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Methods for Detecting Silicones in Biological Matrixes

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Methods for analyzing for silicon and silicone in biological matrixes were developed. A silicone-specific technique involved microwave digestion of samples in acid solution to rapidly break down the biological matrix while hydrolyzing silicones to monomeric species. The resulting monomeric silanol species were then capped with trimethylsilyl groups, extracted into hexamethyldisiloxane, and analyzed by gas chromatography. In serum, positive identification of silicone species with detection limits below 0.5 μg of Si/mL are possible with this technique. The technique is compared with a silicone-specific technique, ^{29}Si NMR, and a non-silicone-specific technique, ICP-AES. ^{29}Si NMR was far less sensitive, with a detection limit of only 64 μg of Si/mL in serum when analyzing for one compound with a single sharp resonance. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) has potentially lower detection limits, but the technique is not silicone-specific and suffers from species-dependent responses.

Because of the increased use of silicones as biomedical materials, there has been interest in developing methods for detecting silicone in biological matrixes. Unfortunately, many of the methods suffer serious limitations. Atomic spectroscopy techniques such as inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or atomic absorption (AA), are not silicone-specific, but rather analyze for silicon as a surrogate for silicone. With proper controls, this approach is useful, however, it is important to realize that silicon is naturally present in tissues and silicon levels can be influenced by factors other than the presence of silicone. Silicon intake in a typical U. S. diet has been estimated as 20−50 mg per day, but can range substantially higher.[1] The consequences of high silicon intakes can be dramatic. Silicon intakes exceeding 1400 mg/day from ingestion of magnesium trisilicate antacids were linked to the formation of silica stones.[1] Other factors can also affect comparisons based purely on silicon levels. For example, silicon output in urine varies with the time of day.[2] It has also been found that patients undergoing hemodialysis exhibit significantly elevated serum and urine silicon concentrations.[3] These are just a few of the factors that make comparisons based purely on silicon levels difficult.

The ubiquitous presence of silicon also introduces silicon analysis problems. As shown in Table 1, reported silicon concentrations for normal blood and serum vary over a considerable range.[3,4,5,6,7,8,9] At least part of the variability may be due to contamination from environmental sources of silicon or silicone. Dust is a source of silicon contamination, and silicone may be introduced from the surface of laboratory items such as plasticware and syringes. In addition, human contact may introduce contamination from silicone that is present in many personal care products. Great care must be taken to avoid sample contamination. The importance of avoiding sample contamination may be reflected in the fact that the lowest normal blood−silicon levels were reported in ref 9, a work in which the authors acquired blood samples using silicone-free syringes and processed samples under class 100 clean room conditions to avoid contamination.

Atomic spectroscopic techniques have also been used for analyzing biological tissues. Typically these analyses involve acid digestion followed by analysis for total silicon.[10,11] These methods cannot distinguish silicones from inorganic silicates in tissue. Extraction techniques have been used in an attempt to preferentially extract organosilicon compounds into organic solvents prior to the analysis.[12] However, even this approach cannot preclude the possibility of extracting complexed silicates.

Trace silicone analysis is difficult. Many of the potential problems surrounding analyte losses, contamination, sample preparation, and sample analysis have been enumerated in reviews.[13−15] Losses may occur as a result of strong adsorption of silicones at surfaces or because of volatilization of low molecular

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Table 1. Reported Normal Silicon Levels in Blood or Blood Components

matrix	mean μg of Si/mL	technique	ref
blood	6.24	AA	4
blood	1.29	digestion/colorimetric analysis	5
blood	0.5[a]	DC−PES	6
plasma	0.17	GF-AAS	7
plasma	0.14	DC−PES	3
serum	0.13	ICP-AES	8
blood	0.0250	GF-AAS	9

[a] Approximate median.

weight species. Losses may also occur during sample digestion procedures as a result of the volatility of low molecular weight silicones or silanes that may be generated during acid digestions. Instrumental concerns include the materials of construction of a spectrometer which can cause problems through interaction with silicon compounds or leaching of silicon compounds as contaminates.

