

trolled by the acidic synthesis conditions. The structural, optical, and electrical properties of the molecular doped mesoporous silicates were examined. The process can be extended to a wider chemical synthetic route for various functional materials for electrochromic devices, nonlinear optical switching materials with organized molecules, and photoconversion materials based on energy and carrier migration through the mesochannels.

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Bioelectronic Glasses: Electrical Addressability of Sol-Gel Immobilized Biomolecules**

By Ying Tang and Bakul C. Dave*

Redox-active biomolecules are electronic elements built to process information using electron transfer as a signal-

transducing mechanism.^[1] The unique functional responses of electroactive biomolecules offer heretofore untapped opportunities for designing novel materials for molecular bioelectronics.^[2] However, most biomolecules that are freely mobile in solution are dimensionally unoriented, mechanically fragile, and thermodynamically only marginally stable under normal conditions.^[3] Assembling these molecules into a geometric structure that provides rigidity, optical and/or electronic addressability, and stability is critical for practical applications. An approach that has been shown to be quite successful in this direction is based on encapsulation in an inorganic oxide glass that is synthesized using the sol-gel process.^[4-6] The rigid inorganic matrix not only restricts molecular motion and fixes these molecules in space but also, by virtue of its framework, provides mechanical strength and improved long-term stability. Recent examples of proteins and enzymes in SiO₂ sol-gel matrices have shown initial success in using biomolecules for practical applications. The transparent silica sol-gels are effective matrices for optical applications, and the encapsulated biomolecules can be addressed by means of photons.^[7] Optical signal transduction using silica sol-gels has also been used to design biological and/or chemical sensors.^[8-14] However, the transparent silica glasses are limited to optical addressability and signal transduction, and electronic means of communication with the encapsulated biomolecules is not feasible.

In order to be able to elicit bioelectronic responses, we modified silica glasses with Sn to obtain an electroactive host matrix that facilitates transport of electrons between the immobilized biomolecule and the electrodes. These novel materials make use of the unique bioelectronic properties of redox-active biomolecules in a solid-state framework. The biomolecular materials are characterized by a) an electroactive host matrix, b) the existence of ohmic contacts between the host matrix and the redox-active biomolecule for electron transfer, c) the presence of locally restricted areas for physical separation of molecules in different oxidation states, and finally d) the feasibility of electrode contacts with the matrix at the bulk level for connection to the external electronic circuit. These new materials exploit the electroactivity of the modified sol-gel matrices to provide bioelectronic responses.

The Sn-doped silica sol-gels [Sn/SiO₂] are electroactive matrices that can be used for encapsulating biomolecules, and electron-transfer events can occur between the redox-active biomolecule and the matrix. The Sn-doped sol-gel matrices, therefore, act as carriers of electrons from the electrodes to the redox-active sites of the biomolecules. In this way, it is possible to address the redox state of encapsulated biomolecules by means of an externally applied potential, as well as to measure electrons liberated from the encapsulated biomolecule as an increase in current flowing through the material.

We used a modified sol-gel protocol for preparing Sn-doped silica sol-gels.^[15] The two-component sol-gels were

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synthesized by mixing two hydrolyzable precursors to form Si–O–Si and Sn–O–Sn networks. Tetramethyl orthosilicate (TMOS) and $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ were used as precursors for preparing Sn-doped silica glasses. The glasses were formed by mixing the precursors either in water or acetate buffer (pH 4.6, 0.1 M) followed by sonication at room temperature. The sol formed by hydrolysis is allowed to gel in polystyrene cuvettes. The gelation times range from 1 to 5 days. The Sn/SiO₂ sol-gels formed this way are light yellow in color. Using this protocol, both monolithic as well as thin-film configurations can be obtained.^[16]

Our initial focus was to establish the feasibility of electron transfer from the electrode to the encapsulated biomolecules. For this purpose, flavin mononucleotide (FMN) was used as a model biomolecule because of its photoactivity and redox activity.^[17] Encapsulation of FMN in Sn/SiO₂ glasses results in yellow-colored samples.^[18] These glasses exhibit the optical absorption and emission properties characteristic of FMN. The initial conditions of this synthesis are quite acidic, and the optical absorption spectra reveal that encapsulated FMN is protonated within the Sn/SiO₂ matrix. A comparison of the optical spectra of solution and sol-gel suggests a near-total retention of structure on encapsulation. The initially formed gels were allowed to age to enhance their mechanical properties. The aged sol-gel materials containing FMN are stable under ordinary conditions for extended periods and were, therefore, used for spectroscopic and electrical measurements.

