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Influence of the sol–gel chemistry on the activity of a lipase encapsulated in a silica aerogel

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Abstract

Silica aerogels have been shown to be efficient encapsulation media for the lipase of *Burkholderia cepacia*. The present study has focused on the encapsulation of this lipase in an aerogel made from 80% tetramethoxysilane (TMOS) and 20% methyltrimethoxylsilane (MTMS), dried by the supercritical CO_2 method. By varying different parameters in the synthesis chemistry of such materials, the structure and texture of the resulting gels can be significantly affected. The aim of the present study was to examine the possible existence of correlations between modifications of the gel's synthesis procedure, and the catalytic activity of the gel-encapsulated lipase in the esterification reaction of lauric acid with 1-octanol. The synthesis parameters studied included aging of the wet gels in different solvents, variation of the solvent used during gel synthesis, variation in the molar ratio of hydrolysis water to silicon precursor and replacement of MTMS by another alkoxide. The biocatalytic activity was found to depend significantly on these different treatments. The results were analyzed in the light of the gel texture and structure characterization, respectively analyzed by nitrogen adsorption isotherms and ^{29}Si, ^{1}H and ^{13}C NMR. These results suggest that the main role of the aerogel is to maintain the enzyme dispersed as it would be in an aqueous solution, even though it is used in an organic solvent where the lyophilized enzyme is insoluble. The nature of the gel surface groups, in particular their capability to modify the substrates concentration inside the gel, close to the active site of the enzyme, by comparison with the concentration in the solvent outside the gel, seem to have a more secondary effect.
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1. Introduction

Recently, silica xerogels dried by evaporation for which no shrinkage had been observed, as well as aerogels dried by the CO_2 supercritical method, were shown to be efficient enzyme encapsulation media for lipases [1–3]. Moreover, the catalytic efficiency of lipases has been shown to be very dependent on their local environment, in particular on the hydrophobic–hydrophilic balance. Nonetheless, much remains to be known on the specific interactions between such gels and the enzymes. The aim of the present study was therefore to encapsulate a lipase, presently from *Burkholderia cepacia*, in aerogels prepared with a fixed molar ratio of TMOS:MTMS that is to say 80:20. Using the same ratio all long the study brought a fixed proportion of hydrophobic methyl groups, and made it possible to investigate the relative importance of other aerogel characteristics for biocatalytic activity. These characteristics could be due to changes in the gel texture, i.e. pore shape characteristics, or in the surface structure, such as the nature of functionalities present on the aerogel pore surface, other than $Si–CH_3$. The former characteristics could be analyzed by nitrogen adsorption isotherms, while the latter could be analyzed by combined ^{29}Si, ^{1}H and ^{13}C NMR.

Besides the precursor proportions, many other synthesis parameters can be varied in the encapsulation protocol. Presently, the wet gels containing the encapsulated enzyme, were submitted to different aging treatments before drying with supercritical CO_2. The gels were aged in different solvents for increasing aging periods, the liquids used were: the mother liquor, aqueous phosphate buffers at different pH, organic solvents, including acetone which is used as an exchange liquid before CO_2 supercritical drying. The nature of the organic solvent used for the gel synthesis, as well

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as the molar ratio r_w of hydrolysis water to silicon precursor were also examined. Finally, different sol–gel precursors were used to replace the MTMS in the same proportion (20% molar).

2. Experimental part

2.1. Materials

The precursors used in this study were: tetramethoxysilane (98%, MW 152.22, *d* 1.033); methyltrimethoxysilane (98%, MW 136.2, *d* 0.955) from Aldrich, vinyltrimethoxysilane (148.23 g/mol, 97% ABCR), phenyltrimethoxysilane (PhTMS, 198.29 g/mol, 97%, ABCR), isobutyltrimethoxysilane (iBTMS, 178.30 g/mol, 97%, ABCR), bis-trimethoxysilane hexane ($H(TMS)_2$, 326.54 g/mol, 97%, ABCR), methyltriethoxysilane (MTES, 178.30 g/mol, 99%, Aldrich). The other chemicals used were aqueous ammonia solution (0.1 M) from R.P. Normapur-Prolabo; methanol (R.P. Normapur for analysis 99.8%,); dioxane (Fluka, 99.5%), 2-methyl-2-butanol (Fluka, 98%), acetonitrile (Fluka, 99.5%), toluene (SDS, 99.5%), polyvinyl alcohol (MW 15 000) and technical grade acetone from Fluka, the water used was prepared with an ELGA PURELAB UHQ water purification system.

The enzyme was the lipase from *B. cepacia*. Some work presented in this paper, limited to partial drying by evaporation before supercritical drying, and aging in water at different pH and in organic solvents, was done with the lyophilized lipase from Fluka. In this case, enzyme solutions were prepared from the powders by dispersion in water, followed by centrifugation at 2700 rpm for 15 min, and stored at 4 °C before use. The protein concentration in the supernatant liquid was determined with the "BCA-200 Protein Assay Kit" of Pierce. Concentrations of typically ≈3 mg/ml were obtained and the pH of the solution was ≈6.5. However, all other experiments were actually made with the lyophilized lipase gracefully provided by Amano (lipase AYS). In this case, the protein solutions obtained after separation of the insoluble part by centrifugation were similar to those from Fluka (≈3 mg/ml). These protein solutions were moreover purified by precipitation with ammonium sulfate, then dialyzed in Tris–HCl buffer (pH ≈ 7), as described in a previous report [4]. Solutions with ≤0.5 mg/ml purified protein were finally obtained. That is to say, only ≤1% of an initial mass of lyophilized powder was finally used as a purified protein solution in water. The enzymatic activity of all parts eliminated during the protein solution preparation (centrifugation sediment, precipitation) was assessed. It corresponded to ≈40% of the initial lyophilized activity.

2.2. Biocatalytic assay

To determine their catalytic behavior, all the gel-encapsulated lipases were tested in the esterification of 1 mmol 1-octanol with 0.5 mmol lauric acid in 10 ml water saturated isooctane at 30 °C. Before use, the aerogel-encapsulated enzymes had been kept at a water activity $a_w \approx 0.4$. The dry gels were ground to powders with a particle size < 200 μm and agitation was done in a shaking water bath at 180 rpm. In these conditions and for the mass of enzyme dispersed in the gels, it was shown that no limitation of the chemical reaction by diffusion (internal or external) occurred. The reaction kinetics were followed by GC analysis. Fifty microlitre samples were taken from the reaction medium after 1 and 5 h (it was checked that the initial reaction rate could be calculated from the 1 h conversion) and analyzed on a Shimadzu gas chromatograph GC14 equipped with a polar capillary column (SGE BP21, 12 m, 0.22 mm i.d.) with the following temperature program: 5 min at 100 °C, 10 °C/min up to 200 °C, 4 min at 200 °C. The injector and FID detector temperature were 200 and 220 °C, respectively, and nitrogen was used as the carrier gas.

Because the different enzyme solutions prepared always had different protein concentrations, depending on enzyme batch and storage time, a reference aerogel was always made altogether with each series of aerogels scheduled to study the influence of a parameter. A typical reference aerogel sample had a mass ≈200 mg and contained ≈0.1 mg purified protein before supercritical drying, which had been introduced as an aqueous solution with the hydrolysis water. Some of these proteins in the larger pores were lost during CO_2 supercritical drying. Washing of the autoclave pipes and measurement of the protein concentration in the washing liquid provided an estimate of 20% protein loss. On the other hand, no loss was detected in the dialysis acetone, or in the solvent after a catalytic test.

Regarding the influence of the enzyme loading in a reference aerogel, it was found that in the range of 0–0.125 mg purified protein loading, the total activity of a sample was proportional to the mass of purified protein. This showed that no activity limitation was induced by an excessive mass of enzyme. A similar result was observed with the lyophilized enzyme from Amano, in the range from 0 to 60 mg lyophilized powder.

2.3. Reference aerogel synthesis

The reference aerogel synthesis protocol was chosen next to a preliminary study [3] where it turned out to provide an equivalent lipase activity to that obtained when using the most efficient protocol published by Reetz and Zonta [1], in spite of a much lower proportion of hydrophobic silica precursors (only 20% MTMS). Hence, it offered the possibility to really apprehend the effect of supercritical CO_2 drying, contrary to the gels with a much higher MTMS content which are quite similar to each other whether they are dried by evaporation or by supercritical drying (no shrinkage). The present protocol, derived from a protocol by Schwertfeger et al. [5] used methanol to maintain in solution all

precursors as well as the hydrolysis water, but methanol was found to not deactivate the selected lipase in agreement with other recent reports [6]. Indeed, not only the relatively long overall gelation time (typically ≈6 h) was found to not be harmful to the enzyme, but aging in the mother silica precursor liquor was even found to improve the enzyme activity as reported further on. A Lewis base such as NaF which accelerates drastically the condensation kinetics of silica is absolutely necessary to achieve gelation when a high proportion of MTMS is added: this was not the case here. Besides, fast silica condensation induces a significant heating of the enzyme and requires energetic mixing on a vortex agitator. In turn, this makes it very difficult to design the shaping of such a heterogeneous catalyst (e.g. small shaped monolith, membranes, etc.), which was not the case with the present protocol. The wet gel was also translucent, opening the possibility to directly measure the protein concentration inside the gel, except that the gel turned white after supercritical drying and that this idea was not further pursued. Overall, a number of gel synthesis parameters were investigated in the present study, but many more parameters could be important. This could be the case of the gelation catalyst or the precursor proportions. That is to say, it would not be surprising if sol–gel encapsulation showed some further progress in the future.

