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Liquid- and solid-state high-resolution NMR methods for the investigation of aging processes of silicone breast implants

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Abstract

To investigate aging processes of silicone gel breast implants, which may include migration of free unreacted material from the gel and rubber to local (e.g. connective tissue capsule) or distant sites in the body, chemical alteration of the polymer and infiltration of body compounds, various approaches of multinuclear nuclear magnetic resonance (NMR) experiments (²⁹Si, ¹³C, ¹H) were evaluated. While ²⁹Si, ¹³C, and ¹H solid-state magic angle spinning (MAS) NMR techniques performed on virgin and explanted envelopes of silicone prostheses provided only limited information, high-resolution liquid-state NMR techniques of CDCl₃ extracts were highly sensitive analytical tools for the detection of aging related changes in the materials. Using 2D ¹H, ¹H correlation spectroscopy (COSY) and ²⁹Si, ¹H heteronuclear multiple bond coherence (HMBC) experiments with gradient selection, it was possible to detect lipids (mainly phospholipids) as well as silicone oligomer species in explanted envelopes and gels. Silicone oligomers were also found in connective tissue capsules, indicating that cyclic polysiloxanes can migrate from intact implants to adjacent and distant sites. Furthermore, lipids can permeate the implant and modify its chemical composition.

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Keywords: MR-spectroscopy; Silicone implants; Aging; Solid-state NMR; Connective tissue capsules

1. Introduction

Silicone (polydimethylsiloxane) gel-filled implants have been widely used for breast reconstruction and augmentation mammoplasty for more than 30 years. Although they are considered chemically and biologically inert and have been implanted into more than two million women, there are conflicting reports about possible health risks associated with these devices [1].

Silicone gel-filled implants consist of an outer thin envelope (200–300 μm) made of silicone elastomer (highly crosslinked silicone blended with amorphous silica (SiO₂) for reinforcement) that is filled with silicone gel. The gel is a copolymer made of dimethyl- and methylvinyl-siloxane, weakly crosslinked to form a three-dimensional polymer network. The crosslinking is achieved by the reaction of vinyl groups present in the copolymer chains [2].

Analysis of virgin silicone gel-filled implants revealed that the crosslinking reaction is not complete. Free, unreacted material can be removed from the gel by solvent extraction [3]. This free material is most likely responsible for the observed silicone leakage (“bleeding”) from intact implants [4]. It was claimed in 1967 that silicone is engulfed by macrophages and degraded chemically [5]. Silicone degradation was also suggested in 1993 on the basis of ²⁹Si nuclear magnetic resonance (NMR) spectroscopy. Degradation may involve substitution of the methyl and vinyl side groups with hydroxyl groups of the silicone polymer as intermediate steps. This would result in oxidation and breakdown of the polymer and might lead to hydrolyzed silicone, silica and highly coordinated complexes [6–9].

Another possible aging process of silicone implants is the infiltration of body compounds (primarily lipids) into envelopes and gels. The absorption of lipids causes swelling of the polymer network and changes in physical and chemical behavior. Especially, the tensile strength of the envelopes decreases over long time implantation [10–14]. This leads to the decline of the envelope and

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allows the diffusion of macroscopic quantities of gel and unreacted material into local and distant body sites. Silicone has been found in the liver of women with silicone breast prostheses [7].

Recent ^{29}Si NMR spectroscopic studies of envelopes, gels, lymph nodes and tissue capsules have shown that ^{29}Si spectroscopy is a potentially suitable technique for monitoring the presence of silicone species and changes in its chemical composition caused by decay during long time implantation [6,10]. In the present study, we used ^{29}Si and ^{13}C CP/MAS, ^1H and ^{13}C MAS solid-state spectroscopy on envelopes and various liquid NMR spectroscopic techniques (^1H , ^{13}C , ^{29}Si 1D and 2D experiments) on extracts of envelopes, gels and connective tissue capsules. Our goal was to evaluate these methods, which might yield additional information about infiltration of body compounds and monitoring of aging processes.

2. Materials and methods

2.1. Silicone gel-filled breast prostheses and connective tissue capsules

A virgin implant (Dow Corning, Arlington, USA) and two 3 year old implants (Dow Corning, Arlington, USA), both the latter from one patient, were studied. One implant was intact, the other had histologically proven severe “gel bleed”. All were so-called third generation implants, manufactured around the mid-1980s. Compared to their predecessors, implants of this generation were made with a thicker shell and with a textured surface to decrease the incidence of capsular contraction [1]. In addition, three connective tissue capsules were investigated. The corresponding implants were intact. Implantation times were 4 and 16 years, respectively.

2.2. Solid-state NMR experiments

The envelopes were separated from the gels with a scalpel. To remove remains of gel from the inside of envelopes, envelope samples were treated with chloroform (CHCl_3), then samples cut into small pieces and stacked into 7 mm ZrO_2 rotors (Bruker, Germany). Magic angle spinning (MAS) experiments on gels were not reliable due to difficulties in keeping a constant spinning rate over a longer period of time. The same was true for implant shells.

^1H MAS, ^{29}Si CP/MAS, ^{13}C CP/MAS (crosspolarization, CP) and ^{13}C MAS (high-power decoupled) NMR spectra were acquired on a Bruker DSX 400 NMR Spectrometer (Bruker, Karlsruhe, Germany) operating at ^1H frequency of 400.15 MHz. The resonance frequencies for ^{29}Si and ^{13}C were 79.49 and 100.63 MHz.

Experiments were carried out with a 7 mm CP/MAS probe. The 90° pulse lengths were $3.7\ \mu\text{s}$ (^1H MAS), $7.2\ \mu\text{s}$ (^{29}Si CP/MAS), $4\ \mu\text{s}$ (^{13}C CP/MAS) and $5\ \mu\text{s}$ (^{13}C MAS). Relaxation delays were 4, 15, 6 and 4 s, respectively. The number of scans was 100, 3000, 3000 and 1800, respectively. The contact time for all CP experiments was 10 ms.

Normally, ^{29}Si and ^{13}C have long relaxation times. Crosspolarization combined with MAS (CP/MAS) was used to reduce measuring time due to long relaxation times and for signal/noise enhancement. In this CP-technique, magnetization is transferred from protons to carbons or other dilute nuclei, thus taking advantage of the shorter relaxation times of protons [15].

All the MAS spectra were obtained at a constant temperature of 25°C with a spinning speed between 2000 and 5000 Hz to eliminate broadening from dipole–dipole coupling and chemical shift anisotropy. The free induction decays were multiplied with an exponential function (line broadening parameter 5 Hz) prior to Fourier transformation. The chemical shifts were referenced relative to external tetramethylsilane (TMS) for ^1H , ^{13}C and ^{29}Si .

