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Encapsulation of deoxyribonucleic acid molecules in silica and hybrid organic-silica gels

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The encapsulation of small DNA molecules was attempted in pure silica and in hybrid polyvinyl alcohol-silica gels. The materials which were obtained were examined by nitrogen adsorption, and by ^{29}Si and ^{31}P NMR spectroscopy. The extraction of the DNA molecules from the gels was examined in a buffer aqueous solution as well as in an acidic medium. The results suggested that the DNA molecules remained trapped inside the gels due to a permanent bonding to the gel network.
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1. Introduction

Recently, new developments in bio-inorganic materials have appeared, in which biomaterials such as enzymes, bacteria or cells were encapsulated inside sol-gel materials. Many such biosensors are being studied in this way, as well as biocatalysts such as lipases encapsulated by Reetz *et al*. in hybrid silica-organic gels [1].

In the present study, we examined the encapsulation of DNA molecules in silica and hybrid polyvinylic alcohol-silica gels. The object was to examine if such biomolecules could be stored in inorganic or hybrid organic-inorganic gels and if they were denaturated by such a process or if they could be extracted further on without destruction.

2. Experimental procedure

The reactants used in the present study were: polyvinyl alcohol with average molar mass 15,000 (termed PVA in this paper), deoxyribonucleic acid from salmon sperm, low molecular weight, both from Fluka, methanol R. P. Normapur, ammonia min 28% R. P. Normapur, hydrochloric acid min 36% R. P. Normapur, sodium fluoride technical grade, a phosphate buffer solution pH 7.2 all from Prolabo, methyltrimethoxysilane 98% (MTMS), propyl-trimethoxysilane 98% (PTMS), poly (dimethoxysiloxane), 200, viscosity 50 cSt (PDMS) and tetramethoxysilane 98% (TMOS), all from Aldrich.

Four types of gels were prepared. The first ones (termed T) were made from an adaptation of a process used by Blyth *et al*. [2]. For this purpose, a silica sol was first made by adding 1.75 ml of distilled water and 0.05 ml of a 0.1 mol l^{-1} aqueous HCl solution, to 7.38 ml of TMOS. Separately, a 20 mg/ml DNA solution in pH 7.2 phosphate buffer was prepared. The final sol was obtained by mixing 800 μl of this DNA solution and 500 μl of the silica sol. Mixing was done by vigorous hand agitation for 5 s, followed by cooling in an iced bath for a few minutes as the hydrolysis and condensation reactions was exothermic, then followed by a gentle hand shaking for a few min. A turbid solution was first obtained. It slowly cleared with time and produced gel monoliths with a glassy characteristic overnight.

The three other types of samples were made by processes adapted from protocols designed by Reetz *et al*. [1]. For sample M gels, 100 μl of a 1 M NaF aqueous solution and 200 μl of a 4% (by weight) PVA aqueous solution, was mixed in 664 μl of the previous DNA buffer solution. After gentle hand agitation, 857 μl of MTMS was added to the solution. During hand agitation, turbidity developed in the sample and some heat was released. The sample was then placed in an ice bath until gelation was achieved. White monoliths which did not shrink during drying were obtained.

For samples of type M/D, pure MTMS was replaced by 434 μl of PDMS plus 643 μl of MTMS. In this case complete drying was never achieved. The final materials were white monoliths which remained partially wet.

For samples of type T/PT, 700 μl of the DNA solution was mixed with 100 μl of the 1 M NaF solution and 200 μl of the 4% PVA solution. After agitation, 857 μl of PTMS and 164 μl of TMOS were added. White monoliths which did not shrink during drying were obtained.

The porous texture of the samples which were obtained were characterized by nitrogen adsorption

according to the Brunauer, Emmett and Teller (BET) method. The network structure and a possible interaction between the silica network and the nucleic acids in the DNA were analyzed by ^{29}Si and ^{31}P solid-state NMR spectroscopy, on a Bruker DSX-400 spectrometer at 400 MHz. The samples were spun in the magic angle at *ca.* 10 kHz. The pulse technique was used with a pulse interval time of 5 s. Free induction decays of 4096 and 779 were taken for the ^{29}Si and ^{31}P spectra, respectively.

