materials for long-term stabilization of the enzymes in solid form as well as their utility in controlled release applications in detergent systems for normal fabric care.

The amino-functionalized organosilica sol−gels provide several advantages that make them suitable for dissolution-controlled release of encapsulated molecules. First, these gels exhibit water-dependent reversible gelation and dissolution, which is not possible with other sol−gel-derived materials. Second, these materials form a loosely held network characterized by weak interactions between sol−gel particles that can be easily and rapidly disrupted by dissolution in water. Third, these gels are completely soluble in water and as result guarantee 100% release of dopant entities. Fourth, these gels are hydrophilic and therefore compatible with the aqueous environment typically used in controlled release applications. Finally, the basic environment of the gels is especially compatible with alkaline proteases, which have optimal activity and retain their native conformation under basic conditions. These characteristics make these materials potentially suitable as dissolution-controlled release systems in fabric care applications. Through a judicious choice of precursor composition, it is possible to optimize the composition for effective stabilization of the enzyme as well as efficient release when dissolved. Up to 100% of the enzyme can be released from these gels when placed in water in a very short period on the order of 10 min. The encapsulated enzyme is found to be stable in these gels for extended periods on the order of years. These characteristics give these materials appealing prospects for potential applications in fabric care detergents wherein the enzyme is mostly utilized for stain removal. The powdered gels containing the enzyme may be mixed with powder detergents to incorporate the enzyme in the detergent formulations. The ease of preparation, feasibility of sol−gel synthesis under ambient conditions, cost-effectiveness, and compatibility of these sol−gels with other industrially relevant enzymes make these systems particularly suitable for practical applications related to controlled delivery and release systems in general.

Conclusions

In conclusion, we demonstrate the practical feasibility of a strategy based on using amino-functionalized organosilica sol−gels in dissolution-controlled release of encapsulated species including biomolecules. The gels formed by these materials are due to aggregation of loosely interacting particles, which can be easily dissolved when placed in water, leading to a fast release of dopant entities. In addition, we demonstrate that it is possible to encapsulate, stabilize, and subsequently release biomolecules such as alkaline protease subtilisin from these gels. Through a judicious choice of precursor composition, it is possible to optimize the composition for effective stabilization of the enzyme as well as efficient release when dissolved. The encapsulated enzyme is found to be stable in these gels for extended periods on the order of years, suggesting a potential utility of these materials in stabilization of different molecular entities, specifically biomolecules. Finally, the results indicate potential utility of these gels as versatile controlled release and delivery matrixes where the release of actives can be initiated by water-induced dissolution of these organosilica sol−gels. Further studies on extending this approach to other industrially relevant biomolecules are currently under way in our laboratory.

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