The focus of this paper is the detection of silicones. Poly(dimethylsiloxane) (PDMS) is by far the most common form of silicone produced commercially, and it is also the form of silicone most widely used in medical applications. Therefore, any test for detecting silicones in biological tissues should be sensitive to the dimethylsiloxane moiety. In discussing such tests, it is convenient to use a silicone shorthand notation that is commonly used in the industry. In this notation, M, D, T, and Q denote $(CH_3)_3SiO_{1/2}$, $(CH_3)_2SiO$, $CH_3SiO_{3/2}$, and $SiO_{4/2}$ respectively. Thus, MDM, M_3T, and M_4Q are short for $(CH_3)_3SiO(CH_3)_2SiOSi(CH_3)_3$, $((CH_3)_3SiO)_3Si(CH_3)$, and $((CH_3)_3SiO)_4Si$, respectively. Using this notation, the common trimethylsilyl-terminated poly(dimethylsiloxane) would be denoted as MD_nM.

Radiolabeling methyl groups of poly(dimethylsiloxane) with ^{14}C provides a very sensitive and specific tag for detecting silicone in tissues. Silicone labeled with ^{14}C was used to monitor silicone migration from gels implanted in rats.[16] A technique involving radiolabeled octamethylcyclotetrasiloxane (D_4) has been developed for use in distribution and metabolism investigations.[17] Obviously, the technique is only useful if the silicone source has been labeled with an appropriate tag.

Silicone-specific spectroscopic techniques such as ^{29}Si NMR, FT−IR, and Raman have been used to detect silicone in biological tissues. ^{29}Si NMR was used to detect silicone degradation products in the blood of women with implants.[18] However, reported silicon levels were 4−5 orders of magnitude higher than levels reported by atomic spectroscopy techniques. As a result, the reported high levels in blood have been challenged,[19,20] and the original authors have since published an erratum stating that the signal-to-noise ratios in their spectra could not be accurately determined.[21] While short of a retraction, it does raise questions concerning the utility of ^{29}Si NMR, a technique with intrinsically low sensitivity compared with those of other techniques. Raman and FT−IR microscopy have been used to identify silicone in capsular tissue and in regional lymph nodes of women with implants.[22,23,24,25] A limitation of IR and Raman spectroscopy is that, as applied, they do not give quantitative information.

Extraction in conjunction with gas chromatographic techniques was used to follow migration of low molecular weight cyclics (D_3 to D_7) following injection in mice.[26] Silicones were identified by use of mass spectral or atomic emission detectors. Whereas these techniques unequivocally identified various cyclics in tissue, the injected doses of approximately 10 g/kg of body weight would be equivalent to injecting a human with half a kilogram of cyclics. Since medical devices consist of polymeric silicones with only traces of the volatile low molecular weight cyclics, the extraction and GC techniques have limited practical utility.

A technique which can specifically detect dimethylsiloxane oligomers and polymers is known in the silicone industry as the Aqueous Silanol Functionality Test (ASFT).[27] In this procedure, an aqueous sample containing silicone species is digested by acidifying to 10−15 wt % HCl and shaking for 1−7 days. During this period, silicones are hydrolyzed to the monomeric units: $(CH_3)_2Si(OH)_2$, $CH_3Si(OH)_3$, and $Si(OH)_4$. A small amount, typically 2 mL, of hexamethyldisiloxane (MM) is then added to the digested sample, and the sample is shaken an additional 1−7 days. During this time, all silanol species are capped with trimethylsilyl groups and extracted into the hexamethyldisiloxane layer. Thus, $(CH_3)_2Si(OH)_2$, $CH_3Si(OH)_3$, and $Si(OH)_4$ are converted to MDM, M_3T, and M_4Q, respectively. The hexamethyldisiloxane layer is then isolated and analyzed by GC versus appropriate standards. The technique is capable of detecting sub ppm levels of $(CH_3)_2SiO$ in aqueous samples.

The great sensitivity of the ASFT is attributed to several factors. Capping silanols with trimethylsilyl groups eliminates the tendency for the analytes to undergo condensation reactions in the GC. Also, capping results in a significant mass increase, which greatly enhances sensitivity when using a GC/FID detector. For example, in the case of the D unit, capping increases the mass from 74 to 236. Lastly, depending on the ratio of the original sample to the hexamethyldisiloxane phase, significant concentration enhancements are possible by extraction of the analytes of interest into a smaller volume. Another great advantage to the technique is that, if the hydrolysis can be run under conditions where methyl

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cleavage does not occur, then it is possible to determine the type of units that were originally present in the polymer, i.e., the absolute concentrations of D, T, and Q species.