2.3. Liquid NMR experiments

To isolate lipids and free unreacted silicone from the polymer network and tissue capsules, samples were extracted for 48 h with an excess of CHCl_3 . The solution was filtered and evaporated. After addition of deuterated chloroform (CDCl_3), the solution was filled into 5 mm NMR tubes.

As a reference sample for lipids, cholesterol (Aldrich, Milwaukee, USA), and a mixture of phospholipids (80% phosphatidylserine; brain extract; Aldrich, Milwaukee, USA) were used. In addition, cyclic and linear siloxane oligomers such as hexamethylcyclotrisiloxane (D_3), octamethylcyclotetrasiloxane (D_4), decamethylcyclopentasiloxane (D_5), hexamethyldisiloxane (HMDS) and dodecamethylpentasiloxane (L_5) were studied for reference. All of these materials were purchased from Aldrich, Milwaukee, USA and studied without further purification.

One-dimensional ^1H , ^{29}Si and ^{13}C spectra, as well as two-dimensional correlation experiments (^1H – ^1H COSY and ^{29}Si – ^1H heteronuclear correlation, HMBC) were applied on extracts of envelopes, gels and connective tissue capsules at 25°C .

All the spectra were acquired on a Bruker DRX 300 spectrometer. One-dimensional spectra were measured using a Bruker 5 mm QNP probe at 300.13 MHz (^1H), 75.44 (^{13}C) and 59.63 MHz (^{29}Si). Ninety degree pulse lengths were 10.5, 8.6, and $9.8\ \mu\text{s}$, respectively. Relaxation delays were 1, 2 and 30 s for ^1H , ^{13}C and ^{29}Si experiments. Between 16 and 2048 scans were acquired for the spectra. Prior to Fourier transformation,

exponential line broadenings of 0.3 (^1H)-1 Hz (^{13}C , ^{29}Si) were applied to the FIDs.

The ^1H - ^1H COSY experiments were carried out with a Bruker TBI probe with a shielded z-gradient. Transients were acquired into 2048 data points with 72 scans per increment and 512 increments in the F_1 -axis, which was zero filled to 1024 before FT. The spectra were symmetrized and no line broadening was applied. The relaxation delay between successive pulse cycles was 2 s. In addition, gradient pulses (1000 μs) were used for selection.

The ^{29}Si - ^1H HMBC experiments were also applied to the connective tissue capsule extract, using the Bruker 5 mm TBI probe. Peak type selection using gradient pulses with coherence selection was applied. A relaxa-

tion delay of 2 s was used between pulse cycles. Typically 1024 data points with 32 scans per increment and 128 experiments (F_1) were acquired. No exponential line broadening was applied.

3. Results and discussion

3.1. Solid-state NMR experiments

^{29}Si CP/MAS spectra (measured with a contact time of 10 ms) of the virgin and two explanted envelopes (Fig. 2a–c) revealed only peaks of polydimethylsiloxane (D unit) at -22.4 ppm, the M^{Q} unit at 12.4 ppm and the Q unit (-101 to -110 ppm) (cf. Fig. 1 for the standard notation used here).

The presence of the M^{Q} unit indicates that the amorphous silica surface used for the preparation of the envelopes had been silylated with HMDS. This treatment serves to increase hydrophobicity and prevents further thickening of the material [15]. M^{Q} units were also detected with ^{29}Si MAS by Picard et al. [11].

The reinforcement of silicone elastomers by blending silica fillers prior to the crosslinking reaction has been the common process for producing tough silicone envelopes. The ^{29}Si CP/MAS spectra exhibited a broad peak extending from -88 to -115 ppm, which can be attributed to the Q^2 , Q^3 and Q^4 units arising from the amorphous silica component. Polydimethylsiloxane (D; 1.6 ppm) was also detected with ^{13}C CP/MAS in the virgin and explanted envelopes (Fig. 3a–c). In contrast to ^{13}C CP/MAS spectra it was possible to detect diphenylsiloxane (at 127.8, 130.1, 134.6, 136.2 ppm) in all three samples with ^{13}C MAS, these are most likely

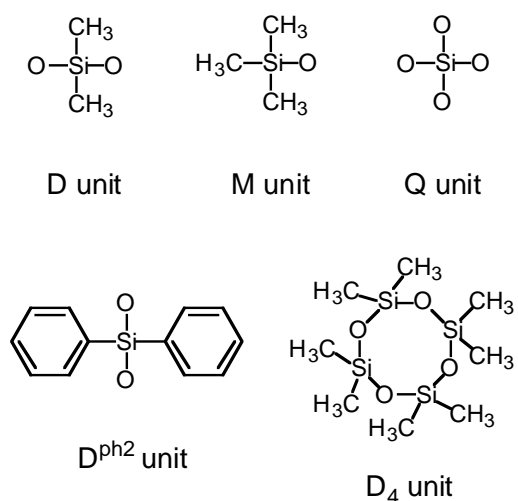


Fig. 1. Chemical structures of different linear and cyclic siloxanes detectable by NMR spectroscopy.

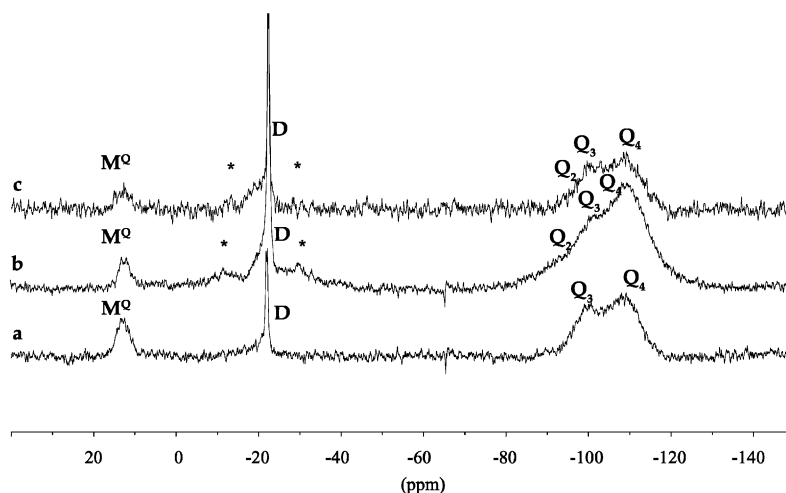


Fig. 2. ^{29}Si solid-state CP/MAS NMR spectra of a virgin implant envelope (spectrum a) and a 3 year old envelope of an implant without (spectrum b) and with gel bleed (spectrum c). 3000 FIDs were recorded with a 7 mm CP/MAS probe, contact time was 10 ms. *—spinning side bands, D—polymethylsiloxane, Q_2 - Q_4 —amorphous silica compounds, M^{Q} —HMDS.