The behavior of FMN in Sn:SiO₂ matrices under an applied potential was investigated using elemental platinum electrodes that were attached to monolithic samples. The potential was applied using a potentiostat (EG&G Princeton Applied Research, Model 283) with respect to a Ag/AgCl electrode used as a reference electrode. The working and counter electrodes were elemental platinum foils of 0.3 and 0.1 cm² area, respectively. Physical pressing on contacts with the electrodes was used for bulk monolithic samples with approximate dimensions of 0.6 × 0.6 × 1.3 cm³. On applying a negative potential of –0.5 V to the working electrode, formation of a red color is observed. This red color begins to spread with time under the applied potential, and ultimately half of the sample turns red (Fig. 1). These results are consistent with the formation of two half-cells within the solid monolith, with reduction occurring in one half and oxidation in the other half. The distinct formation of two halves along with the separation of two colors is a clear indication that the FMN molecules are localized within the pores of the glass and are unable to move within the network (Fig. 2). The formation of a new peak in the absorption spectrum at 490 nm (Fig. 3a) and the development of red color are consistent with generation of the one-electron reduced form FMN^{•-}. The absorption spectra of the reduced form match closely with the previously reported spectra for FMN^{•-}.^[19] The process is reversible, and on switching the potential to +0.6 V the original spectrum is restored.

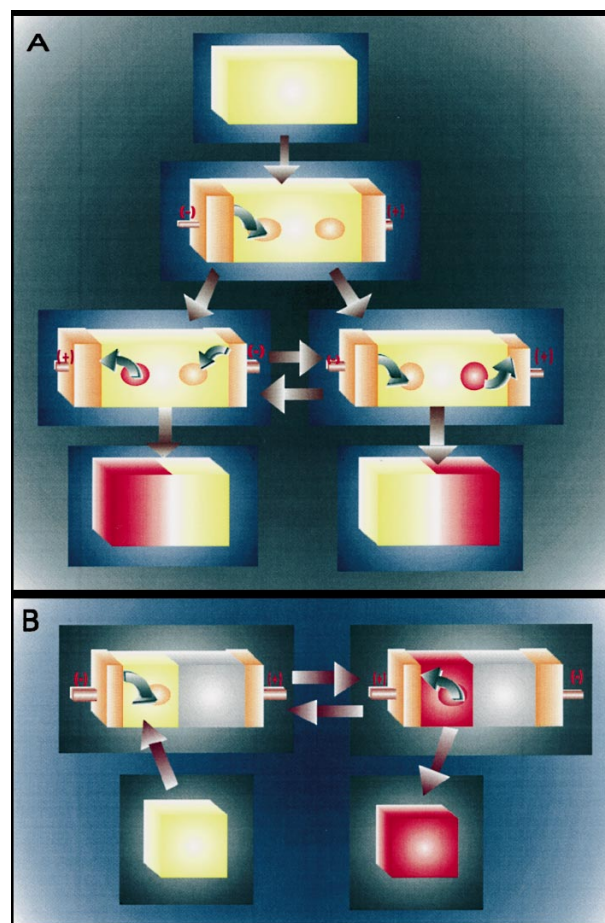


Fig. 1. Schematics of different bioelectronic processes taking place in Sn/SiO₂ sol-gels containing biomolecules as dopants. a) The electronic process occurring when a single sol-gel sample is used. b) The electronic processes occurring when two sol-gels in physical contact with each other are subjected to an applied potential.