The technique does have a few drawbacks. Detection of other functional groups on silicon requires that they be stable under the hydrolysis conditions and that the analyst has appropriate standards to detect and quantify the capped species. Also, it is not possible to detect Me_3SiOH unless a capping agent other than the trimethylsilyl group is used. For most analyses, these are not considered significant problems because the dimethylsiloxane unit comprises the bulk of commercial silicones. The analyst must always be wary of the possibility of methyl cleavage. Although methyl cleavage was not observed in carrying out the standard ASFT on spiked aqueous samples,[27] it is essential to run appropriate recovery experiments to ensure adequate recovery of the analyte and to ascertain whether methyl cleavage occurs.

Unfortunately, the ASFT does not work as well with biological samples. Proteins precipitate upon addition of the HCl, and the solids may interfere with the hydrolysis of entrapped silicones. In addition, some proteins are extracted into the hexamethyldisiloxane layer, leading to formation of gels. The gels can be dissolved in methanol for analysis, but dilution results in a loss in sensitivity.

A modified version of the ASFT was published for analysis of biological tissues.[28] In this variation, capsular tissue from a woman with breast implants was first digested in alcoholic KOH. The sample digestion was completed in strong acid, followed by capping with hexamethyldisiloxane. Presumably, the more rigorous digestion technique hydrolyzes proteins to water-soluble amino acids, thus eliminating both the formation of solids and the extraction of proteins into the hexamethyldisiloxane layer. The disadvantage of the technique is the need to digest sequentially in strong base and acid. Detection limits for this technique were estimated to be 6 μg ($(CH_3)_2SiO$)/g of tissue; however, recoveries for the technique were not reported.

This paper describes the detection of silicones in biological matrixes using silicon- and silicone-specific techniques. The intent is to alert researchers as to some of the pitfalls and limitations of techniques such as ICP-AES and ^{29}Si NMR. In addition, a modified version of the ASFT technique will be presented which allows for faster sample throughput and routine analysis of biological samples.

EXPERIMENTAL SECTION

Reagents. Methyltris(trimethylsiloxy)silane (Gelest), tetrakis-(trimethylsiloxy)silane (Gelest), dimethyldimethoxysilane (Dow Corning), methylene chloride, pig serum (Sigma Aldrich), 34% hydrochloric acid (Fisher Optima Grade), 70% nitric acid (Fisher Optima Grade), 350 cst. poly(dimethylsiloxane) (Dow Corning 200 fluid), 100 cst. poly(dimethylsiloxane) (Dow Corning 360 fluid), and pentadecane (Aldrich) were used as received. National Institute of Standards and Technology (NIST) traceable sodium silicate standards were obtained from SPEX CertiPrep, Inc. Hexamethyldisiloxane (Dow Corning OS-10) was treated with activated charcoal, was filtered, and then a center cut was obtained from distillation on a glass helices packed 36" column outfitted with an electronic reflux ratio controller. Deionized water with a resistivity of 19.2 MΩ-cm was used.

ICP Analysis. Analyses were carried out using a Fisons Accuris ICP system 20412 equipped with a low-power, low-flow torch design. Samples were introduced at a controlled flow rate using a peristaltic pump. Background-corrected intensities were determined by subtracting off-peak intensities from the raw peak intensity at 288.16 nm.

^{29}Si NMR. NMR spectra were acquired on a Varian Unity Plus 400 MHz spectrometer equipped with a 16-mm Nalorac probe capable of 850:1 s/n ratio in a single scan for ^{13}C on the ASTM standard, 60% d_6-benzene/40% *p*-dioxane. Signal-to-noise determinations were performed on a 0.0312% solution of $(CH_3)_2Si(OH)_2$ in pig serum. Spectra were acquired with 256 scans in 17 h and 10 min using the following parameters: 240-s pulse delay, 90° pulse width, D_2O lock reference, and 1.5 Hz of line broadening with ^{1}H decoupling applied during the acquisition period only to avoid nuclear Overhauser effects.