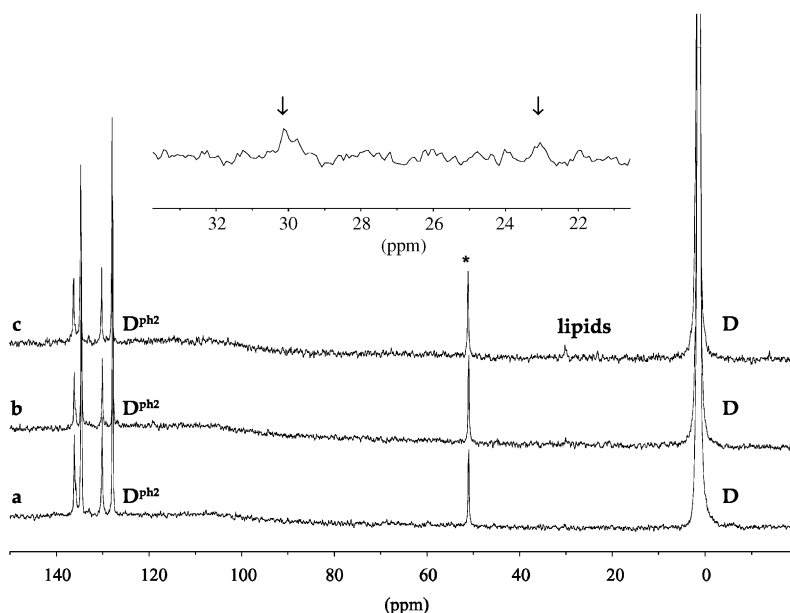


Fig. 3. ^{13}C MAS solid-state NMR spectra (decoupled) of a virgin implant envelope (spectrum a) and a 3 year old envelope of an implant without (spectrum b) and with gel bleed (spectrum c). 1800 FIDs were recorded with a 7 mm CP/MAS probe with a 5000 Hz spinning speed. The upper part of Fig. 3 shows the expanded spectra from 21.0 to 33.0 ppm containing lipid resonances. *—spinning side bands, D—polymethylsiloxane, $\text{D}^{\text{ph}2}$ —diphenylsiloxane.

not degradation products (Fig. 3a–c). In this case, ^{13}C -MAS spectroscopy is more effective than ^{13}C CP/MAS. Due to the fast rotation of the methyl protons, the polarization transfer is not sufficient and therefore no signal increase occurred. Diphenylsiloxane was also found in explanted shells by Picard et al. [11]. The ^1H MAS spectra also revealed signals of $\text{D}^{\text{ph}2}$ at 7.0 and 7.4 ppm. Moreover, signals of lower intensity in the area between 5.6 and 6.0 ppm, arising from protons of vinyl groups, were found in all samples. This finding is interesting, since reactive vinyl groups are not expected to be present in silicone envelopes due to the cross-linking process. Usually, if the crosslinking reactions were complete, all the polymer chains should be chemically attached to the network. In the latter case, silicone migration would be minimized.

When assessing advantages and disadvantages of using different MR-techniques one should apply them to the same samples for a better comparison. Thus our solid-state results are presented here, even though no new information compared to Picard et al. [11] was revealed. In summary, we were not able to detect and characterize degradation products with solid-state NMR. As a consequence, we decided to extract envelopes and gels with CHCl_3 to perform liquid NMR spectroscopy.

3.2. Liquid-state NMR experiments

3.2.1. Extracts of envelopes in CDCl_3

The ^1H spectrum of the virgin sample (Fig. 4a) exhibits the expected signals at 7.2–7.6 ppm (diphenyl-

siloxane) and 5.7–6.0 ppm (vinyl groups). The chemical shifts at 1.2 and 0.8 ppm may be assigned to small oligomers, the signal at 0 ppm to the D unit. The strong peak is flanked by satellites due to ^{13}C - ^1H and ^{29}Si - ^1H spin-spin coupling. Additional resonances can be found in spectra b and c, due to infiltration of phospholipids (Fig. 5), mainly phosphatidylserine (0.6, 1.0, 1.8, 3.4–3.7, 4.1–4.3 and 5.3 ppm). Peak assignments are summarized in Table 1.

Compared to the main resonance of the D unit, the intensities of the lipid resonances are very low, between 0.01% and 0.22%. The infiltration of phospholipids into the envelope was also demonstrated by Adams et al. [12]. By using thin layer chromatography they detected phospholipids, mainly phosphatidylserine and phosphatidylethanolamine, and triglycerides in the envelopes of explanted silicone gel breast implants.

Fig. 6a shows the ^1H - ^1H COSY spectrum of the virgin implant (0–8 ppm). The protons of the $\text{D}^{\text{ph}2}$ unit, the vinyl groups and the D unit correlate only among themselves. In addition, the signals at 0.8 and 1.2 ppm correlate.

The ^1H - ^1H COSY spectrum of the explanted shell (3 years; Fig. 6b) exhibits additional correlations that are summarized in Table 2. The same additional correlations can be found in the ^1H - ^1H COSY spectrum of the 3 year old explanted shell with gel bleed (not shown).

Comparison of Fig. 6b with the ^1H - ^1H COSY spectrum of the reference sample (a mixture of phospholipids, 80% phosphatidylethanolserine) (Fig. 6c) suggests that the additional correlations are due to protons of phospholipids. The multiplet around