To synthesize a reference silica aerogel, 328 μl (2.216 mmol) tetramethoxysilane (TMOS), 82 μl (0.574 mmol) methyltrimethoxysilane (MTMS) were dissolved in 220 μl methanol (5.43 mmol). Methanol is very often used as a common solvent for alkoxide silicon precursors and water which are otherwise insoluble. The mixture of alkoxides in methanol is then added to an aqueous solution containing 192 μl of lipase solution, and 150 μl of a 4% w/w polyvinyl alcohol (PVA) (total water content 19 mmol). It took about 2 h before the emulsion disappeared and the sol started to form leading to a homogeneous, clear solution. The molar ratio of water to Si for hydrolysis and condensation was $r_w \approx 6.8$. It took about 4 h before gelation occurred. Once the gel was formed, it was left at rest in a closed vessel for about 36 h during which aging of the gel occurred. The aerogels used as a reference in this study were dialyzed for ≈24 h in acetone before supercritical CO_2 drying (CO_2 critical point temperature and pressure $T_c = 31.4\,^{\circ}C$, $P_c = 7.37$ bar). This dialysis was found necessary in order to exchange the solvents present inside the gel (water and methanol) for a solvent miscible with liquid CO_2, used during the drying procedure. When recycled or used for characterization after biocatalytic assay, the gels were washed in acetone after the catalytic test. In all studies summarized after, only one parameter of the reference protocol was modified at a time.

2.4. Synthesis parameters investigated

With the lipase from Fluka, series of wet gels were aged in water at pH ≈ 6.1, ≈7.3 or ≈8.3, for increasing times up to 720 h before supercritical drying. Other aging solvents were used: either hydrophobic isooctane or hydrophilic: dioxane containing 0.63% (per volume), 2.8 or 50% water. Also, the effect of a partial drying by evaporation in the laboratory ambient conditions before CO_2 supercritical drying was examined. The evaporation time ranged from 0 (aerogel) to 144 h (xerogel).

With the lipase from Amano, a first series of wet gels were submitted to aging in their mother liquor for increasing times up to 156 h before supercritical drying. Another series of gels aged for 24 h in their mother liquor were then dialyzed in acetone for times varying from 1 to 96 h before supercritical drying. As mentioned before, such a dialysis step is necessary to further exchange acetone for liquid CO_2 before drying in supercritical conditions. In all these gels, the encapsulated lipase was from Amano, except for the gels aged in water at different pH. As mentioned before, methanol is often used as a common solvent for the silicon precursors and the hydrolysis water necessary to form the sol, silicon alkoxides being insoluble in water. The influence of the nature of this common solvent was evaluated by replacing it with other solvents such as acetone, dioxane, 2-methyl-2-butanol and acetonitrile. The influence of the water to siloxane molar ratio r_w was also examined, namely $r_w \approx 3.4$, 10.2 and 13.6 (reference aerogel $r_w \approx 6.8$). In this series, the relative proportions of the three liquid components, i.e. CH_3OH, aqueous enzyme solution and 4% PVA aqueous solution, were kept identical to those used in the reference aerogel. At last, the influence of the nature of the hydrophilic group was evaluated by replacing the 20% MTMS with 20% (molar ratio) of vinyltrimethoxysilane (VTMS), phenyltrimethoxysilane (FTMS), isobutyltrimethoxysilane (iBTMS), bis-trimethoxysilane hexane ($H(TMS)_2$)or methyltriethoxysilane (MTES).

2.5. Samples characterization

All the samples were characterized in the dry state, after supercritical drying. The pore texture of most samples was analyzed by recording their nitrogen adsorption isotherms after desorption under vacuum during 6 h at 180 °C, either after or before catalytic test. For all the gels, only surface end groups could contain H atoms possibly belonging to $Si–CH_3$, $Si–OCH_3$, Si–OH, or adsorbed water molecules. Hence ^1H MAS NMR appeared as an interesting technique to study the surface. These spectra were obtained on a Bruker DSX-400 spectrometer at 400 MHz. For this purpose, the samples were spun in the magic angle at ca. 10 kHz, with pulse interval time 5 s and pulse duration 2 μs. The chemical shifts were measured using $Si(CH_3)_4$ as a reference. To quantify the ^1H NMR data, the integrated peaks were fitted with Gauss–Lorenz curves using WINNMR and XPLOT software. These data were completed by ^{29}Si CP MAS NMR spectra, for which the samples were spun in the magic angle at ca. 5 kHz with a contact time of 5 ms and a relaxation time of 5 s, which provided information on the gel network

connectivity. In some cases ^{13}C CP MAS NMR spectra were also obtained. In this case the samples were spun at 10 kHz, for a contact time of 3 ms. And a relaxation time of 2 s.

3. Results and discussion

3.1. Reference aerogel

In the model esterification reaction used to determine the biocatalytic activity, the initial esterification rates obtained with reference aerogels ranged from 650 to 950 U mg^{-1}(purified Amano before supercritical drying), where 1 U is defined as 1 μmol product h^{-1} initially produced, determined per milligram of enzyme introduced in the wet gels. Given the approximate loss of 20% protein upon supercritical drying, this is equivalent to 800–1200 U mg^{-1} (purified protein in the dry gel). This large variability has been found to mostly depend on the particular enzyme solution being used and on its storage time at 4 °C. On the other hand, for a series of reference samples made simultaneously from the same solution, the standard deviation has been found to be much lower, of the order of 5%. This is the reason why, for each parameter of the present study, a reference aerogel was always made and all the gels of the series were compared to this reference.

With the Fluka as well as with the Amano lipase simply centrifuged before encapsulation, the aerogel activity per milligram of protein was about 10 times lower than with the purified lipase. The aerogel activities made from purified enzyme, also compared with an activity for the as received lyophilized enzyme powder $\nu_0 \approx 2.5$ U mg^{-1}(lyophilized), which was similar for the Fluka lipase and the Amano lipase. That is to say the two enzyme sources provided consistent results.

Considering that a proportion ≈1% of the as received lyophilized enzyme could be introduced in the wet gel after purification, as previously. Considering moreover that ≈40% of the active proteins from the lyophilized powder was eliminated during preparation of the protein solution, and another ≈20% protein was lost during supercritical drying, the activity of the lyophilized enzyme per equivalent mass of purified protein can then be estimated to be $\nu_0 \approx$ 100 U mg^{-1}(equivalent purified protein). That is to say, the aerogel encapsulation improved the enzyme activity by a coefficient of the order of ≈10 per milligram of purified lipase. This magnification factor is indeed consistent with a previous value obtained by direct encapsulation of the lyophilized Fluka enzyme inside an aerogel [7].

A typical nitrogen adsorption isotherm obtained for a reference aerogel is reported in Fig. 1 ($r_w \approx 6.8$), altogether with the isotherm for a similar wet gel dried by evapo-

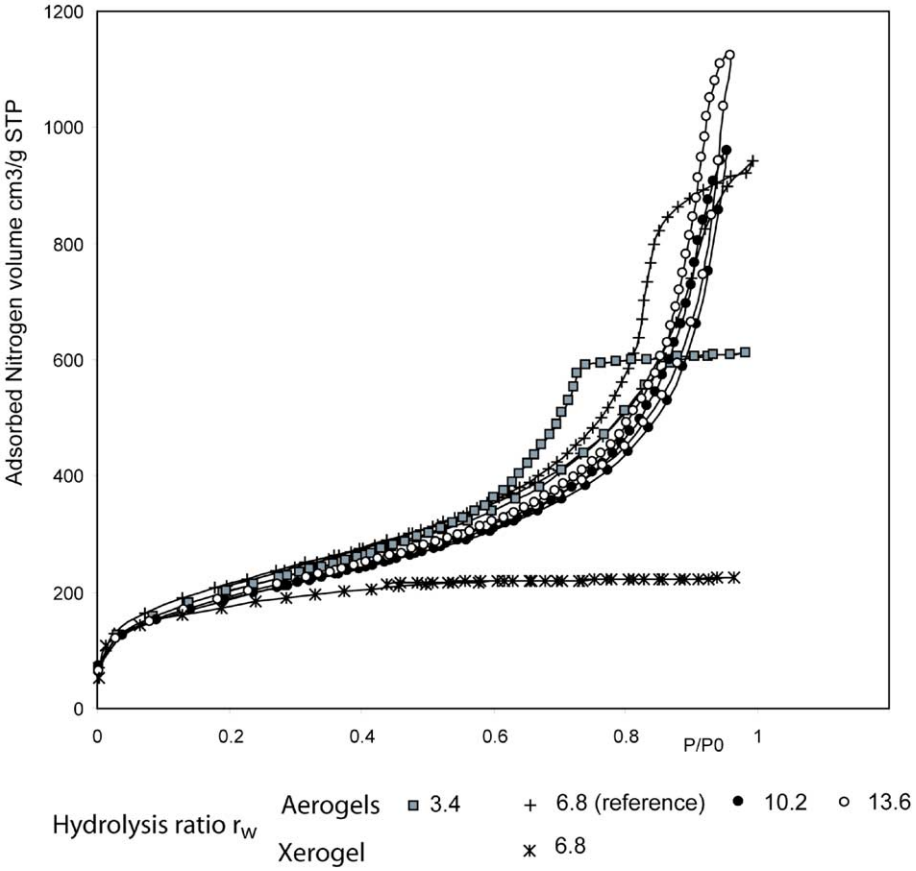

Fig. 1. Nitrogen adsorption isotherms of aerogels made with an increasing proportion of silicon hydrolysis water r_w (purified lipase from Amano).