Synthesis of Dimethylsilanediol. A procedure originally developed by Compton was used.[29] $(CH_3)_2Si(OCH_3)_2$ (26.60 g) and acetic anhydride (2 μL) were weighed into a three-neck 100-mL flask outfitted with a stir bar, addition funnel, thermometer, and short path distillation head. While stirring vigorously, 15.9 g of deionized water was added to the flask. An immediate exotherm was noted, with the pot temperature rising to 40 °C. After stirring 15 min, the reaction mixture was vacuum-stripped to remove methanol. A total of 16.73 g of crude $(CH_3)_2Si(OH)_2$ was isolated (82% yield). Crude $(CH_3)_2Si(OH)_2$ was recrystallized from acetone, washed with cold pentane, and then dried in a vacuum desiccator (15 min at 0.01 Torr). The product consisted of white platelets (mp 94.5−97 °C).

Microwave Digestion. Microwave digestions were carried out using a CEM model MDS-2100 microwave digestion apparatus fitted with as many as twelve 100-mL Teflon-lined pressure vessels. Temperature or pressure was controlled or monitored through sensors attached to a control vessel. In some early experiments involving HCl digestions of spiked sera, carryover contamination was evident. The problem was eliminated by washing the digestion vessels and centrifuge tubes with detergent. In addition, the digestion vessels were subjected to a microwave digestion treatment with 35% HNO_3 to remove residues. The vessels and centrifuge tubes were then rinsed with deionized water and acetone.

Nitric Acid Digestion of Spiked Pig Serum. Teflon pressure vessels outfitted with rupture disks were loaded with 0−20 μL of either 350 cst. PDMS in THF or sodium silicate in water. To each vessel, 1.5 mL of pig serum and 8 mL of 70% nitric acid were added. Samples were digested using a five-step pressure program where the pressure was ramped to 10, 20, 30, 50, and finally 75 PSI with 5-min holds at each pressure. Pressure vessels were cooled in dry ice, vented, and the solutions then transferred to 30-mL Teflon centrifuge tubes. Transfer was facilitated by rinsing the pressure vessels with two 3-mL portions of a 2% boric acid solution. The boric acid served to significantly lower background intensities in the ICP-AES when analyzing solutions of strong acid. Samples were analyzed by ICP-AES against sodium silicate standards in solutions containing 37% HNO_3 and 0.66% boric acid.

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Table 2. Response Factors for Analysis of Silanes in Serum

compound	response factor[a]
NIST silicate	1.00
$MeSi(OCH_3)_3$	0.894
$(CH_3)_2Si(OH)_2$	0.890
$(CH_3)_3SiOH$	0.060

[a] Corrected ICP intensity = response factor × intensity.

Aqueous Silanol Functionality Test. A typical analysis of a sample spiked with PDMS was carried out as follows. Teflon pressure vessels outfitted with rupture disks were loaded with 0−50 μL of a silicone solution in CH_2Cl_2. The CH_2Cl_2 was permitted to evaporate, after which the vessel was loaded with 0.25 mL of serum, 14.50 mL of deionized water, and 5.25 mL of concentrated HCl (34 wt. % HCl). Sealed vessels were subjected to a microwave program involving a 10-min ramp to 140 °C followed by a temperature hold for 2 h. Samples were transferred to 30-mL Teflon centrifuge tubes. To each sample was added 2 mL of hexamethyldisiloxane containing 100 μg/mL pentadecane. The samples were tightly capped, shaken for 24 h on a Burrell Scientific Model 75 wrist shaker, and centrifuged at 3000 rpm for 30 min in a Beckman Model GS-6 centrifuge. The hexamethyldisiloxane layer was then analyzed for MDM, M_3T, and M_4Q by GC.

Gas Chromatography. Samples were analyzed on a HP 6890 GC outfitted with an autosampler, a 30 m × 0.25 mm ID × 0.1 μm film thickness DB1-HT column, and an FID detector. Injections of 3 μL were run split (100:1) with a total gas flow of 109 mL/min and a constant column flow of 1 mL/min. Samples were analyzed versus MDM, M_3T, and M_4Q standards with pentadecane as an internal standard.

Designed Experiment. To optimize the microwave digestion conditions, a 3-factor Box-Behnkin designed experiment was set up and analyzed using Design-Expert 5.0.7 (Stat-Ease Corp.). Data were fit to a quadratic model.