The overall process of redox events occurring in the glasses is shown in Figure 1. The application of an electrical potential gives rise to the formation of two half-cells, with reduction of FMN occurring at the negative electrode. The applied potential is not enough for oxidation of FMN to occur at the positive electrode. On applying a potential to the sample, bulk electroreduction of FMN occurs at the cathode with the formation of red-colored FMN^{•-}. This process is reversible when the electrode potential is switched. The reduced form of FMN is reoxidized to yellow-colored FMN, while at the other end reduction results in the formation of red FMN^{•-}. Overall, starting with a yellow-colored sample, a sol-gel with two halves containing both FMN and FMN^{•-} can be obtained. The electronic processes occurring when two sol-gels in physical contact with each other are subjected to an applied potential are shown in Figure 1b. In this case a blank Sn/SiO₂ sol-gel (without any dopant) is used as a reference in contact with a sol-gel sample containing FMN as a dopant. On applying a negative potential to the side containing FMN, electroreduction of FMN occurs in the sample, while the charges are balanced by the

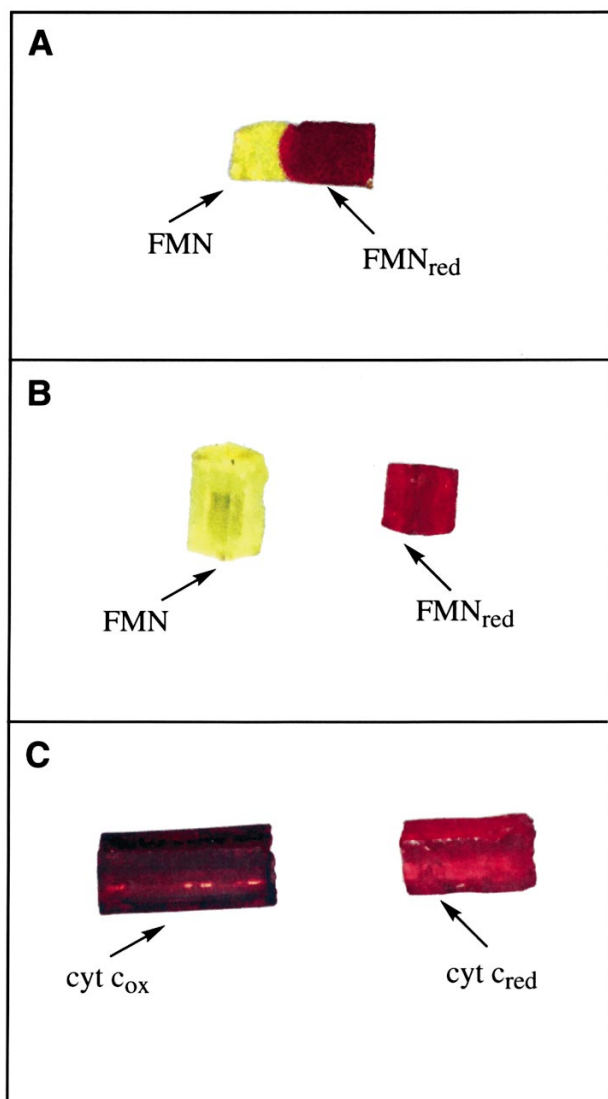


Fig. 2. FMN- and cyt C-doped Sn/SiO₂ samples in their oxidized and reduced forms. a) Monolithic samples of Sn/SiO₂ sol-gels with two half-cells containing electroreduced and oxidized forms of FMN. b) Electroreduced and oxidized bulk samples of FMN obtained using the two-gel configuration shown in Figure 1b. c) Electroreduced and oxidized forms of cyt C sol-gels obtained using the two-gel configuration.

reference sample. The overall process is reversible and, on switching the applied potential, the electroreduced sample can be reoxidized. In this way, one can obtain bulk samples containing both reduced and oxidized forms.

The behavior of films containing FMN in Sn/SiO₂ glasses was also investigated.^[20] For this purpose, films with approximate thickness of 150 μm were used. The response of color change and reduction in the thin films at -0.5 V is substantially faster compared to the bulk sol-gels. The overall color change takes place in ca. 20 min. On reversing the potential to +0.6 V, the original form is regenerated (Fig. 3b). The change in color of the film with respect to the applied potential shows that these materials are electrochromic in nature, where the active response is generated by the redox-active FMN biomolecule.