Extraction of the DNA from the sol-gel materials was then attempted, to examine the stability of encapsulation products. For an extraction operation, a gel sample was crushed in a mortar. About one third of the resulting powder was placed in a neutral pH buffer solution and the quantity of DNA which was liberated was measured during several hours. Part of the crushed powder was also submitted to an acid extraction operation, in conditions where DNA is degraded in 5 h. The size of the different DNA molecules was measured by agarose gel electrophoresis.

3. Experimental results

Analysis of the DNA material used in this study showed that it had a relatively broad size distribution, with most of the molecules having a small size comprising less than 50 base pairs and one component having a large size. All DNA molecules which were extracted had a small size and they did not seem to be degraded. However, when the crushed powder was placed in the neutral buffer solution, only part of the encapsulated material was immediately dissolved, but then it stopped almost completely as shown in Fig. 1a. Actually, extraction in these conditions was the most important in T gels (about 45% DNA extracted), while the second most important amount extracted (about 25%) was from mixed T/PT gels. In acidic medium, a progressive and complete dissolution occurred in about 5 h (Fig. 1b) which corresponded to a complete degradation of DNA by comparison with the effect on free DNA. Pore texture analysis by the BET method showed that T gels had adsorption isotherms of the type I in the Brunauer, Deming, Deming and Teller (BDDT) classification [3], which corresponds to microporous materials (Fig. 2). Hence they were almost exclusively microporous with pore size < 2 nm as shown in Fig. 3, although their specific surface area Asp was high, of the order of 550 m^2/g. On the other hand, all samples made by the hybrid PVA-silica procedure were non-porous, with a very low specific surface area of the order of 1 m^2/g, in a range of values where the exact data number is unreliable. As an indication, the data for samples T/PT are reported in Figs 2 and 3.

In Fig. 4, ^{29}Si NMR are reported. All peaks could be indexed in terms of the Q^4, Q^3, T^3, T^3, and D^2 coordination configurations for Si atoms, as summarized for instance by Glaser *et al.* [4]. The nature of these configurations and their chemical shifts in ppm by reference with Tetramethylsilane (TMS) are indicated in Fig. 5.

In Fig. 6, ^{31}P NMR results are reported for the initial DNA molecules and for the encapsulated DNA. The chemical shifts are measured by reference with orthophosphoric acid. The initial DNA showed a signal at ≈ -3 p.p.m. which is typical of P atoms in the DNA phosphate groups [5]. This signal was still present in the encapsulated DNA although slightly displaced at ≈ 2 to 2.6 p.p.m. in all hybrid PVA-silica type materials (samples M, M/D and T/PT), while in samples T it was displaced at ≈ 1.1 p.p.m. Moreover, two small signals appeared in gel encapsulated DNA samples, by comparison with the initial DNA: a peak at ≈ -1.1 to -1.6 p.p.m. in hybrid type materials, and one at ≈ -11 to -13 p.p.m. in T, M/D and T/PT samples (not in M samples).

4. Discussion

It appeared that hybrid silica gels made with PVA had no porosity as shown by the BET data. Hence, it can be considered as normal that only a moderate proportion of DNA, of the order of 10 to 15%, could be extracted in a buffer solution. It can be considered that the DNA part which re-dissolved immediately corresponded to small molecules located on the surface of the powder made by crushing a sample for the operation of extraction. In comparison, the buffer solution extraction was increas-