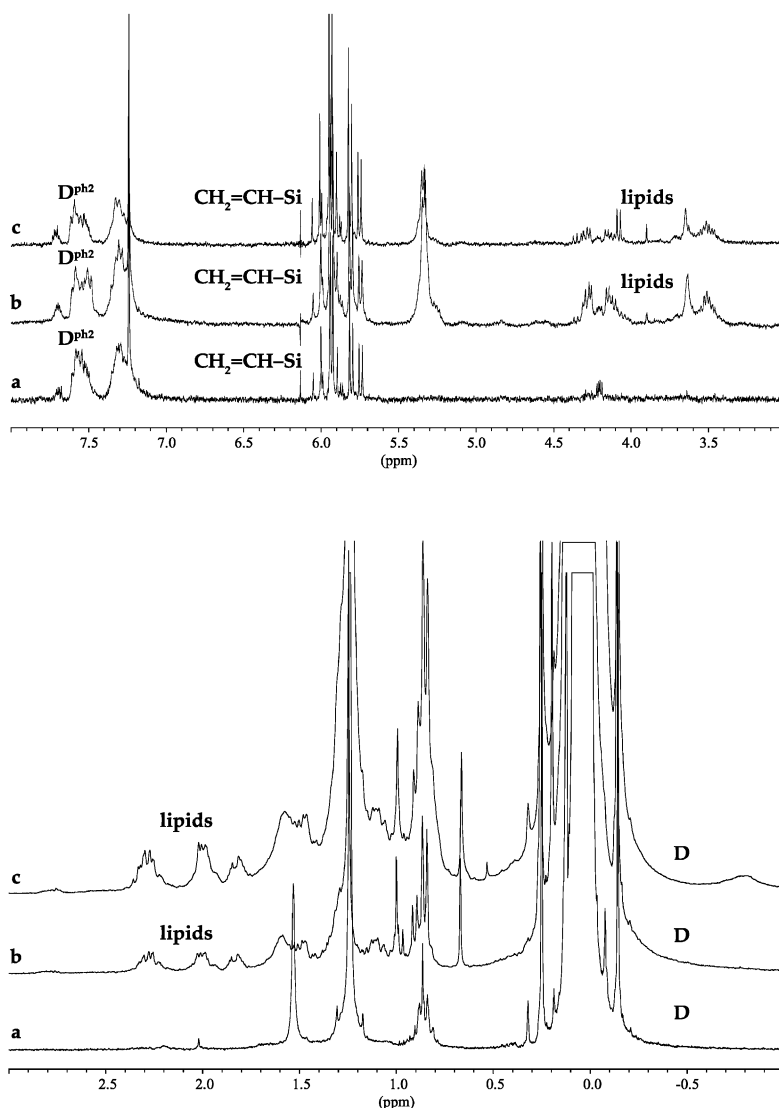


Fig. 4. ^1H liquid NMR spectra of a virgin implant (spectrum a) and 3 year old implant without and with gel bleed (spectra b and c). 16 and 80 (spectrum c) FIDs were recorded with the 5 mm TBI and QNP (3 year old implant) probe. The lower part of Fig. 2 shows the expanded spectra from -1.0 to 3.0 ppm. Peaks at 2.8 ppm are also present in explanted samples, but with very low intensity.

4.1 ppm is due to the glycerol backbone. The resonances in the area from 0.6 to 2.8 ppm are typical of the CH_2 and CH_3 groups of the fatty acids. Protons close to the double bonds have chemical shifts at 2.0 and 2.8 ppm ($\text{CH}_2\text{-C}=\text{C}$ and $\text{C}=\text{C-CH}_2\text{-C}=\text{C}$) [16]. The correlation with the olefin protons near 5.3 ppm confirms this assignment. The signal of the CH_2 group adjacent to the carboxyl unit of the fatty acid is observed at 2.3 ppm [16]. The multiplet near 3.4 ppm is assigned to the serine residue. The chemical shifts at 1.2 and 0.8 ppm can be assigned to oligomer species. The 3 year old implant with gel bleed contains the highest amount of these units (4.6%), which may be a sign of degradation of the polymer network resulting in the formation of small polymer chains not connected with the network (Table 1).

The ^{29}Si spectrum of the virgin implant (Fig. 7a) revealed chemical shifts at 7.4 and -21.7 ppm. The chemical shifts at 7.4 ppm are due to HMDS, an end blocker added to the polymer matrix during manufacturing [11]. The base of the main resonance at -21.7 ppm is broadened due to D groups with different chemical environments resulting in different chemical shifts.

In the spectrum of the 3 year old explanted envelope (Fig. 7b) signals of cyclic polysiloxanes with low intensity are visible: at -10.3 ppm due to hexamethylcyclotrisiloxane (D_3 , 0.34%), octamethylcyclotetrasiloxane (D_4 , 0.18%) at -19.0 ppm and decamethylcyclopentasiloxane (D_5 , 1.0%) at -20.5 ppm. In addition, the spectrum reveals the signals of silicon bonded to vinyl groups at -35.6 ppm (D^{V} , 0.33%),

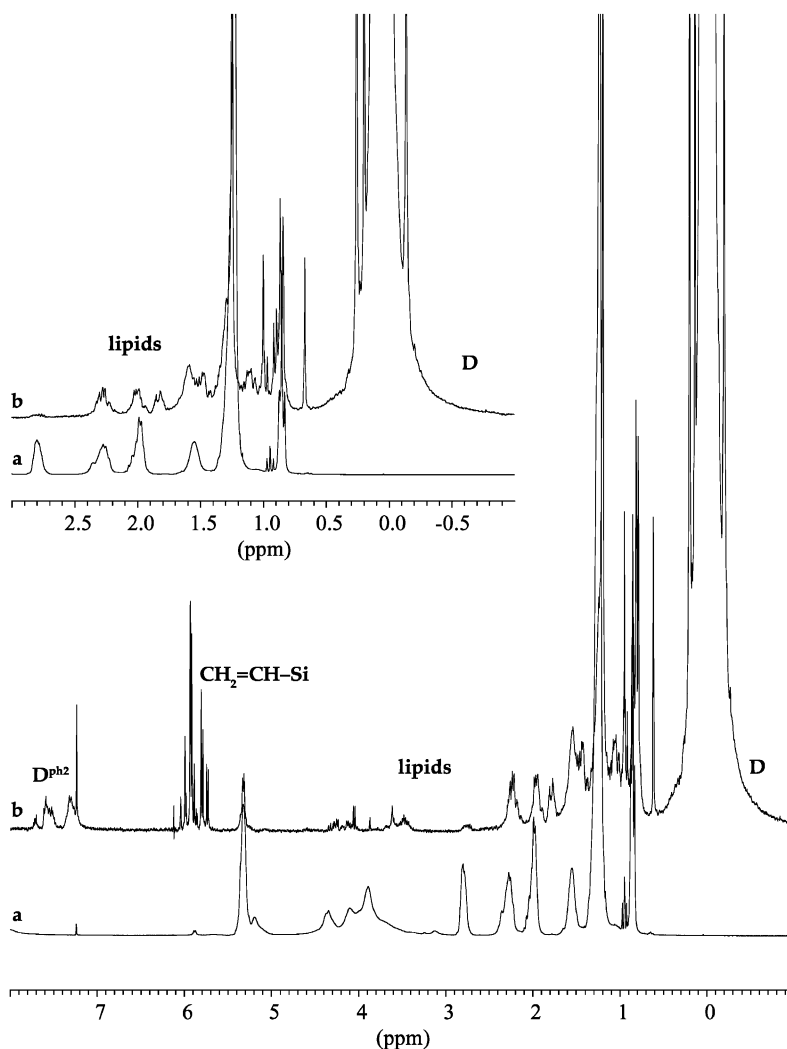


Fig. 5. ^1H solution NMR spectra of a phospholipid mixture (80% phosphatidylserine) (spectrum a) and a 3 year old sample without gel bleed (spectrum b). For experimental conditions see legend Fig. 2. The vertical inset shows the expanded area from 3 to -1 ppm.