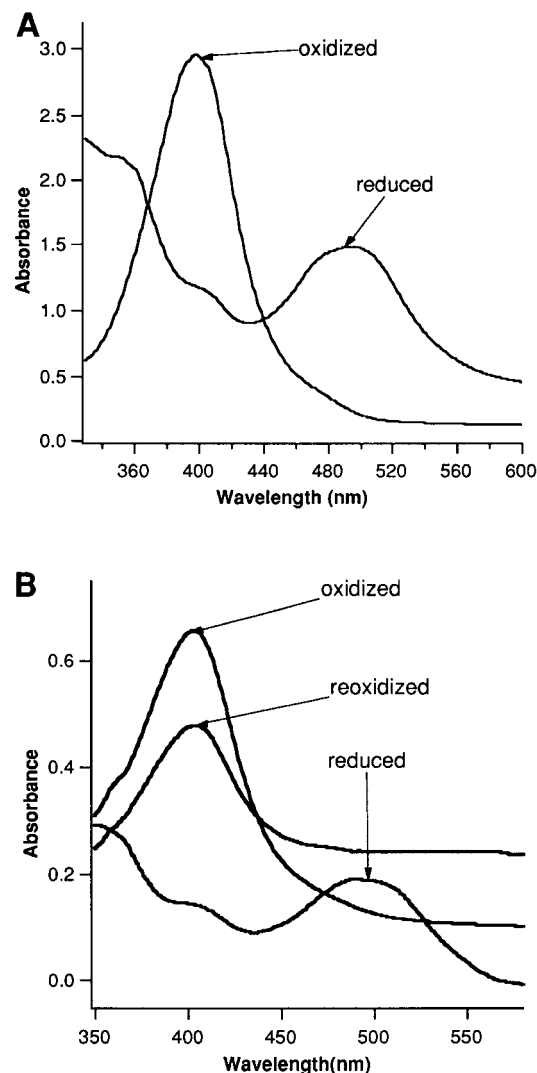


Fig. 3. Optical absorption spectra of FMN-containing sol-gels with monolithic and thin-film samples. a) Optical absorption spectra of monolithic Sn/SiO₂ sol-gels containing FMN in the electroreduced and reoxidized forms. b) Electrochromic properties of Sn/SiO₂ thin films containing FMN as a dopant. Optical absorption spectra of thin films show the original electroreduced and electrooxidized forms of FMN.

We also investigated the feasibility of electronic addressability of a globular protein by encapsulating cytochrome C (cyt C) in Sn/SiO₂ matrices.^[21] Cyt C is an important redox protein (MW = 12 500) that contains the heme group with Fe³⁺ at the active site in the oxidized form.^[22] On reduction, the Fe³⁺ center is converted to Fe²⁺. This reduction process accompanies changes in the optical absorption spectra: the Soret band shifts to a higher wavelength, and new peaks at ~525 and 550 nm are observed, which are absent in the spectra of the oxidized form.^[23] The bulk gels containing the oxidized form of cyt C are reddish-brown in color. On electroreduction at -0.5 V (vs. Ag/AgCl), the overall color of the gel changes to orange-brown. The changes in optical spectra with respect to the applied potential are shown in Figure 4. The formation of new peaks centered at 525 and 550 nm is characteristic of the reduced form of cyt C. The

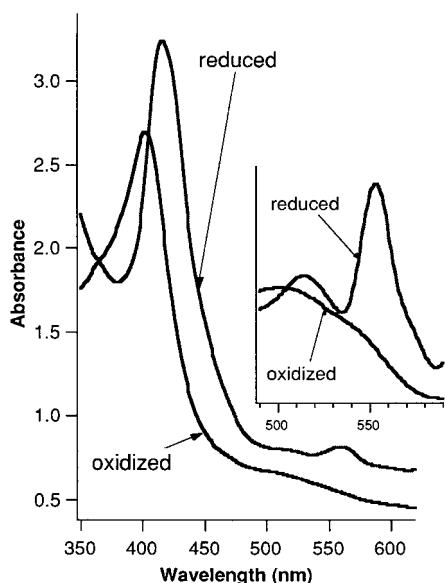


Fig. 4. Optical absorption spectra of cyt C-containing sol-gel monoliths with oxidized and reduced forms of cyt C. The inset shows the spectra in the 500 nm region that are characteristic differences in optical properties of the oxidized and reduced forms of cyt C.

absorption spectra show that overall bulk electroreduction of cyt C takes place in Sn/SiO₂ sol-gels when a negative potential is applied. Our data thus demonstrate that it is possible to address and switch the oxidation state of a protein by electronic means.