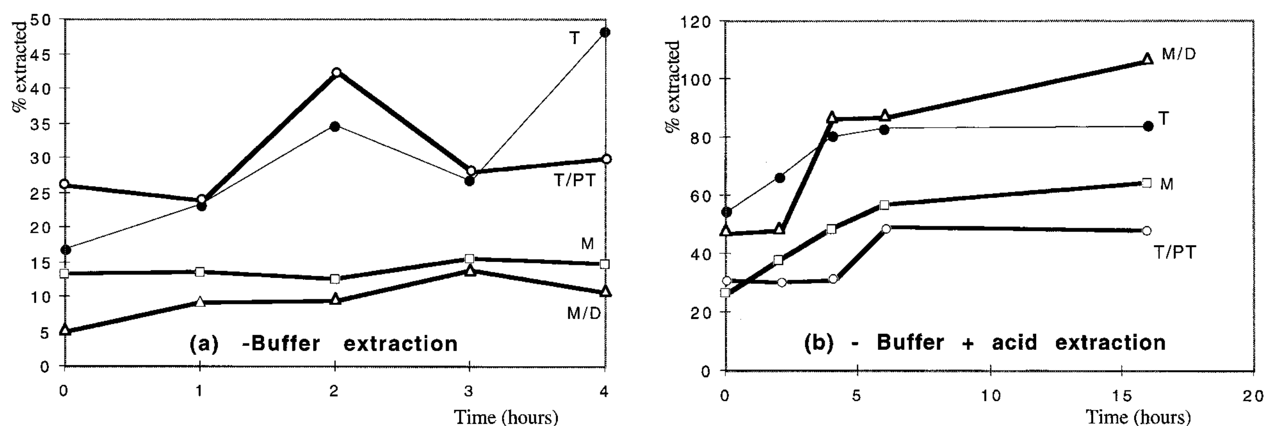

Figure 1 % DNA extracted from gel samples: (a) in a neutral buffer solution; (b) in a neutral buffer solution followed by extraction in acid medium.

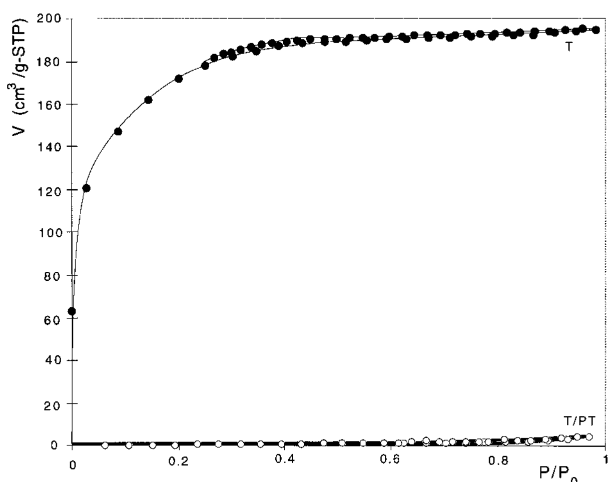

Figure 2 Nitrogen adsorption by the BET method, of sample of types T and T/PT.

ingly efficient as the proportion of TMOS used to make the gels increased. This can be related to the fact that T gels had a high specific surface area mostly due to abundant micropores, from where the smaller size DNA molecules could be relatively well extracted. The remaining part of the DNA could only be extracted from its matrix after degradation. The net result is that larger size molecules remained trapped in such sol-gel materials and could not be easily recovered. A possibility to alleviate this problem could consist in choosing a sol-gel process leading to larger mesoporous materials.

According to the ^{29}Si NMR data (Fig. 4), the silica networks for the four types of gels were different. In T gels made from TMOS, most Si atoms were engaged in Q^4 and Q^3 coordination as described in Fig. 5, that is to say they were linked to either 4 or 3 other (OSi $\equiv$) groups. Q^4 sites were also present in the next samples partially made with TMOS: the T/PT ones. However these sites were present in a much smaller proportion, in relation with a smaller TMOS proportion. In these T/PT samples, all other Si atoms were in T^3 and T^2 sites. As the T^2 sites were in larger proportions than the T^3 ones, it appears that significant linear segments were present in the silica network. Obviously, there was a direct correlation between the ratio of DNA molecules recovered in a buffer solution and the relative ratio of Q^4 sites. On the other hand only T^2 and T^3 sites were present in M gels and the T^3 sites were prominent. As T^3 sites were sufficient to insure a three-dimensional silica network, it explains that materials with a solid rubbery consistency similar to that of T/PT gels were obtained, while T gels were very brittle. The situation is yet very different in M/D gels where D^2 sites corresponding to long linear siloxane segments dominated, while T^3 interconnection sites were scarce. This can explain why these samples had a permanently viscous consistency. However, the ^{29}Si NMR data did not make it possible to suggest or reject a possible bonding between siloxane chains and the DNA or PVA molecules.

On the other hand, the ^{31}P NMR data in Fig. 6 show two small new peaks in the encapsulated DNA samples, by comparison with the initial DNA. The main peak present in pure DNA corresponded to the phosphryl configuration (O_2C,P=0) where one phosphorus atom is linked to three O atoms by a single bond and to one oxygen atom by a double bond (Fig. 7). The moderate and negative chemical shifts of the new small peaks in the encapsulated DNA, suggest that they also corresponded to modified phosphoryl type configurations for the P atoms. Actually, the compilation of data by Tebby [5] show that many phosphoryl groups correspond to chemical shifts in this range.