RESULTS AND DISCUSSION

Many groups have used ICP-AES to analyze for silicon in blood or serum. Most of these studies have involved diluting the sample and then analyzing the solution directly. For the most part, these procedures should give reasonable estimates of total silicon, but the analyst should be aware that, if organosilicon compounds are involved, there is the potential for species-dependent responses which could significantly impact the accuracy of the result. For example, serum samples containing spikes of $(CH_3)_3SiOH$, $(CH_3)_2Si(OH)_2$, or $CH_3Si(OCH_2CH_3)_3$ (which presumably hydrolyzes to form $CH_3Si(OH)_3$) were diluted 4:1 with dilute HNO_3 solution and then analyzed by ICP-AES. Table 2 shows response factors for each silane relative to spiking with a NIST silicate standard.

Clearly, the ICP-AES analysis using NIST silicate standards would give reasonable estimates of the concentration of silicon arising from dimethylsilanediol or methylsilanetriol. However, trimethylsilanol gave a response nearly 17 times that of the NIST standard. Thus, if trimethylsilanol were present, it would result in a significant overestimate of the silicon concentration.

To eliminate species-dependent response, it is common to subject samples to an ashing procedure. Wet ashing by microwave digestion of PDMS or sodium silicate spiked pig serum in HNO_3 was conducted in order to determine the appropriateness of the technique for sample preparation for total silicon determination. Samples were analyzed by ICP-AES. Background-corrected intensities were converted to silicon concentrations on the basis of calibration curves made up from NIST traceable silicon standards. Figure 1 shows a plot of actual silicon levels based on spiking with PDMS or sodium silicate versus the concentration as measured by ICP-AES. As one might expect, serum samples spiked with sodium silicate give a slope near one, demonstrating the appropriateness of the standards for that determination. The nonzero intercept is indicative of a substantial amount of native silicon that is present in the particular bottle of pig serum (approximately 6.6 μg/mL serum). In contrast, the slope of the line for the PDMS spiked samples is nearly 2.3. This demonstrates a species-dependent response for the digested PDMS samples, resulting in an overestimate in the amount of silicon present. This might be caused by incomplete digestion of the PDMS producing species which influence sample introduction into the plasma. Doubling the microwave digestion time did not eliminate the elevated response for PDMS samples in the ICP-AES. It is likely that this problem could be overcome by running the digestion using HNO_3/HF or HNO_3/HCl/HF as the digesting acid. Unfortunately, this would require the use of HF, a very hazardous acid.

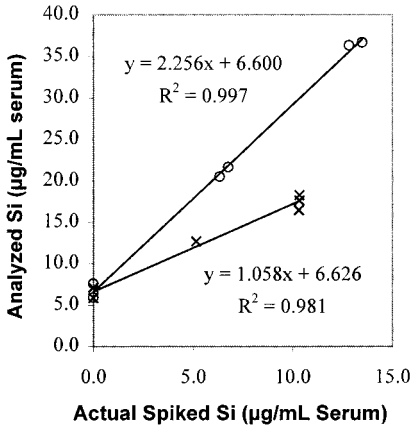

Figure 1. ICP-AES analysis of sample prepared by microwave digestion of poly(dimethylsiloxane)-spiked pig serum in HNO_3. ○ − poly(dimethylsiloxane)-spiked serum, x − silicate-spiked serum.

Clearly, many of the techniques in use based on ICP-AES can detect elemental silicon, but absolute quantitation would not be possible unless species-dependent responses are eliminated. Even then, ICP-AES does not reveal anything about the origin of the silicon species.

NMR does reveal information about the species present in the sample; however, NMR is not nearly as sensitive as atomic spectroscopic techniques. This is especially true of ^{29}Si NMR which has the added disadvantages of long spin−lattice relaxation times (T_1), a negative magnetogyric ratio, and a low natural abundance of the ^{29}Si isotope. The long T_1 relaxation times are particularly troublesome since obtaining quantitative information typically requires using pulse delays of five times the longest T_1. Normally, T_1 relaxation times can be significantly reduced by the inclusion of a paramagnetic relaxation agent, but common agents have low water solubility. In addition, paramagnetic relaxation is strongly distance-dependent and only effective at close proximity

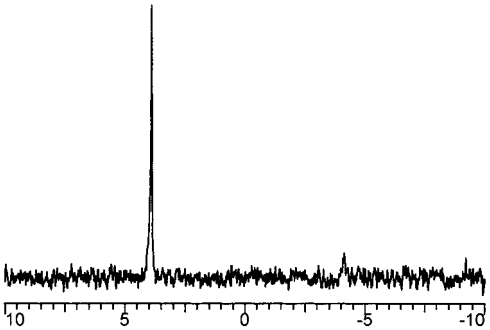

Figure 2. ^{29}Si NMR spectrum of a 0.0312% solution of $(CH_3)_2Si(OH)_2$ in pig serum. Spectrum was acquired with 256 scans in 17.1 h. Peak at 3.9 ppm is dimethylsilanediol, s/n = 34.6. The peak at −4.2 ppm arises from trace levels of the condensation product, tetramethyldisiloxanediol.

to the nuclei of interest. It might not be effective at all or effective to varying degrees in a complex biological matrix.