Finally, we investigated the feasibility of electron transport from the biomolecule to the electrodes. The photoredox properties of FMN were exploited to generate excess electrons by means of excitation with light. The glasses containing FMN were exposed to light from a 40 W tungsten lamp. On exposure to light, an excited state of FMN is formed, which is characterized by a low reduction potential.^[24] From this excited state the transfer of electrons can take place to the Sn/SiO₂ material. Indeed, the samples containing FMN demonstrate a photoconductive behavior. A direct correlation of light exposure to change in amperometric response can easily be observed (Fig. 5). There is an

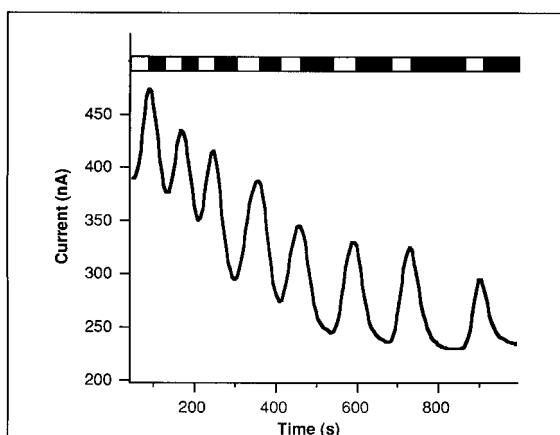


Fig. 5. Photocurrent response of monolithic sol-gels with FMN as a dopant.

increase in current flowing across the material when the light is turned on. On switching the light off, a slow decay of current is observed. The decay in peak maxima is consistent with the decay of FMN in the sample, which is partly due to reaction of the excited state of FMN with atmospheric oxygen. Overall, a direct correlation between the photoconductive response and illumination is observed.

Although at present a detailed understanding of the principal modes of interactions between redox-active biomolecules and Sn/SiO₂ sol-gels remains to be established, it is apparent that the electron-transfer events are mediated by the electroactive host matrix. Current results indicate that the redox-active biomolecules are immobilized within the sol-gel. Although FMN is a smaller molecule, significant non-covalent interactions with the matrix render it largely immobilized. On the other hand, the larger molecular dimensions of cyt C severely restrict the translational degrees of freedom within the porous gel and, therefore, cyt C molecules are trapped in the gel. The immobilization is confirmed by the fact that no leaching of the dopant biomolecules is observed when the gels are placed in water. In view of the immobilized nature of the biological dopants, it is prudent to assume that diffusion-related events are attenuated in the sol-gel matrix. Doping of silica with Sn leads to the formation of a mixed binary oxide network, which is likely to be conducive to electron-hopping pathways for electron movement through the sol-gel. The Sn/SiO₂ sol-gels contain both Si–O–Si and Sn–O–Sn linkages, as established by Fourier transform infrared spectroscopy (FTIR) spectra (not shown), which show the characteristic Sn–O–Sn vibrations at 550 and 625 cm⁻¹.^[25] In such a system wherein the dopant biomolecules are immobilized, the observation of bulk redox switching is consistent with a matrix-mediated electron transfer such that electron movement occurs through the matrix.

In conclusion, our experiments demonstrate that it is possible to establish electronic communication with a redox-active biomolecule that is immobilized within the porous structure of a sol-gel. Redox-active biomolecules encapsulated within these materials can be made to undergo oxidation–reduction processes under an applied potential. The transport of electrons is through the electroactive matrix, which acts as a carrier of electrons between the redox-active biomolecule and the externally placed electrodes. The presence of electron-transfer pathways between the redox-active biomolecule and the matrix allows mimicking of biological signal processing based on electron transfer, but using the addressing mechanisms of artificial electronic components. These materials are used as signal transducers for converting an electrical signal to an optical output (electrochromism), as well as for converting an optical signal to an electronic output (photoconductivity). The feasibility of integration into a structural matrix with rigidity, stability, processability, and addressability (optical/electronic) provides a scientifically and technologically viable

approach to the long-sought-after aim of utilizing proteins and enzymes for bioelectronic device applications.