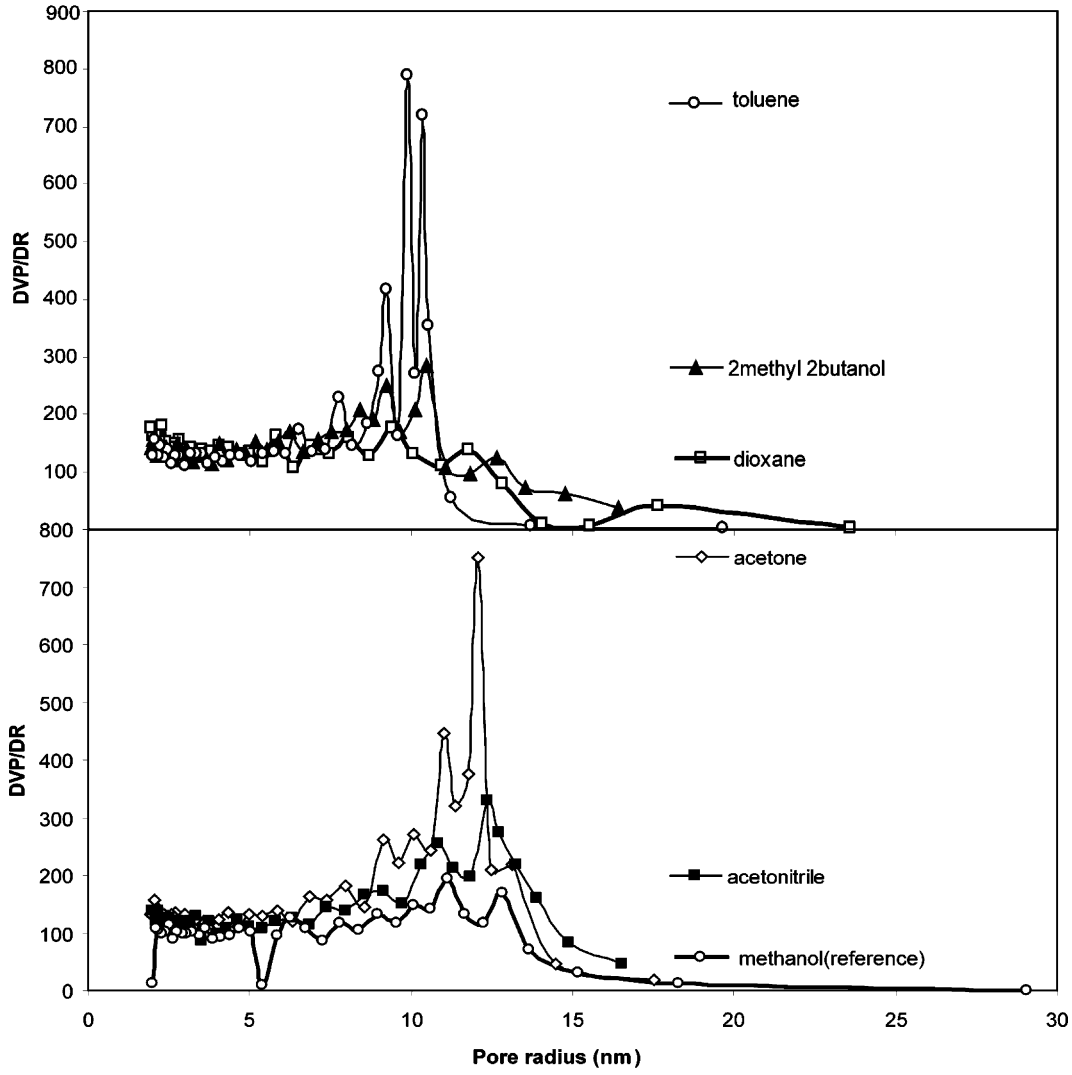

Fig. 2. Pore size distributions derived from the desorption branches of the nitrogen adsorption isotherms, by the Roberts method [23,24] for the reference aerogels synthesized in different solvents (purified lipase from Amano).

ration to produce a xerogel. The aerogel isotherms were very similar to each other, whether encapsulation was done with the Fluka or the Amano lipase. Isotherms for aerogels prepared with different hydrolysis ratio r_w are also shown in the same figure. These isotherms are all of type IV according to the classification by Brunauer, Deming, Deming and Teller (BDDT) [8]. The so-called mesopores, which are pores with a radius between 2 and 50 nm, are responsible for the observed adsorption–desorption hysteresis loops. That is to say, they make the major contribution to the specific surface area A_{sp}, as well as an important contribution to the specific pore volume V_{sp}. Nonetheless, caution must be used regarding the significance of V_{sp} as determined from these isotherms, because in silica aerogels the major contribution to the volume is not due to mesopores but to larger pores for which the adsorption–desorption characteristics are too close from $P/P_0 = 1$ (P_0 is the saturation pressure of nitrogen over a flat interface) hence can only be observed with very special equipment. In spite of this, the pore incremental distribution defined as the contribution dV_p to pore volume due to pores with a radius between r and $r + dr$, that is to say the derivative dV_p/dr, shows a slight maximum contribution due to pores with a radius in the range 10–12 nm (Fig. 2). As shown by Fig. 2, this maximum can be enhanced by using other sol–gel synthesis solvents than methanol. The specific surface area of a reference aerogel area was typically $\approx 850\ m^2/g$, mainly due to mesopores.

For the xerogel in Fig. 1, the specific pore volume V_{sp} ($\approx 0.35\ cm^3/g$) was much lower than in the aerogel ($\approx 1.45\ cm^3/g$), the cylindrical sample radius shrinkage being of the order 7% for the aerogel, instead of 70% in the xerogel. On the other hand the specific surface area A_{sp} decreased much less, from 850 to $600\ m^2/g$. Indeed, while the aerogel was mostly composed of mesopores (pore of radius from 2 to 50 nm contributing to over 80% of V_{sp}),

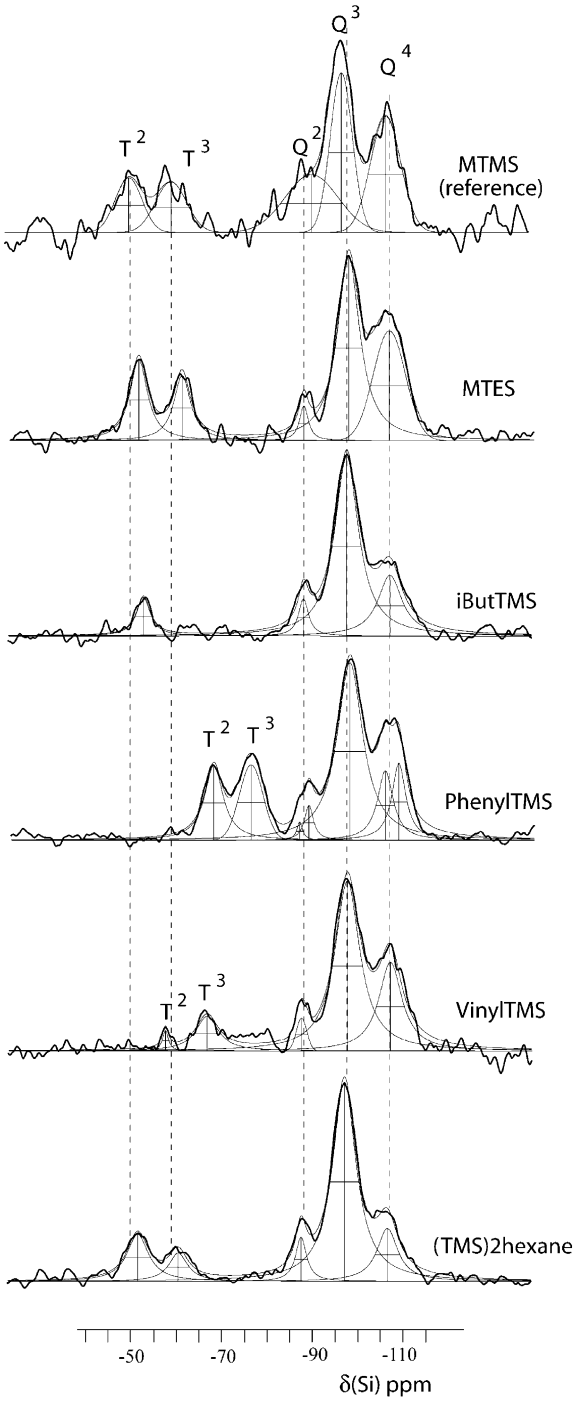

Fig. 3. ^{29}Si NMR spectra of aerogels in which MTMS was replaced by another silica precursor (purified lipase from Amano).

the xerogel was mostly composed of micropores (pore of radius <2 nm contributing to over 90% of V_{sp}).

The nature of the gel solid backbone which explains the occurrence of such high specific surface areas, is illustrated in the ^{29}Si NMR spectra in Fig. 3 where the spectra of a reference aerogel (containing MTMS) is reported, altogether with the corresponding spectra of similar aerogels in which the 20% MTMS were replaced by another silicon precursor. According to known chemical shifts [9,10], this spectra shows the presence of an excess of Q^3 sites (i.e., $Si(OX)(OSi{\equiv})_3$ sites in which X can be any terminal group without Si, with a lower proportion of Q^4 and Q^2 sites (i.e., respectively, $Si(OSi{\equiv})_4$ and $Si(OX)(OX')(OSi{\equiv})_2$). T^3 and T^2 sites (i.e., respectively, $SiCH_3(OSi{\equiv})_3$ and $SiCH_3(OX)(OSi{\equiv})_2$) due to hydrolysis and condensation of MTMS are also present. In the case of reference aerogels, the relative area of T^2+T^3 to all T^n and Q^n signals is ≈23%, which is globally consistent with the proportion of MTMS used as the silicon precursor. The high specific surface area comes from the Q^2, Q^3, T^2 and T^3 sites which involve a large majority of the Si atoms, while the densely cross-linked SiO_2 network only corresponds to Q^4, present in a lower proportion.