Table 1

Summary of the observed ^1H chemical shifts (δ_{iso}) in the silicone shells. The areas of all signals are set to 100% for the determination of the single area

	Virgin envelope	3 year old envelope	3 year old envelope with gel bleed
δ_{iso} ($\text{C}_6\text{H}_5\text{-Si}$) (ppm)	7.2–7.7	7.2–7.7	7.2–7.7
(signal intensity %)	(0.27)	(0.14)	(0.22)
δ_{iso} ($\text{CH}_2=\text{CH-Si}$) (ppm)	5.7–6.1	5.7–6.0	5.7–6.0
(signal intensity %)	(0.16)	(0.15)	(0.15)
δ_{iso} ($\text{Si-CH}_2\text{-C}$) (ppm)	1.2	1.2	1.2
(signal intensity %)	(2.12)	(0.82)	(3.49)
δ_{iso} (Si-CH_3) (ppm)	0.8	0.8	0.8
(signal intensity %)	(0.52)	(0.45)	(1.12)
δ_{iso} (D) (ppm)	0.0	0.0	0.0
(signal intensity %)	(96.86)	(97.65)	(94.01)
δ_{iso} (additional resonances) (ppm)	—	0.6, 1.0, 1.8, 2.8, 3.4–3.6, 4.3–4.8, 5.3	0.6, 1.0, 1.8, 3.4–3.6, 4.0–4.4, 5.3
(signal intensity %)	—	(0.09, 0.13, 0.08, 0.00, 0.03, 0.01, 0.04)	(0.16, 0.09, 0.12, 0.01, 0.06, 0.06, 0.09)

HMDS (7.4 ppm, 1.00%) and PDMS (-21.7 ppm). The assignments and signal areas are summarized in Table 3.

The ^{13}C spectrum of the virgin sample (Fig. 8a) reveals chemical shifts at 29.7, 14.8 and 1.0 ppm. The

chemical shift at 1.0 ppm is due to the carbons of the methyl groups of the D unit. The peaks at 29.7 and 14.8 ppm can be assigned to CH_2 and CH_3 groups of siloxane oligomers. These peaks also support the

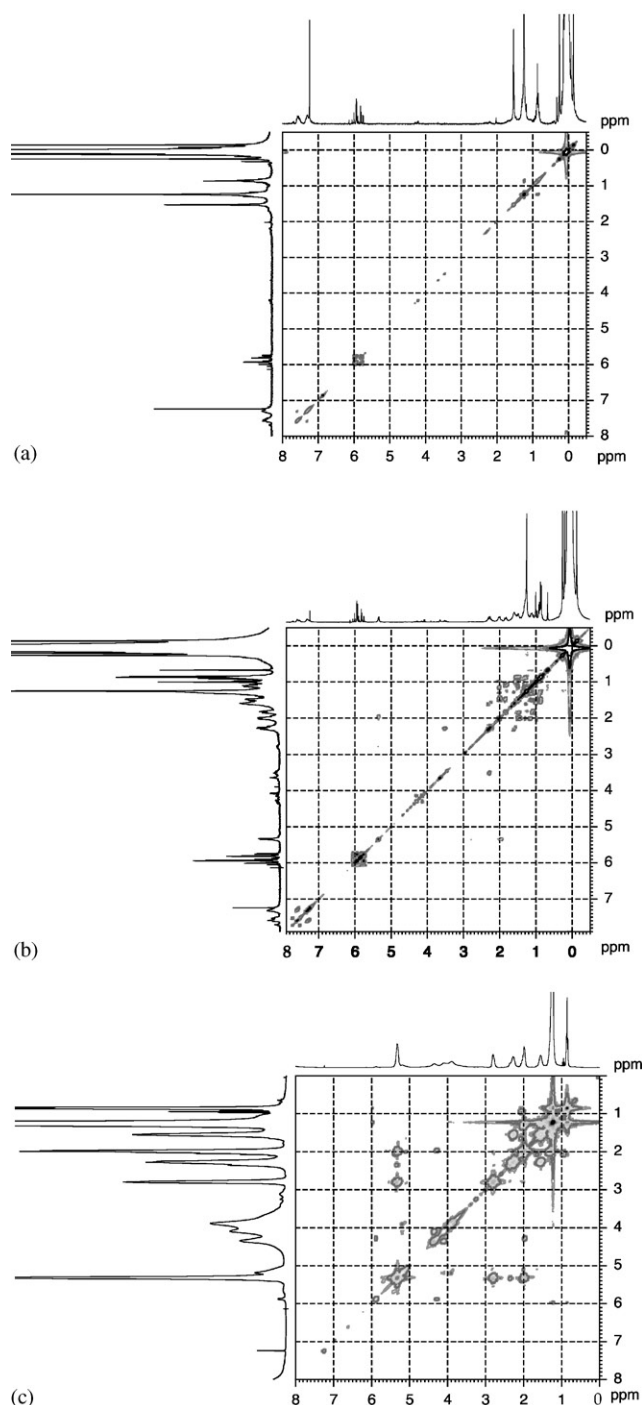


Fig. 6. (a) ^1H , ^1H COSY spectrum of the virgin sample (0–8 ppm), (b) 3 year old implant without gel bleed (0–8 ppm) and (c) phospholipid mixture as a reference sample (0–8 ppm). The ^1H , ^1H COSY experiments were carried out with a Bruker TBI probe. Transients were acquired into 2048 data points with 72 scans per increment and 512 increments in the F_1 -axis, which was zero filled to 1024 prior to FT. The spectra were symmetrized and no line broadening was applied. The relaxation delay between successive pulse cycles was 2 s. In addition, gradient pulses (1000 μs) were used for selection.

assignments of the signals at 0.8 and 1.2 ppm due to free silicone found in the ^1H solution NMR spectrum (Fig. 4). The same signals can be found in the spectra

Table 2

Correlations of the ^1H , ^1H COSY experiment of the 3 year old implant

δ_{iso} (^1H) (ppm)	δ_{iso} (^1H) (ppm)
0.9	1.2
2.0	1.1, 1.3, 1.4
2.3	1.6
4.1	4.3
4.3	4.1
5.3	2.0
5.7–6.0 ($\text{CH}_2=\text{CH-Si}$)	5.7–6.0

of the explanted envelope (Fig. 8b). In addition, the spectrum of the 3 year old implant (Fig. 8b) exhibits additional resonances due to phospholipids, as confirmed by the comparison with the ^{13}C NMR spectrum of the phospholipid mixture.