The biological matrix itself may impose limitations on the NMR experiment. Biological fluids such as serum commonly have higher dielectric constants than usual organic NMR solvents, like $CDCl_3$, resulting in poorly tunable probes and reduced pulse power delivered to the sample. Lower effective pulse power means longer 90-degree pulse widths with concomitant reductions in sensitivity. Long pulse widths also limit the size of the spectral window that can be observed while ensuring that all nuclei see the same effective pulse width which is required for quantitative analysis. On a VXR400 NMR spectrometer equipped with a 16-mm probe, typical 90-degree pulse widths of 30 μs for $CDCl_3$ solutions were increased to 45−50 μs for serum samples. This is too long for good T_1 measurement and severely limits the frequency range available for quantitative acquisition. Solvent effects can be minimized by using a lower volume probe, but this also reduces the sample size and ultimate sensitivity. To balance these factors, the experiments were moved to a Unity Plus 400 MHz spectrometer that handles higher pulse power yielding useful 25.5-μs 90-degree pulse widths for ^{29}Si on the 16-mm probe. In a recent paper, Knight demonstrated the potential advantages of high magnetic field strengths and polarization transfer pulse sequences in ideal nonaqueous systems.[30] Extrapolation to biological matrixes has not been demonstrated and may be problematic for large sample sizes since polarization transfer experiments are highly dependent on effective pulse power.

The value of T_1 for dimethylsilanediol at room temperature in pig serum was measured as 48.4 ± 0.9 s using fast inversion recovery.[31] As a result of the long relaxation time, the acquisition time for 256 quantitative scans for determination of the detection limit required over 17 h. On the basis of the definition of the detection limit as requiring a signal-to-noise of three, the detection limit for dimethylsilanediol in pig serum was determined to be 64 μg Si/mL. One should keep in mind that this is the detection limit for a discrete water-soluble organosilicon compound with a single sharp resonance, Figure 2. In actual biological samples, there would be the potential for numerous species of varying solubility and resonance frequencies. In addition, if the organosilicon compounds were bound to other species in the matrix, substantial line broadening and a subsequent loss in sensitivity could occur.

Table 3. Designed Experiment to Optimize Microwave Digestion Conditions for ASFT Analysis

HCl (wt. %)	serum (mL)	temp (°C)	*N*	$(CH_3)_2SiO$ recovery (%)	std. dev.	CH_3 cleavage[a] (%)	std. dev.
5	0.250	130	3	41.8	13.8	0.23	0.09
5	0.625	120	3	30.0	20.8	0.13	0.14
5	0.625	140	3	36.4	10.7	0.58	0.86
5	1.000	130	3	12.4	7.6	0.55	0.92
10	0.250	120	3	46.3	18.3	1.34	0.16
10	0.250	130	3	75.1	15.3	1.28	0.20
10	**0.250**	**140**	**3**	**88.2**	**13.6**	**1.14**	**0.44**
10	0.625	130	15	55.2	18.9	0.9	0.3
10	1.000	120	3	51.7	32.7	0.33	0.02
10	1.000	140	3	76.9	18.2	0.69	0.57
15	0.250	130	3	42.4	38.1	1.74	0.98
15	0.625	120	3	20.2	6.3	1.16	0.22
15	0.625	140	2	55.5	45.1	1.38	0.25
15	1.000	130	3	16.9	15.9	0.76	0.04

[a] Percent of D units undergoing methyl cleavage, estimated from the concentration of D and T units.

Another technique which can measure silicon levels and reveal limited information on the form of silicon is the ASFT test. Unfortunately, the test generally takes a long time to run and may not work well for biological samples. A solution to both these problems is to accelerate the test by carrying out the digestion at a higher temperature in sealed vessels. This serves to rapidly hydrolyze both the silicone and the proteins that constitute the sample. This is most easily carried out by acid digestion of the samples in microwave pressure vessels.