The local environment which is likely to influence the enzyme activity, in a gel, is related to the nature of the silica

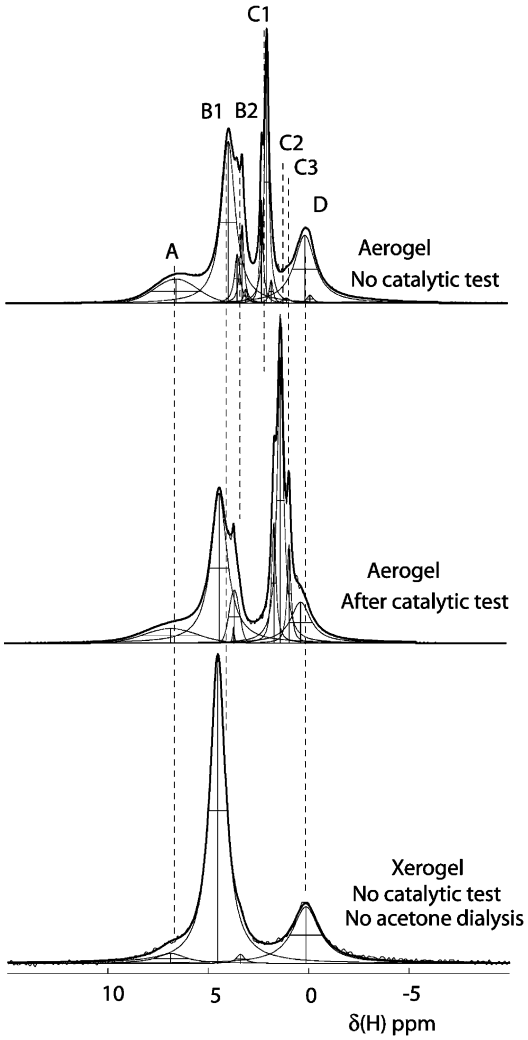

Fig. 4. ^{1}H NMR spectra of a reference aerogel after acetone dialysis and CO_2 supercritical drying before a catalytic test; a similar aerogel after a catalytic test; and a xerogel similar to the reference aerogel but dried by evaporation without dialysis in acetone, before a catalytic test (purified lipase from Amano).

Table 1
Assignation of the main signals in ^{1}H NMR of silica gels (in bold, the most intense signals)

Molecule	Chemical functionality	δ (ppm)	Reference
Gel synthesis medium			
Acetone $(CH_3)_{2A}CO$	**CH_3(A)**	**2.162**	SDBS 319 [11]
Methanol $CH_{3B}–OH_A$	OH(A)	3.66	SDBS 3302 [11]
	CH_3(B)	**3.43**	
MTMS $CH_{3B}–Si–(OCH_3)_{3A}$	**CH_3(A)**	**3.558**	SDBS 3963 [11]
	CH_3(B)	0.124	
TMOS $Si–(OCH_3)_A$	**CH_3(A)**	Unknown	
H_2O		1.58	[12]
Silica gels			
Si–OH	Calculation	2–2.2	[13]
	Empirical	2.3	[14]
	Empirical	1.7–2.1	[15]
	Empirical	3.5	[16]
Si–OH···O	Calculation	Mostly 5–8	[13]
	Empirical	3.0	[15]
	Empirical	3.5	[15]
$Si–OH\cdots OH_2$	Empirical	3.5–3.8	[16]
$Si–O–CH_3$	Empirical	**3.5**	[16]
$Si–OH\cdots OHCH_3$	Empirical	3.5	[16]
Reaction medium			
1-Octanol $CH_{3E}–(CH_2)_{4D}–(CH_2)_{2C}–CH_{2A}–OH_B$	CH_2(A)	3.604	SDBS 1938 [11]
	OH(B)	2.40	
	CH_2(C)	1.55	
	CH_2(D)	1.48	
	CH_3(E)	**1.29**	
		0.884	
Isooctane $(CH_3)_{2C}–CH_A–CH_{2B}–C(CH_3)_{3D}$	CH(A)	1.659	SDBS 2353 [11]
	CH_2(B)	1.122	
	CH_3(C)	0.907	
	CH_3(D)	**0.891**	
Lauric acid $CH_{3E}–(CH_2)_{8D}–CH_{2C}–CH_{2B}–COOH_A$	COOH(A)	11	SDBS 1497 [11]
	CH_2(B)	2.34	
	CH_2(C)	1.64	
	CH_2(D)	**1.46**, **1.07**	
	COOH(E)	0.88	

gel network terminal groups, which happen to be the only one to actually involve H atoms. Hence they can be studied by solid state ^{1}H NMR, although the corresponding spectra were found to be much more difficult to interpret. Actually, these spectra were found to largely depend on the history of a gel. Indeed, Fig. 4 shows the ^{1}H NMR spectra of aerogel reference samples, before being submitted to a catalytic test (reminder: each wet gel is dialyzed in acetone before supercritical drying), then after being tested in a catalytic test (a sample used in a catalytic test was systematically washed in acetone and dried by evaporation before recording its ^{1}H spectra). Moreover, a ^{1}H NMR spectrum for a xerogel (for which the wet chemistry is similar to the reference aerogel, except that it was directly dried by evaporation without dialysis in acetone) is joined. These ^{1}H NMR spectra clearly show four groups of signals, which are labeled A, B1 + B2, C1, C2 + C3, and D in the present paper. In order to interpret them, the main chemical shift attributions regarding either the reactants in the gel synthesis solvent, the esterification reaction medium, or attributions from other reports for silica gels, were gathered in Table 1. It must be noted that the values quoted for silica gels were either obtained by ab initio calculations, or were empirically reported in papers without real sound justification.

The ^{1}H NMR signal D located at a chemical shift from 0 to 0.16 ppm can without any controversy be attributed to $Si–CH_3$. First, the proton chemical shifts were determined with respect to $Si(CH_3)_4$, hence values close to 0 ppm. Secondly it corresponds to the shift of this side group in MTMS. Thirdly, its magnitude was found to increase with the proportion of MTMS in the gels, in another study [17]. Finally, the solid state ^{13}C MAS NMR spectra, in spite of their general poor quality, clearly show a sharp peak near 0 ppm attributable to this group (Fig. 5), which is normal because chemical shifts are also measured by comparison with $Si(CH_3)_4$ in ^{13}C NMR. It must be pointed out that the poor quality of the ^{13}C NMR spectra is normal, because this is solid state NMR, and only a relatively low proportion of the gel terminal groups comprised C atoms.

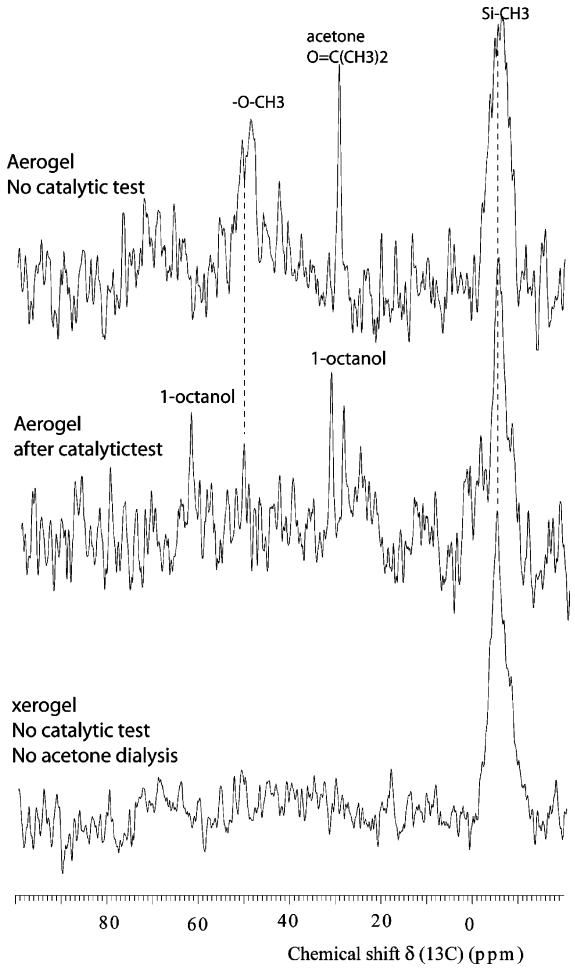

Fig. 5. ^{13}C NMR spectra of the same gels as in Fig. 4 (purified lipase from Amano).

The group of chemical shifts C2 + C3 (0.7–1.5 ppm) was only observed for aerogels which had been used as catalysts in esterification reaction, and when the test was found to be efficient. This suggests that the signals are due to the strong adsorption of one or several of the substrates (1-octanol, lauric acid) or product (octyl laurate ester). Indeed, the ^{1}H NMR spectra in liquid medium of these compounds have their main chemical shifts near this value. We also checked that these compounds adsorb significantly on silica aerogels. This adsorption is strong enough so that these products cannot be desorbed easily by washing with acetone. This is indeed consistent with the fact than in silica gas chromatograph columns, acetone passes through quickly while the carboxylic acid and ester are retained a longer time. Besides, the ^{13}C NMR spectra do show significant peaks corresponding to the known liquid medium chemical shifts for the 1-octanol (≈63 ppm and group near 32 ppm) or lauric acid (group near ≈31 ppm).

The sharp C1 signal at ≈2 ppm, is mostly observed in aerogels dialyzed in acetone before supercritical drying, and not used in catalytic tests. It drastically attenuates in aerogels which have been stored for a long time in open air in the laboratory ambient conditions. It is not observed in xerogels dried by evaporation in air, hence it can be explained by the adsorption of acetone, the main signal of which happens to be near that chemical shift (Table 1). This explanation is comforted by ^{13}C NMR which in this case shows the main sharp signal of acetone near ≈30 ppm. Note that, according to ab initio calculations as well as empirical proposals (Table 1), isolated Si–OH also have a chemical shift near this position. Hence it is possible that both shifts overlap onto each other, which might explain that a small peak always remains after a long storage time. Considering that acetone probably adsorbs on Si–OH (i.e. Si–OH $\cdots$ O=C$(CH_3)_2$), and that each acetone molecule brings six hydrogen atoms by comparison with one for Si–OH, the suggestion appears to be self consistent.