Diphenylsiloxane and vinyl groups can only be detected in the spectra of the explanted device: 120.7, 126.5, 131.7, 133.3, 136.1 and 140.0 ppm (Fig. 8b). The presence of lipids and diphenylsiloxane in silicone shells was also monitored with ^{13}C solid-state NMR spectroscopy by Picard et al. [11].

Note that the liquid-state NMR reveals a number of additional details concerning the presence of oligomers and lipids that are not visible in the solid-state NMR spectra. This discrepancy may most likely be attributed to the CHCl_3 washing step prior to the solid-state NMR experiments.

3.3. Liquid-state NMR spectroscopy on extracts of connective tissue capsules

Thirty to forty percent of all patients have connective tissue capsular contracture around their implants. The tissue around the capsule is often solidified. After some time, the inside of the capsule may be calcified [17,18]. Moreover, the implant may also be compressed and ruptured [19].

The capsular contracture is described as a defense of the body against silicone. It is supposed that the phagocytosis of silicone results in fibroblast proliferation with subsequent capsular contracture [20,21].

We used ^{29}Si , ^1H HMBC inverse spectroscopy, in contrast to simple ^{29}Si NMR, to gain sensitivity. Here, with the help of inverse spectroscopy, the resonances of the sensitive nucleus (^1H) are detected, resulting in reduced measuring time and an increase in sensitivity. The sensitivity of these kind of experiments is eight times higher compared to ^{29}Si NMR spectroscopy.

Five silicon peaks can be found in the ^{29}Si , ^1H HMBC spectrum of an extract of the tissue capsule in C_6D_6 corresponding to a 4 year old implant (Fig. 9): 7.5, -10.0 , 0.0 , -22.0 and -24.0 ppm (Table 4). The correlated proton signals are at 0.32, 0.43, 0.10 and

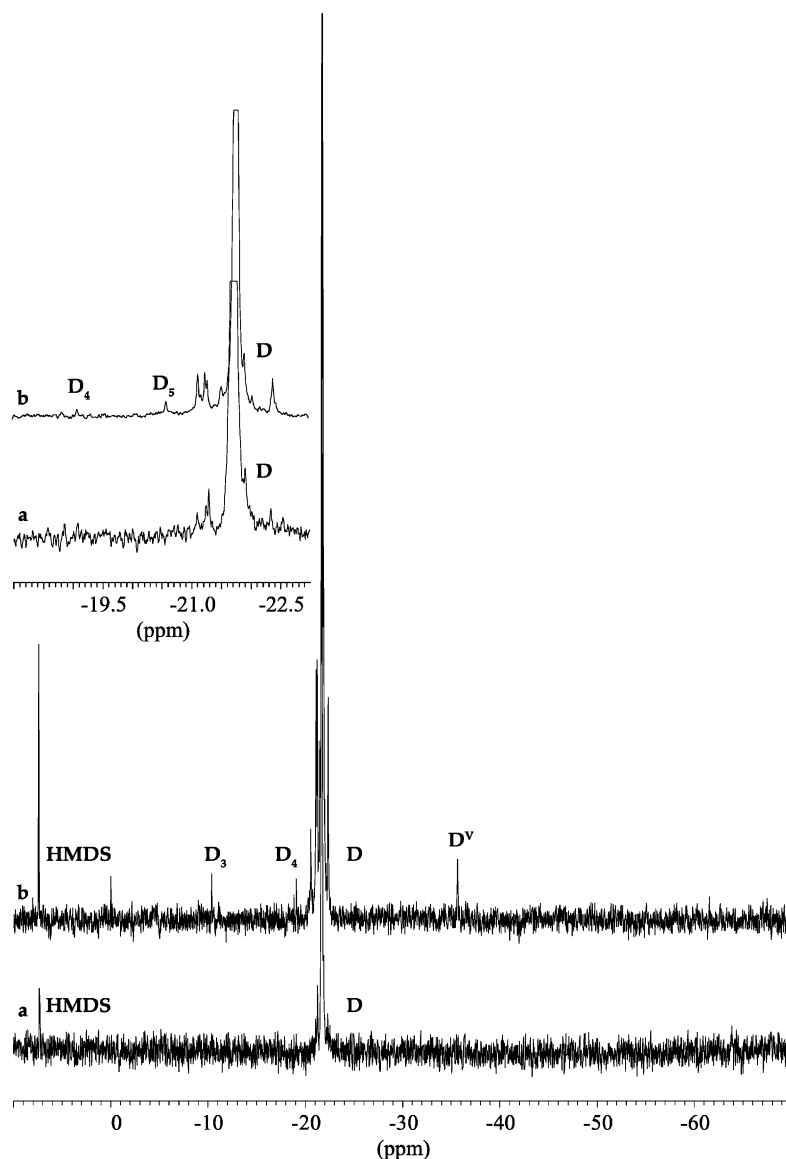


Fig. 7. ^{29}Si liquid NMR spectra of a virgin sample (spectrum a) and a 3 year old sample (spectrum b) without gel bleed (10 to -70 ppm). 1024 scans and a relaxation delay of 30 s were applied. The vertical inset shows the area from -18 to -23 ppm.

Table 3

Summary of the ^{29}Si chemical shifts (δ_{iso}) of silicone envelopes and gels. The areas of all signals are set to 100% for the determination of the single area

	Virgin envelope	3 year old envelope	Virgin gel	3 year old gel
δ_{iso} (HMDS) (ppm)	7.4	7.4	7.4	7.4
(signal intensity %)	(1.00)	(1.00)	(0.49)	(0.40)
δ_{iso} (D_3) (ppm)	—	-10.3	—	-10.3
(signal intensity %)	—	(0.34)	—	(0.04)
δ_{iso} (D_4) (ppm)	—	-19.0	—	-19.0
(signal intensity %)	—	(0.18)	—	(0.11)
δ_{iso} (D_5) (ppm)	—	-20.5	—	-20.5
(signal intensity %)	—	(1.00)	—	(0.28)
δ_{iso} (D) (ppm)	-21.7	-21.7	-21.7	-21.7
(signal intensity %)	(99.00)	(97.00)	(99.45)	(98.96)
δ_{iso} (D^{V}) (ppm)	—	-35.6	-35.6	-35.6
(signal intensity %)	—	(0.33)	(0.06)	(0.11)
δ_{iso} (T) (ppm)	—	—	—	-67.0
(signal intensity %)	—	—	—	(0.10)