A designed experiment was carried out to determine the optimum conditions for recovery of dimethylsiloxane from pig serum spiked with PDMS while minimizing methyl cleavage. For practical purposes, digestion conditions were sought that could be accomplished in 2−3 h. This allows the analyst to prepare the samples and then run the capping portion of the experiment overnight.

A 3-level Box-Behnken designed experiment was used to optimize the digestion conditions. The factors chosen for study were the HCl concentration, the volume of serum, and the temperature. Serum, water, and HCl were added such that the total volume added was fixed at approximately 20 mL. The amount of PDMS spiked into each serum sample was also held constant. Thus, decreasing the volume of serum also had the effect of increasing the concentration of PDMS relative to serum, but held it constant relative to the final matrix. Capping with hexamethyldisiloxane was carried out by shaking at room temperature for 24 h. Increasing the capping time seemed to have little impact on the recovery. The responses analyzed were the recovery of $(CH_3)_2SiO$ and the amount of CH_3 cleavage. Methyl cleavage was determined from the concentration of M_3T. The results of the experiment with this design are shown in Table 3.

A response surface for recovery as a function of sample size and temperature at a fixed HCl concentration of 10 wt. % is shown in Figure 3. From this figure, it is immediately apparent that recovery increases with increasing temperatures and decreasing serum sample size. Unfortunately, methyl cleavage increases with

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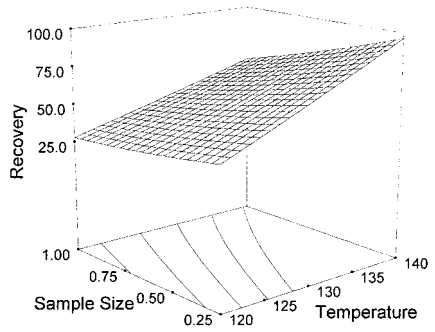

Figure 3. Response surface for recovery as a function of sample size and temperature during microwave digestion of poly(dimethylsiloxane)-spiked pig serum in 10 wt. % HCl.

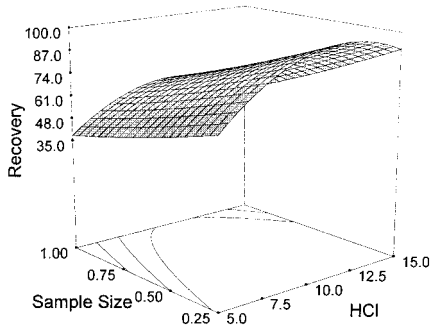

Figure 4. Response surface for recovery as a function of sample size and HCl concentration during microwave digestion of poly(dimethylsiloxane)-spiked pig serum at 140 °C.

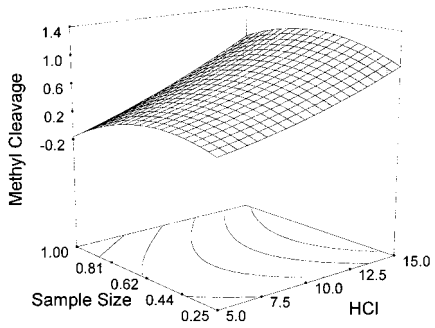

Figure 5. Response surface for methyl cleavage as a function of sample size and HCl concentration during microwave digestion of poly(dimethylsiloxane)-spiked pig serum at 140 °C.

increasing temperature as well. Response surfaces for recoveries and methyl cleavage at 140 °C are shown in Figures 4 and 5. Here it becomes evident that recovery goes through a maximum as the HCl concentration is increased. This may be a direct result of the increased amount of methyl cleavage which is observed at higher temperatures and HCl concentrations.

Software optimization suggested reasonable recoveries with minimal methyl cleavage required an HCl concentration of 8.9−9.5%, a temperature of 137−138 °C, and a sample volume of 0.25 mL. The digestion conditions closest to these optima, as evaluated in Table 3, were 10 wt. % HCl, 140 °C, and 0.25 mL of serum. Under these conditions, 88.2% recovery and 1.1% methyl cleavage were observed. Unfortunately, standard deviations were high, and further optimization may be warranted.