The group of chemical shifts B1 (3.9–4.6 ppm) and B2 (3.2–3.4 ppm) corresponds to values close to –O–CH_3 functionalities which belong either to adsorbed methanol, or to un-hydrolyzed alkoxy ≡Si–OCH_3 (chemical shift near 3.5 ppm for both). Moreover, some empirical attributions to Si–OH $\cdots$ OH_2 in the range 3.5–3.8 ppm have been proposed, while ab initio calculations showed that hydrogen bonds of the type of Si–OH $\cdots$ O usually result in a chemical shift from 5 to 8 ppm (Table 1). Our interpretation is that B2 is due to –OCH_3 groups, which is comforted by the corresponding ^{13}C NMR data. Also, in another study with increasing proportions of MTMS, this peak was found to increase with the proportion of un-hydrolyzed MTMS [17]. Considering that hydrogen bonding of –O–CH_3 groups with neighbor Si–OH $\cdots$ groups are possible, the variable value of the observed chemical shifts can be understood (Fig. 6). Nevertheless, it cannot be decided whether this signal corresponds to adsorbed methanol or to un-hydrolyzed Si–OCH_3.

As for B1, it was very prominent in the xerogel (Fig. 4) whereas ^{13}C NMR did not show the presence of –OCH_3 groups. Hence we assume this must be attributed to hydrogen bonded Si–OH $\cdots$ O. This is also true for the chemical shift $\delta_H(A)$ ≈ 6.5–7 ppm, in agreement with ab initio calculations [13]. Besides, a silica bound OH group can participate in various types of hydrogen bonds, such as with adsorbed water Si–OH $\cdots$ OH_2, or according to the vicinal mode Si–OH–Si (Fig. 6) and it has been shown that the chemical shifts of these silica bound OH can vary significantly depending on the material history and thermal treatment [18].

3.2. Aerogel surface modification by aging in liquid media before drying (lipases from amino and from Fluka)

The aging of wet gels in various liquids before acetone exchange and supercritical drying, for a same proportion of silicon precursors and similar chemical synthesis protocol, can have an important effect on their catalytic activity. Moreover this effect drastically depends on the aging conditions (Table 2). Indeed, wet gels with the amano lipase aged in their mother liquor before supercritical drying showed a

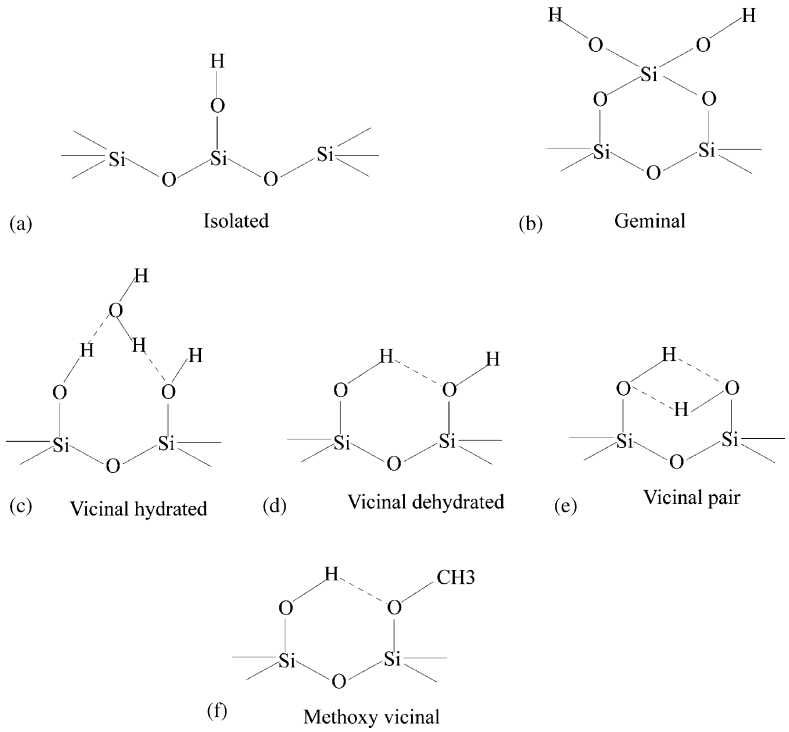

Fig. 6. Various Si–OH surface groups configuration according to Ref. [25], and possible hydrogen bonds with Si–OCH_3 (purified lipase from Amano).

catalytic activity which tended to increase moderately with the aging time. A similar result was found with the Fluka lipase. This was a quite different evolution from that obtained with the Fluka lipase by letting the wet gels stand in water at different pH before acetone dialysis and supercritical drying. In the latter case, the activity drastically decreased during the first aging days until it reached a stable pH-dependant value. For the samples aged in an organic liquid containing a low amount of water, such as water saturated isooctane (virtually no water as the two liquids are immiscible) or dioxane containing 2.8% water, the catalytic activity slightly increased with both lipases (isooctane) or remained unchanged (dioxane with 2.8% water). On the contrary, in dioxane: water 50:50, the catalytic activity rapidly decreased with the aging time, reaching values even lower than those reached in aqueous media. As for dialysis in acetone, which can be considered as a type of aging in acetone before supercritical drying, the activity significantly increased for aging time up to 5 h with the Amano lipase, after which it reached a stable value. Aging in acetone with the Fluka lipase for at least 24 h gave consistent results with that from the Amano.

Table 2
Effect of aging a wet gel in various liquids, before CO_2 supercritical drying, on the catalytic activity of aerogels

Aging liquid	Aging time (h)	ν/ν_{ref}	$\nu/\nu_{lyophilized}$
Mother liquid (reference)	24	1	10
Mother liquor	132	1.36	13.6
Water pH 6.16	265 h	0.5	5
Water pH 7.29	265 h	0.25	2.5
Water pH 8.23	265 h	0.30	3
Isooctane	456 h	1.25	12.5
2.8% water dioxane	456 h	0.95	9.5
50% water dioxane	456 h	0.07	0.7
Acetone	1 h	0.42	4.2
Acetone	5 h	1.01	10.1
Acetone (reference)	24 h	1	10

As explained in the text, comparison with the lyophilized enzyme activity is made assuming an improvement factor of 10.

Possible correlations between the catalytic activity of the aerogels and their texture characterized by A_{sp}, V_{sp} and the pore size distributions derived from the nitrogen adsorption isotherms can first be examined. The texture remained unchanged after aging in the mother liquor. On the other hand, aging in water at different pH had an effect. The specific pore volume, V_{sp}, remained relatively constant while the specific surface area, A_{sp}, only moderately decreased with the aging time. More precisely, the contributions to the specific surface area $A_{sp,Kel}$ and the specific pore volume $V_{sp,Kel}$, due to pores with a radius larger than the Kelvin radius (that is to say roughly the mesopores with a radius >2 nm), increased significantly during the first 4 days, before reaching a relatively higher constant value. Overall, this textural evolution correlated reasonably well with that of the catalytic activity, as shown in Fig. 7. In organic solvents, the aging of aerogels induced an effect similar to aging in aqueous medium on the texture. That is to say the specific surface area, $A_{sp,}$ moderately decreased while the specific pore volume, $V_{sp,}$ moderately increased with time. Also, the contributions to the specific surface area, $A_{sp,Kel,}$ and the specific pore volume, $V_{sp,Kel}$, due to the mesopores, increased. In this case,

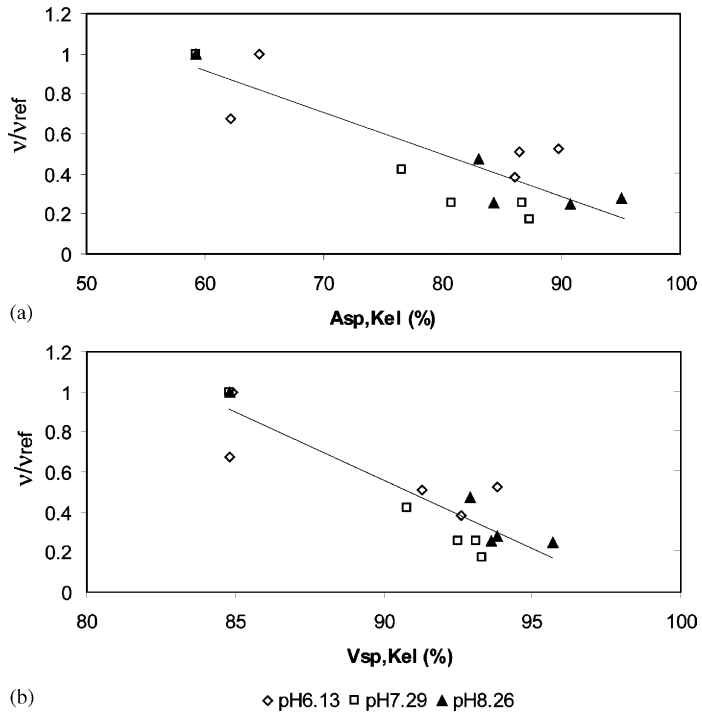

Fig. 7. Correlation between the relative activity of the aerogels, defined as the ratio of aged activity ν to unaged activity ν_{ref} and the mesoporous texture, for aerogels aged in aqueous media at different pH: (a) contribution $A_{sp,Kel}$ of pores with a radius larger than the Kelvin limit to the specific surface area; (b) contribution $V_{sp,Kel}$ of pores with a radius larger than the Kelvin limit to the specific pore volume (lipase solution from Fluka).

no correlation existed with the evolution in the catalytic activity which was opposite in isooctane and in dioxane with 50% water.

On the other hand, a correlation was observed between the catalytic activity and the texture, as a result of different dialysis times in acetone before supercritical drying. A 1 h acetone dialysis time was apparently insufficient to fully exchange the aqueous-methanol solution for acetone, before CO_2 supercritical drying, so that this aerogel was less able to resist the drying capillary stresses. The nitrogen adsorption isotherm was also closer from that of a xerogel, by comparison with the isotherm of a sample dialyzed for 5 h. Acetone dialysis for 5 h was sufficient to perform the appropriate solvent exchange, which resulted in a higher specific pore volume (≈2.5 time higher), as well as a higher contribution of mesopores in the radius range from 6 to 9 nm (Fig. 8).