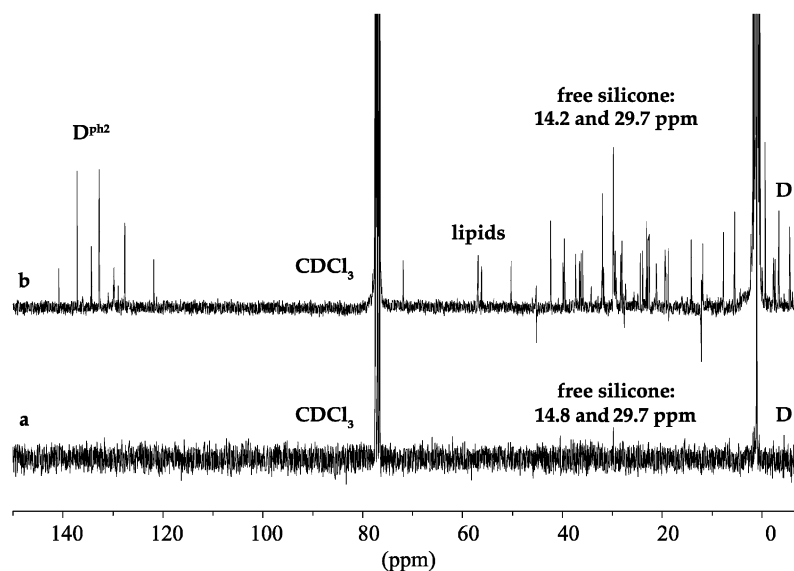


Fig. 8. ^{13}C NMR liquid-state NMR spectra of a virgin sample (spectrum a) and a 3 year old sample (spectrum b) without gel bleed. A relaxation delay of 2 s was used. 272 (spectrum a) and 1024 (spectrum b) scans were accumulated.

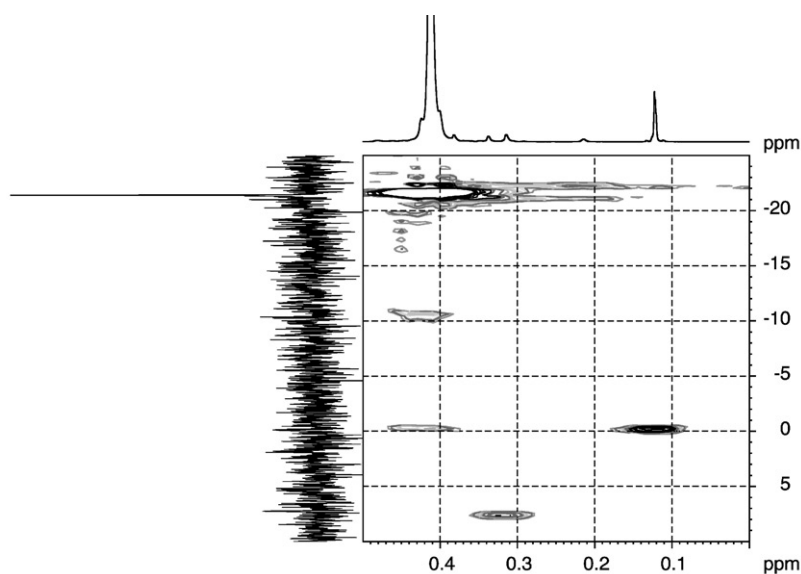


Fig. 9. ^{29}Si , ^1H HMBC spectrum of a 4 year old tissue capsule, measured with a Bruker 5 mm TBI probe. Peak type selection using gradient pulses with coherence selection was used. A relaxation delay of 2 s was used between pulses. Typically, 1024 data points with 32 scans per increment and 128 experiments (F_1), zero filled to 512 prior to FT, were acquired. No exponential line broadening was used.

0.43, 0.41 and 0.21 ppm, respectively. The peaks may be due to HMDS, M^{Q} , probably hydrolyzed silicone, PDMS and D_5 . Other components of the capsule are mainly phospholipids and collagen [12]. Only polydimethylsiloxane and HMDS were detected in the extracts of the other two capsules.

In contrast to the recent solid-state NMR work of Garrido et al. [10] it was possible to detect various silicone species in connective tissue capsules, probably

Table 4

Results of the ^{29}Si , ^1H HMBC spectrum of an extract of a connective tissue capsule in CDCl_3

$\delta_{\text{iso}} (^1\text{H})$ (ppm)	$\delta_{\text{iso}} (^{29}\text{Si})$ (ppm)
0.41	-22.0
0.43, 0.10	0.0
0.43	10.0
0.32	7.5
0.21	-24.0

due to the use of ^{29}Si , ^1H HMBC inverse spectroscopy. In summary, when using advanced NMR techniques, informations about the chemical composition of material bleeding from implants can be obtained. Other techniques such as atomic absorption only allows the determination of silicon levels [18].

3.4. Liquid-state NMR spectroscopy on extracts of gels

We also evaluated whether lipids can migrate through the intact shell into the gel, where they might initiate the aging of the gel.

Liquid-state NMR spectra of explanted gels are quite similar to the spectra of the shells. With ^1H (Fig. 10) and ^{13}C experiments it was possible to detect infiltrated phospholipids. ^{29}Si NMR spectroscopy was useful to reveal the presence of cyclic polysiloxanes (Fig. 11). The amount of free silicone, lipids and cyclic polysiloxanes in the gels was much lower than in the shells (Table 3). In addition, the ^{29}Si NMR spectrum of the explanted gel

revealed the peak of the T group (Table 3) perhaps indicating a more advanced aging process.

^1H NMR experiments of explanted envelopes and gels of model implants revealed also a higher lipid concentration in the shells [9]. The extent of lipid infiltration is associated with the amount of silica in the envelope [10]. A high silica concentration seems to restrict lipid infiltration, as silica hinders polymer chain mobility [9].

In a summary, the suitability of liquid- and solid-state NMR experiments for monitoring degradation processes in silicone breast implant materials has been investigated. The absorption of lipids by silicone envelopes has been suggested to trigger degradation of the elastomers [21]. Increased hydrophobicity makes it easier for lipids to infiltrate the shell [22]. The absorption of the network with lipids induces the so-called plasticizer effect: the silicone envelop swells and the polymer chains become more mobile. As a consequence, the network is more wide meshed than before, so that it is easier for silicone oligomers to migrate into the body.