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- [15] Typical preparation of Sn-doped silica sol-gels involves dissolving 3.4 g SnCl₄·5H₂O in 4 mL of deionized water followed by the addition of 1.55 g of tetramethyl orthosilicate (TMOS). The mixture was sonicated for 15 min to obtain sol. The freshly prepared sol (1 mL) was mixed with the same amount of deionized water and poured into polystyrene cuvettes. Gelation takes place in ca. 2 days. These sol-gels were allowed to age for 2–4 days.
- [16] Monolithic configurations were obtained by the formation of sol-gels in standard 4 mL polystyrene cuvettes. Films were formed by spin coating sol onto glass microscope slides. Typical film thickness was ca. 150 μm. The slides were first cleaned with water. The clean slides were then used for depositing films.
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- [20] The samples of FMN in film configurations were prepared using a slightly modified protocol. The sol was prepared by dissolving 3.4 g of SnCl₄·5H₂O in 3 mL of acetate buffer (0.1 M, pH 4.6) followed by the addition of 1.55 g of TMOS. The mixture was sonicated for 10 min. To 0.5 mL of this sol, 20 μL of 8.25 M NaOH solution was added followed by the addition of 0.1 mL of acetate buffer (pH 4.6). To this mixture, 45 μL of 0.05 M FMN solution was added. Finally, 100 μL of polyvinyl alcohol (2.5 wt.-% in water) was added to enhance the mechanical properties of the films. For film formation, 100 μL of the mixture was used on a clean glass slide.
- [21] Cyt C-containing samples were prepared from the sol as described earlier.^[15] Low-concentration sol-gels used for monitoring the Soret band were prepared by mixing 1.5 mL of sol with 1 mL of deionized water and 0.4 mL of cyt C solution (2 mg/mL in water). Gelation times were on the order of 1 week, and aging was continued for 2–3 weeks. The concentrated samples were prepared by mixing 1.5 mL of sol with 50 μL of 8.25 M NaOH, 0.5 mL of 0.1 M acetate buffer (pH 4.6), 0.5 mL of cyt C solution (6.5 mg/mL in water), and 200 μL of 2.5 wt.-% polyvinyl alcohol solution. Gelation times were about 1 week, and aging was continued for 1 month. The final size of the aged gels used for electrochemical experiments was 0.7 × 0.7 × 1.4 cm³.
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Controlled Nanocrystallization of Organic Molecules in Sol-Gel Glasses**

By Alain Ibanez,* Serguei Maximov, Antoine Guiu, Catherine Chaillout, and Patrice L. Baldeck

On the nanometer scale, material properties can be sensitively modified by quantum size and surface enhancement effects. Many studies concern metallic and semiconductor nanocrystals due to the strong effects of confinement on electron-hole pairs. This type of confinement is not expected in organic crystals because of the small radius of Frenkel excitons. This explains why studies on organic nanocrystals are so scarce.^[1,2] However, size effects may also be significant in organic crystals. For example, a confinement of the molecular exchange interaction should lead to an enhancement of optical non-linearities.^[3,4] Here, we report a simple and generic preparation of stable organic nanocrystals embedded in sol-gel glasses. This monodisperse nanocrystallization is obtained by an instantaneous nucleation followed by a controlled growth of the nuclei. In this process, the gel viscosity reduces the growth rate and avoids coalescence while gel pores act as nanosized growth reactors. Currently, we are designing nanocomposite materials with molecules selected for optical applications. Other types of molecules could lead to new fields of applications such as magnetism.

Previous methods for the preparation of organic nanocrystals were carried out in the liquid phase.^[1,2] Nanocrystals were obtained by precipitation of saturated dye solutions. In this type of process, it is difficult to control the growth and the coalescence of crystals. Since 1984 organic molecules have been dispersed or anchored onto xerogel matrices.^[5–7] This sol-gel technique has been used extensively because of its low temperature processing,^[8] which fits well with the low thermal stability of organic phases. In this work we have extended the sol-gel method to the preparation of stable organic nanocrystals in thin films and bulk gel-glasses. The process is based on the control of the nucleation and growth kinetics of the organic phase. Monodisperse sizes as small as 20 nm have been obtained in this work but lower particle diameters are possible due to the nanometer size of pores in dense gel-matrices.

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