Regarding correlations of the catalytic activity with the aerogel structure, a quantification by Gauss–Lorenz curves of the ^{29}Si NMR spectra indicated an average total area of ≈28% for the T^2+T^3 signals, versus the $Q^2+Q^3+Q^4$ ones, for all gels aged in the mother liquor. This indicates that the degree of silica cross-linking was relatively constant, a result consistent with that reported by Reetz [19]. The only significant structural difference between samples, concerned the relative area of the D signals attributed to Si–CH_3 in the ^{1}H NMR spectra, by comparison with the C signals attributed either to adsorbed acetone or to adsorbed substrates, or even by comparison with the B signals attributed to Si–OCH_3 or Si–OH···O. It must be reminded that such an observation has to be handled with caution, as the relative magnitude of these signals depends on the conditions to which a sample was submitted right before recording the ^{1}H NMR spectra. It is likely that adsorption of acetone or 1-octanol occurs on silanols Si–OH side groups, so that the ratio of signals D to C could provide an estimation of the surface Si–CH_3 sites versus Si–OH ones. For instance, the samples aged in their mother liquor showed a tendency to increase the relative magnitude of the D signals, attributed to Si–CH_3. This would indicate that increasing time in the mother liquor permitted to complete the hydrolysis of residual MTMS precursor molecules, which are more difficult to hydrolyze than TMOS, leading to a better coverage of the aerogel pore surface with Si–CH_3 groups.

Also, the ^{1}H NMR spectra showed an evolution during the first 5 h of acetone dialysis. The relative intensity of the B1 signal attributable to Si–OH···O decreased from ≈76 (1 h dialysis) to ≈40% (5 h dialysis) and then remained constant. After 1 h acetone dialysis, the ^{1}H NMR spectra looked like that of a xerogel, while the spectra after 5 h acetone dialysis was similar to that of a reference aerogel. This evolution correlated well not only with the catalytic activity, but also with the texture evolution. In this case, the correlation can be attributed to an insufficient liquid exchange after 1 h dialysis, before CO_2 supercritical drying, which resulted in a material closer to a xerogel than an aerogel. Of course, a more reliable comparative description of samples would require a more involved standardized sample preparation procedure before recording the NMR spectra, such as a standardized

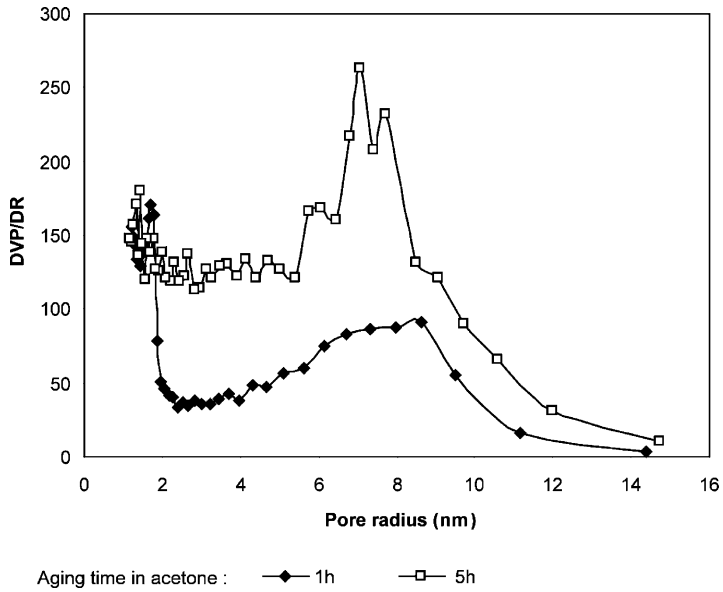

Fig. 8. Pore size distributions of aerogels dialyzed for increasing times in acetone before supercritical drying, derived from the desorption branches of these isotherms according to the Roberts method [23,24] (purified lipase from Amano).

acetone adsorption. Nevertheless, it could be noticed that each time an aerogel-encapsulated enzyme was efficient as a biocatalyst, the B signals attributable to the adsorbed acetone right after supercritical drying, or adsorbed substrates right after a catalysis test, were more prominent.

3.3. Aerogel surface modification by liquid medium synthesis conditions (lipase from Amano)

The solvent used for the sol–gel synthesis also had a significant influence on the catalytic activity, which is summarized in Table 3. This effect may be explained either by a difference in the nature of the solvents which remained adsorbed on the gel, or by an interaction between the solvent and the enzyme during encapsulation, or by a different amount of residual water. As for the quantity of water used to hydrolyze the silicon precursors, the data in Table 3 show that the catalytic activity increased as r_w increased from ≈3.4 to 10.2. Then, it remained relatively constant for $r_w \geq 10.2$.

Table 3
Effect of the sol–gel synthesis solvent and hydrolysis ratio r_w on the catalytic activity of aerogels

Organic solvent	Hydrolysis ratio, r_w	ν/ν_{ref}	$\nu/\nu_{lyophilized}$
Methanol (reference)	6.8	1 (reference)	10
Acetone	6.8	0.74	7.4
Dioxane	6.8	1.12	11.2
2-Methyl-2-butanol	6.8	0.51	5.1
Acetonitrile	6.8	0.60	6
Toluene	6.8	0.67	6.7
Methanol	3.4	0	0
Methanol	6.8	1 (reference)	10
Methanol	10.2	1.32	13.2
Methanol	13.6	1.29	12.9

As explained in the text, comparison with the lyophilized enzyme activity is made assuming an improvement factor of 10.

The nature of the solvent did not change the shape of the nitrogen adsorption isotherms. These isotherms remained of type IV corresponding to mesoporous materials [8], although the specific pore volume V_{sp} and specific surface area A_{sp} could change by up to ≈50% depending on the solvent. Consequently, the pore size distribution curves derived from these isotherms (Fig. 2) all showed a maximum in the same range, from 8 to 14 nm, although the relative intensity of this maximum depended on the solvent. Nonetheless, no real correlation between the catalytic activity ν and the contribution $A_{sp,Kel}$ or $V_{sp,Kel}$ of mesopores to the texture, could be found. The results were different for the aerogels synthesized with a different r_w hydrolysis ratio, for which an evolution of the nitrogen adsorption isotherms and pore size distribution correlated with the activity was observed (Figs. 1 and 9). The adsorption isotherms showed an evolution towards a type IV shape with a reduced hysteresis loop and high specific pore volume for $r_w \geq 10.2$, while the pore radius distribution changed from relatively centered (≈5.1 nm for $r_w = 6.8$) to a rather flat distribution up to 15 nm for $r_w \geq 10.2$.

There was no significant effect of the synthesis organic solvent on the aerogel structure, according to the ^{29}Si NMR spectra. The only difference concerned the B signals attributable to Si–OCH_3 (≈3.5 ppm) or Si–OH···O (3.9–4.5 ppm) in the ^{1}H NMR spectra. This signal was more or less intense relative to the other signals, depending on the solvent, although no correlation to any textural or activity data could be made. On the other hand, the ^{1}H NMR and ^{13}C NMR (Fig. 10) spectra showed decreasing signals for the –OCH_3 groups due either to un-hydrolyzed Si–OCH_3

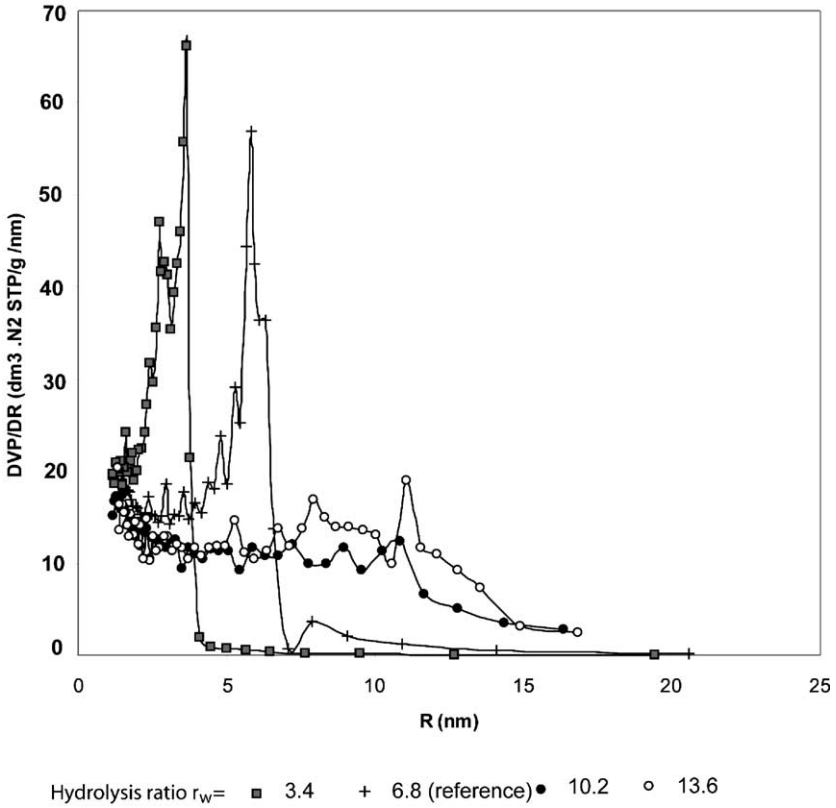

Fig. 9. Pore radius distribution curves derived from the desorption branches of the nitrogen adsorption isotherms, for aerogels hydrolyzed with an increasing hydrolysis ratio r_w (purified lipase from Amano).

or to adsorbed methanol, by comparison with the Si–CH_3 signals, as r_w and the catalytic activity both increased. This can be explained by a better hydrolysis of the residual alkoxy groups as the hydrolysis water ratio increased.