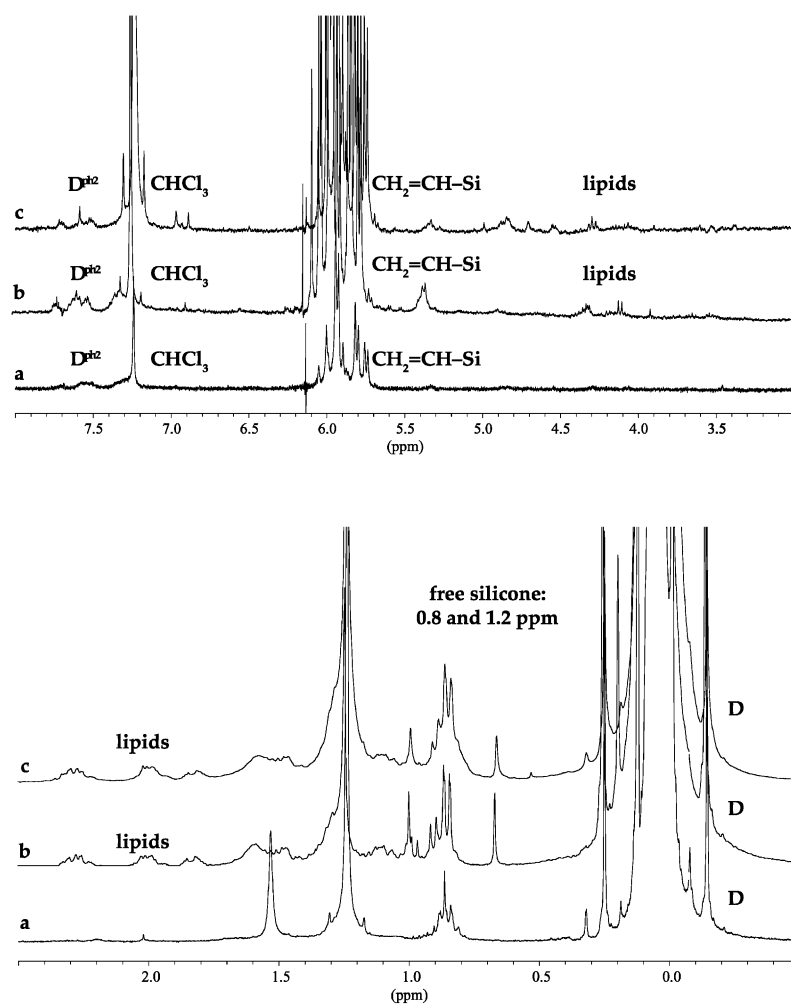


Fig. 10. ^1H liquid-state NMR spectra of the silicone gels of a virgin implant (spectrum a), a 3 year old implant without and a 3 year old implant with gel bleed (spectra b and c). 128 (spectrum a) and 256 (spectra b and c) FIDs were recorded with the 5 mm TBI and QNP (3 year old implant) probe. The lower part of Fig. 9 shows the corresponding expanded area from -1.0 to 3.0 ppm.

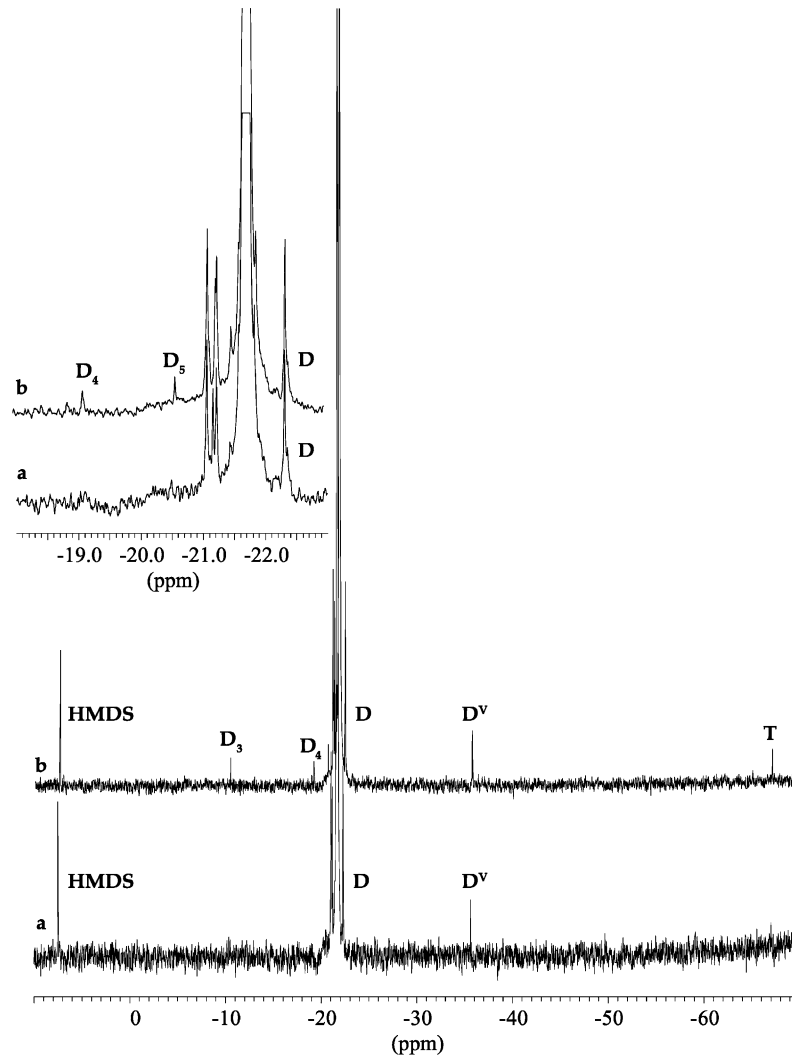


Fig. 11. ^{29}Si liquid-state NMR spectra of the gels of a virgin sample (spectrum a) and a 3 year old sample (spectrum b) without gel bleed (10 to -70 ppm). 1024 scans and a relaxation delay of 30 s were applied. The vertical inset shows the area from -18 to -22.5 ppm.

The loss of elasticity of the silicone shells with increasing implantation time can be demonstrated by stress measurements of silicone envelopes.

The high concentration of cyclic polysiloxanes found in our materials is consistent with previous studies. Flassbeck et al. [23] showed by GC-MS that these compounds can be formed during implantation. Lykissa et al. [24] found that leakage of silicones from implants was greatest when the surrounding medium was lipid rich. Especially, low molecular weight silicones were observed to diffuse into a lipid rich medium like connective tissue capsules [24].

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