The first one of these small peaks is very close to the main DNA phosphoryl peak. It is present in all gels where alkyl groups were directly bonded to Si atoms and also where polyvinylic alcohol was added. This small peak appears as a shoulder on the DNA peak in the T and T/PT spectra while it is well separated in the M/D gel. Hence we suggest that it might correspond to a new P configuration termed P^{OCSi} in Fig. 7, where a new P-O-C$\equiv$(Si) link was established. Its exact position followed the exact position of the main DNA peak as

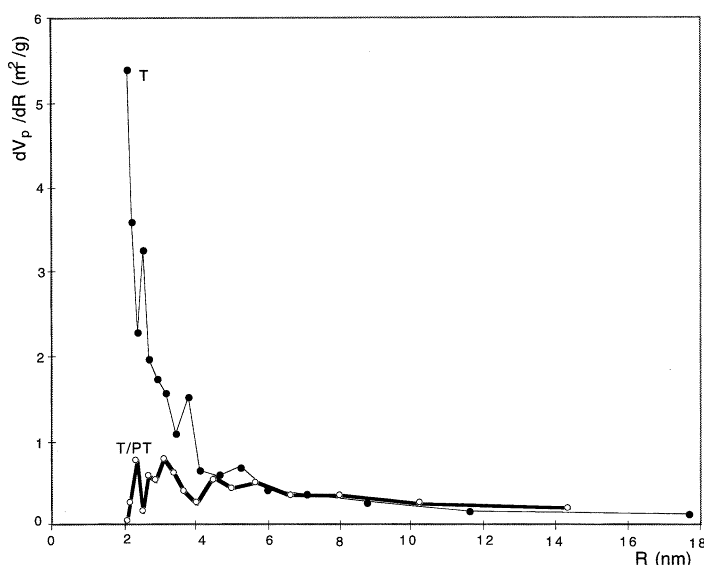

Figure 3 Pore size distribution derived from the isotherms in Fig. 2, in samples of types T and T/PT.

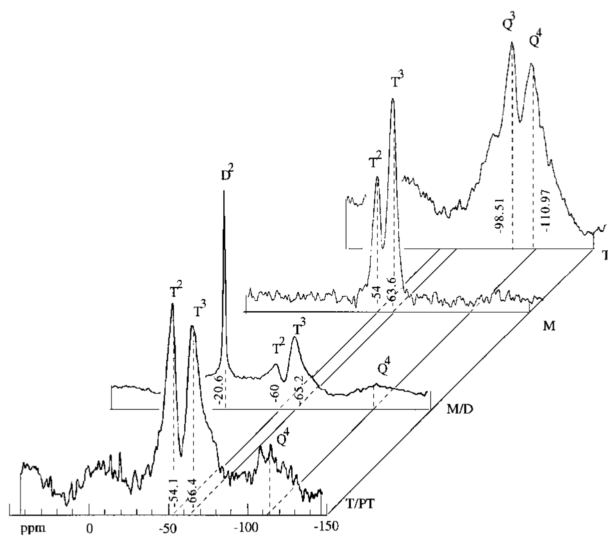

Figure 4 ^{29}Si MAS NMR of encapsulated gel samples of types T, M, M/D and T/PT.

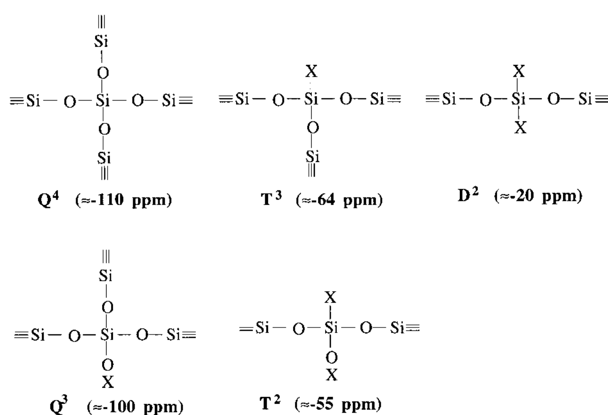

Figure 5 Si coordination configuration and their approximate chemical shift in p.p.m.