3.4. Replacement of MTMS by another silicon precursor (lipase from Amano)

The catalytic activity was found to depend very much on the hydrophobic precursor used to replace MTMS, for the same molar proportion (20%). However, no apparent relationship at all seemed to exist between this activity and the pore texture characteristics, as this is shown in Table 4 and Fig. 11. For instance, the pore size distribution of iButTMS was intermediate between those of VinylTMS and MTMS, and this was true also regarding specific surface area and specific pore volume (Table 4). Yet the former precursor produced aerogels with no activity compared to the two other ones. In the ^{1}H NMR spectra, the C peaks due to acetone or substrate adsorption ($\delta_H \approx 2$ ppm) was present in all aerogels before catalysis except on the sample partly made from bis(TMS)hexane (Fig. 12), which showed no activity. Indeed, it was well present for instance in the samples made with iButTMS which also showed no activity. This would tend to indicate that the reaction was not displaced by modification of the substrate concentration inside the pores of the gels, that is to say in the immediate vicinity of the enzyme, by comparison with the external liquid which surrounds monolithic gel pieces.

3.5. Partial drying by evaporation (lipase from Fluka)

The nitrogen adsorption isotherms of the mixed xeroge–aerogels AX with the encapsulated lipase from Fluka were already reported in a previous publication [7]. Increasing the evaporation up to 144 h before performing supercritical drying, progressively transformed the isotherms from a shape typical of aerogel, to a shape typical of xerogels (Fig. 1). Simultaneously, the catalytic activity decreased and had disappeared in the xerogel. Actually, as shown in Fig. 13, this evolution correlated well with the progressive disappearance of the mesopores which is complete after 120 h evaporation. An exception concerns the two data points for $A_{sp,Kel} > 60\%$ or $V_{sp,Kel} > 80\%$ (solid symbols in Fig. 13), which showed a different correlation, nevertheless consistent with the evolution observed for samples aged in aqueous media at different pH, in the same range of mesopore relative contribution (Fig. 7). Indeed, the xerogels and aerogels made with the lipase from Amano, were similar with respectively the aerogels and xerogels made with the lipase from Fluka. The xerogels had a very small activity compared to the free enzyme.

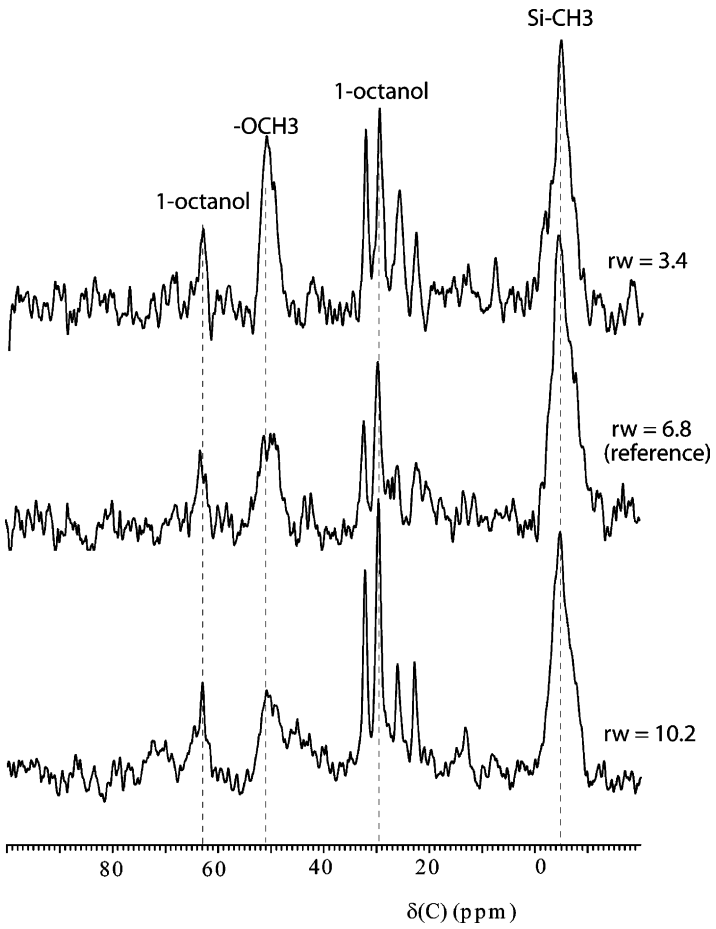

Fig. 10. ^{13}C NMR spectra of aerogels made with an increasing ratio r_w of hydrolysis water to Si (purified lipase from Amano).

3.6. General analysis of the results

Enzymes such as lipase and silica gels both interact with liquid media and have a structure which can be modified by these liquids. Hence it seemed interesting to see if any correlation between the activity of an encapsulated lipase, and the encapsulating aerogel structure could be observed, when submitting both of them to a large range of liquid medium treatments. Basically, the present study showed that such a correlation did not exist at large, although partial correlations could be observed in some cases, for instance when aging in aqueous media at different pH. That is to say simultaneous but independent evolutions of the enzyme and the gel occurred, because of interactions with the liquid. Stated differently, the main influence of the aerogel network on the enzyme activity is different from that due for instance to a liquid solvent.

It is well known that the texture of silica gel network depends significantly on their history in liquid medium. Such evolutions after gelation during aging in aqueous medium were extensively described, in particular by Brinker and Scherer [20]. They comprise: (1) shrinkage under the capillary forces which operate during evaporation; (2) spontaneous shrinkage, termed syneresis, inside the mother liquor

Table 4
Effect of 20% Si precursor other than TMOS, in aerogel synthesized in dioxane, on the initial esterification rate ν, and the textural characteristics (A_{sp}: specific surface area; V_{sp}: specific pore volume; $A_{sp,Kel}$ and $V_{sp,Kel}$: contributions to A_{sp} and V_{sp} due to the pores with a radius larger than the Kelvin limit, calculated from the desorption branch of the isotherm by the Roberts method [23,24])

Precursor material	A_{sp} (m^2/g)	C (BET)	$A_{sp,Kel}$ (% of A_{sp})	V_{sp} (cm^3/g)	$V_{sp,Kel}$ (% of V_{sp})	ν/ν_{ref}	ν/ν_{lyop}
MTMS (reference)	921	92	65	2.13	90	1	10
VinylTMS	716	168	52	1.43	82	1.07	10.7
PhenylTMS	804	125	32	0.97	64	1.12	11.2
iButTMS	750	123	61	1.70	86	0	0
$H(TMS)_2$	68	110	100	0.19	100	0	0
MTES	832	67	87	2.84	91	0.46	4.6

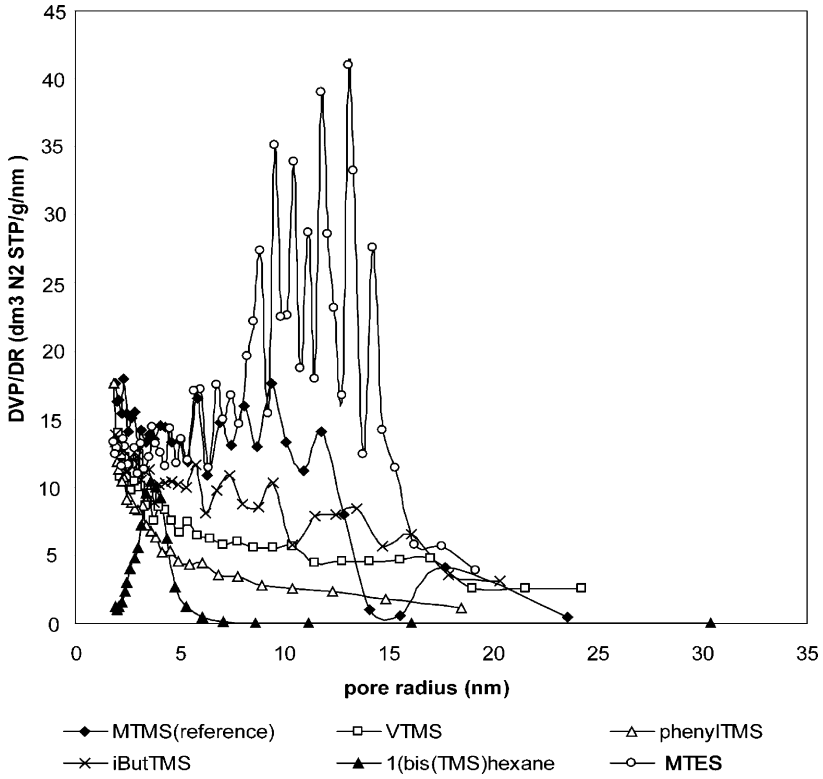

Fig. 11. Pore size distribution, by the Roberts method [23,24] of aerogels in which MTMS was replaced by another silicon precursor, derived from the nitrogen desorption branches (purified lipase from Amano).

and (3) dissolution re-condensation reactions which are responsible of an evolution more specifically termed aging. The latter evolution is linked to the rates of dissolution and condensation which rapidly increase as the pH increases from 2 to 9. All over, the condensation rate remains higher than the dissolution rate, so that the micropores disappear in favor of the mesopores, leading to the formation of a coarser texture made of denser colloidal silica particles, of increasing size. The pore surface is smoothed out and the network rigidified, but the sample volume is little affected. This is a different evolution from drying by evaporation which compresses the gel network.

Regarding the enzyme, it is known that the first factor which can have a drastic influence is the nature of the solvent medium, including the last pH to which the enzyme was submitted. This has to do with interactions of the solvent with the hydrophilic versus hydrophobic surface sites of the enzyme, which in turn can change its conformation. In the present study, this explains that the various solvents or pH, or even types of silicon precursor in which the aerogel-encapsulated enzyme were aged, could have very marked effect on the enzyme activity, unrelated to the gel network structure.

This does not mean that the gel network has no effect on the enzyme, but this is an action of a different nature. Indeed, a first major effect can be mechanical. For instance, shrinking the aerogel network either by partial evaporation, or by insufficient acetone dialysis before CO_2 supercritical drying, certainly compresses the enzyme in the same way as it compresses the gel network. It should also drastically reduce the diffusion rate of the substrates towards the enzyme. Overall, the apparent activity could be expected to be drastically reduced by shrinkage of the gel, in agreement with the present findings. In the present study, we also noticed that the added PVA helped the gel network to resist slightly better to shrinkage (although we could not provide a reliable number), which in itself is sufficient to improve the enzyme compression history during drying. This is particularly true in xerogels of the type made by Reetz et al. from 100% MTMS. Such gels are fully hydrophobic. They float on water and can be dried by evaporation without any shrinkage, because the solid–liquid contact angle is such that the capillary compression on the gel network is strongly reduced.

A second major mechanical effect concerns the dispersion of the enzyme. Lipases are not generally soluble in the organic solvents used in fine chemistry, in which substrates are soluble. Rather, a lyophilized lipase is partially agglomerated in organic solvents. On the other hand this enzyme is soluble in water, provided the pH is not too close from its electrical zero point charge (z.p.c.). This means the enzyme could be dispersed as individual solute molecule in the hydrolysis water used to make the gel. Because the gel network is built around the enzyme, this dispersion state was maintained in the gel, even after drying, and even after introducing the gel in an organic liquid used to perform the reaction, where the enzyme would otherwise be insoluble.

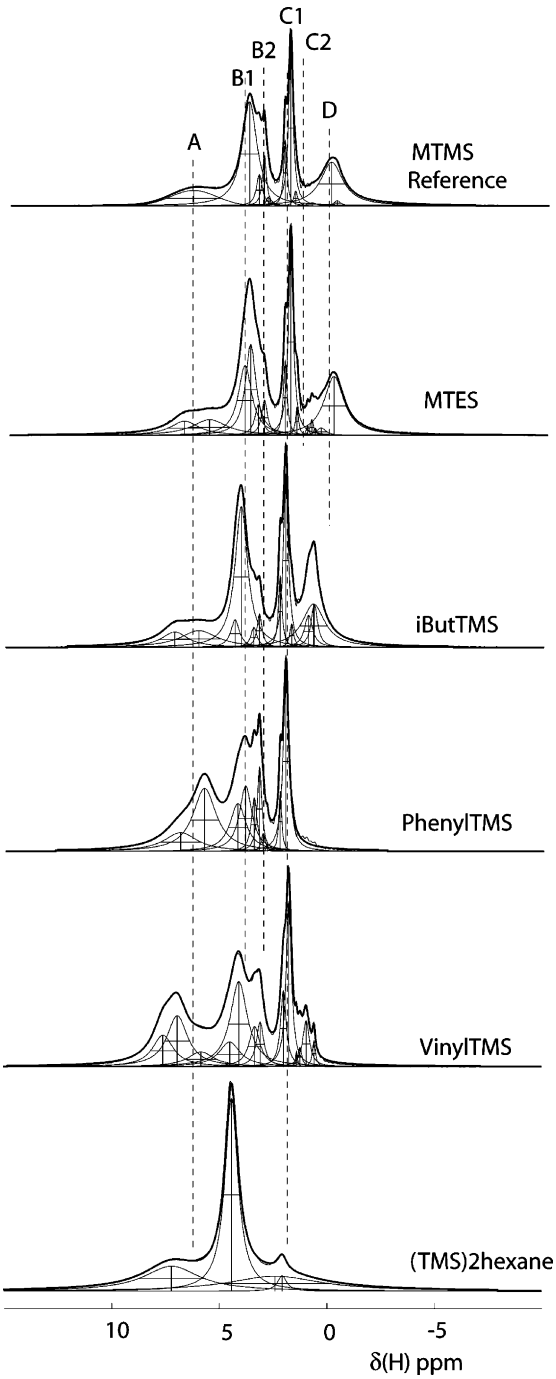

Fig. 12. ^{1}H MAS NMR spectra of aerogels in which MTMS was replaced by another silica precursor (purified lipase from Amano).

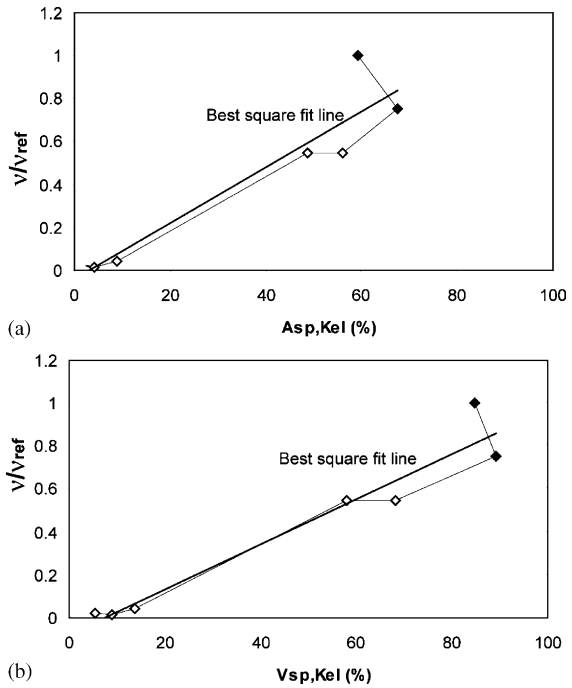

Fig. 13. Correlation between the relative activity of the aerogels, defined as the ratio of the activity after partial evaporation before supercritical drying v to the activity without any evaporation v_{ref} and the mesoporous texture: (a) contribution $A_{sp,Kel}$ of pores with a radius larger than the Kelvin limit to the specific surface area; (b) contribution $V_{sp,Kel}$ of pores with a radius larger than the Kelvin limit to the specific pore volume (lipase solution from Fluka).

In the cases where the gels were made in conditions not harmful to the enzyme itself, another indirect action of the gel network could be to modify the equilibrium concentration of the substrates inside the gel pores, that is to say immediately close to the enzyme, by comparison with the solvent which surrounds gel monolithic pieces. For instance the retention of various polar or apolar compounds, by adsorption on the surface sites of the gel pores, depends on the nature of these sites, either hydrophilic such as Si–OH, or hydrophobic such as $SiCH_3$. However, this phenomenon was found not to be a major one regarding the catalytic efficiency, as shown when replacing the 20% MTMS by other precursors. That is to say it only affected those molecules directly adsorbed on the gel, not the liquid composition inside the pores further from the network walls.

The final aerogel shrinkage after drying was difficult to assess from the final linear dimensions of monoliths, as it ranged from 1 to 3 mm in diameter on cylindrical samples with an initial diameter of 14 mm and height ≈6 mm. Moreover, in the case of aerogels made with an increasing water proportion, the wet gel volume was higher when the hydrolysis water proportion was higher. Hence the corresponding aerogel shrunk more in proportion when the water ratio increased, by comparison with the related wet state. Nevertheless, for a same initial mole number of Si, the final dry aerogel volume was still slightly higher after supercritical drying, although a reliable number would be difficult to provide. Also, no macroscopic shrinkage or syneresis was observed by aging in water or organic solvents, which means that deactivation of the enzyme (or activation with isooctane) was only due to chemical action of the liquid medium on the lipase. Actually, although it is known that the final specific pore volume determined by nitrogen adsorption does not reflect the largest pores (above 50 nm) of aerogels, as this was mentioned before, it can be argued that these large pores are

Table 5
Relationship between relative activity and pore volume with selected synthesis parameters

Synthesis parameter	ν/ν_{ref}	V_{sp} (cm^3/g)	Approximate shrinkage dL/L (mm/mm)
Hydrolysis water molar ratio			
$r_w = 3.4$	0	0.94	[a]
$r_w = 6.8$ (reference)	1	1.45	[a]
$r_w = 10.2$	1.36	1.48	[a]
$r_w = 13.6$	1.29	1.73	[a]
Dialysis time in acetone			
1 h	0	0.8	3/14
5 h	1	1.51	2/14
Aging time in mother liquor			
12 h	0.64	1.58	2/14
156 h	1. 27	1.67	2/14
Xerogel $r_w = 6.8$	<0.05	0.35	9/14

[a] See comment in text.

likely to loose their enzyme molecules during supercritical drying. So that the nitrogen determined specific pore volume somewhat reflects the volume of the mesoporous cages which actually encapsulate the enzyme. And indeed, the data gathered in Table 5 show that, although no unique general correlation between the final aerogel relative activity and this specific pore volume exists, some consistent trends could definitely be observed for a number of parameters. Overall, it again appears that mechanical shrinkage and enzyme deactivation for instance by hydrophilic solvents, are two independent actions which must both be controlled to enhance the enzyme activity. Consistently, the drying of xerogels made from only 20% MTMS shrinkage is important, which explains that the catalytic activity of these materials is very low.

A last aspect of the enzyme–gel interactions concerns the influence of the enzyme on the gel structure and texture. The enzyme loading used in the present study was rather small, but a possible slight improvement of the silica condensation by the enzymes was already mentioned [21]. In another related work the influence of the protein content on the gelation was specifically addressed, the protein solution being prepared from the same lyophilized lipase as here. A clear gelation catalysis effect was reported [22], although it could not be decided whether this was a true enzymatic process or a simple proteinic templating effect. Further work on the subject is actually underway to clarify this point.

4. Conclusions

Silica aerogels have been found to be very adequate encapsulation media for some lipases, possibly improving their activity compared to that of the free enzyme. Besides the facts that enzymes are maintained individually dispersed in gels, and are protected from capillary contractions during drying when dried altogether with the aerogel in supercritical CO_2, the NMR structural data in the present study do not show any obvious correlation between the nature of surface groups on the gel pore surface, and the catalytic activity. This suggest in turn that any aerogel in which drying contraction could be avoided, whatever the nature of this gel, but provided it could be made with solvent or gel precursors not harmful to the enzyme, would be a good encapsulation media for the enzyme.

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