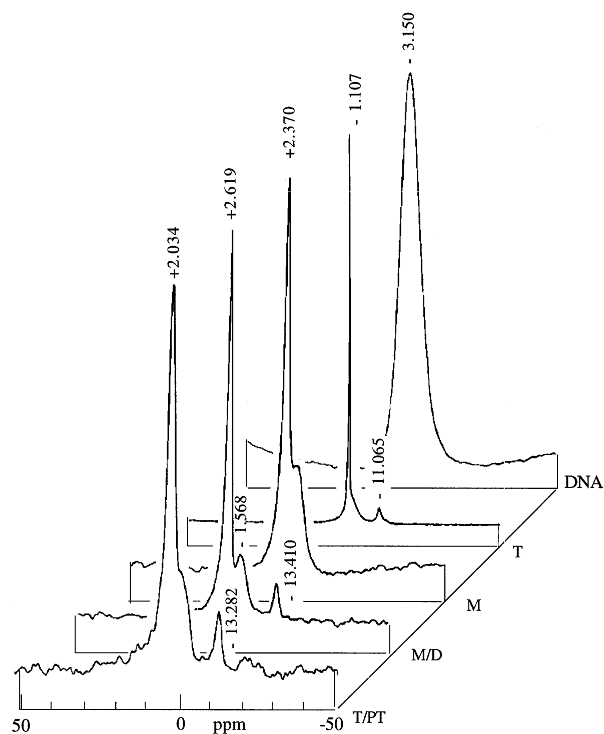

Figure 6 ^{31}MAS NMR of initial DNA and encapsulated DNA in T, M, M/D and T/PT samples.

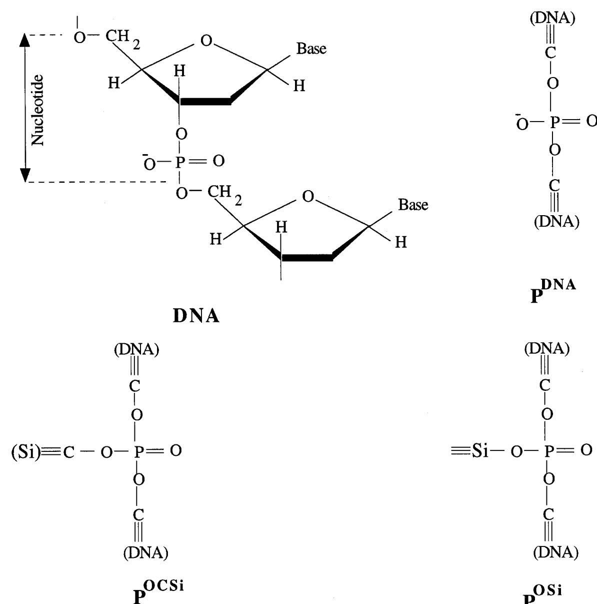

Figure 7 Configuration sites of P atoms in DNA molecules, and possible new configurations in encapsulated DNA materials.

both peaks shifted by about the same amount from sample to sample. A possible interpretation is that the C atom involved in the new link did not belong to PVA but to the alkyl group (methyl or propyl) present in the M, PT or D precursors. As the D precursor has two methyl groups per Si atom, it may explain why this peak was well identified in the M/D materials.

The second new small peak is only present in the T gel (at ≈ -11 p.p.m.) and in the M/D and T/PT gels (at ≈ -13 p.p.m.). We suggest that they could correspond to a new P configuration termed POSi in Fig. 7, where a new P-O-Si$\equiv$ link was established. The fact that it was not observed in M gels only, can be placed in relationship with the fact that only this gel did not show Q^4Si configurations in Fig. 4.

In summary, it appears that the DNA molecules were linked to the Si gel network, either through the intermediate of O-alkyl groups or of O-Si links, or both, which explains the difficulty in recovering these DNA molecules in a buffer solution only.

5. Conclusions

Analysis of small DNA molecules encapsulated in pure silica and in hybrid polyvinyl alcohol-silica gels, in particular by ^{31}P NMR spectroscopy, showed that complexation between the Si network and the DNA phosphate groups probably occurred. This explains that most DNA molecules could not be re-extracted from the gels further on. This result may also be in part due to the microporous pore size in the silica gel network, which was moreover plugged by PVA in the hybrid gels. A possible modification which could make it possible to recover the DNA molecules, would be to examine the DNA encapsulation in silica gels with a larger mesoporous size range.

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