There are tradeoffs in choosing the digestion conditions. For example, increasing the serum volume digested from 0.25 to 1.00 mL leads to a decrease in the recovery to 77%, but the concentration enhancement gained by extraction into the MM layer should lower the detection limit in serum by as much as a factor of 4.

Table 4. Recovery and Methyl Cleavage Observed in ASFT Analysis Following Digestion (3 h, 140 °C, 10% HCl, 0.25 mL of Serum)

spiked serum concn. (μg/mL)		measured concn. in serum (μg/mL)			% D recovery (blank corrected)		% D units undergoing Me cleavage	
D	*n*	D	T	Q	ave.	std. dev.	ave.	std. dev.
1866	3	1634	12.9	1.87	87.5	17.6	0.8	0.3
453	6	389.4	3.89	1.54	85.5	15.4	1.0	0.6
17.7	6	18.5	0.27	1.71	92.4	2.1	1.7	1.6
0.00	6	2.07	0.00	1.78				

Moderately increasing the digestion time had little impact on recovery or methyl cleavage. In the experiment shown in Table 4, 0.25 mL of serum was digested for 3 h in 10% HCl at 140 °C. Spikes of PDMS were varied between 17.7 and 1866 μg $(CH_3)_2$-SiO/mL of serum (equivalent to 6.7 to 707 μg Si/mL of serum). Over this series, recoveries from individual runs ranged from 65 to 101%. It was noted that the precision was poor with samples having higher concentrations of silicone spikes, but precision improved dramatically for the lower concentration samples. This may be due to the insolubility of poly(dimethylsiloxane) resulting in a heterogeneous sample. If a means could be found to stir the sample, the precision might improve dramatically.

Although there was considerable variability in the individual runs, the test is applicable for detection of silicones over a wide range of silicone concentrations. Also, the observed recovery and precision at lower silicone concentrations, 92.4 ± 2.1% at 17.7 μg D/mL, should be acceptable for most analyses. Thus, if replicate analyses give irreproducible results, running the analysis on smaller samples should resolve the problem.

Based on six blank serum analyses, the serum contained 2.07 ± 0.38 μg $(CH_3)_2SiO$/mL of serum. Using the convention of establishing the detection limit from three times the standard deviation of the blank, the detection limit would be 1.1 μg $(CH_3)_2$-SiO/mL of serum or 0.43 μg Si/mL of serum. Detection limits would have improved if lower background serum blanks could have been obtained or if larger volumes of serum had been digested. For critical work near the detection limit, more blank determinations are highly recommended.

Preliminary experiments have shown that the technique is applicable to other tissue samples as well. Digestion of PDMS spiked chicken livers under nonoptimized conditions gave recoveries ranging from 51 to 90%.

A few words of caution about the test are appropriate. ASFT analysis of serum or even deionized water blanks will show traces of dimethylsiloxane. The origin of the dimethylsiloxane species has not been established; however, the species may originate as impurities in the hexamethyldisiloxane or as the result of environmental contamination. Silicones are widely used as antifoam agents, adhesives, lubricants, release agents, etc., and silicone contaminants may be introduced during sampling or processing. It is also possible for species present in the sample to catalyze methyl cleavage from hexamethyldisiloxane during the capping phase of the analysis, thus generating D or even Q units. Therefore, to determine if detected silicone is significant, it is

essential to run a series of matrix-matched blanks or suitable controls in order to determine the detection limit of the technique for the samples of interest.

CONCLUSIONS

Many researchers analyze for silicon as a surrogate for silicone. In these cases, it is advisable to keep in mind that silicon and silicone are commonplace, and contamination can be a significant issue. Running appropriate matrix-matched blanks is absolutely essential. Researchers should also take care to determine whether species-dependent responses may influence their results. This is particularly true of ICP-AES, in which volatile silanes or incompletely digested silicones may lead to overestimates of the silicon concentrations. Positive identification of silicone is possible by a number of spectroscopic techniques; however, most do not yield quantitative information. An exception is ^{29}Si NMR; but the technique lacks sensitivity, particularly if the silicone is present as multiple species.

A sensitive technique for identifying and quantifying silicones in aqueous or biological samples is to use microwave digestion to hydrolyze silicone and proteins followed by analysis of the digested sample using the Aqueous Silanol Functionality Test. Detection limits below 1 μg $(CH_3)_2SiO$/mL of sample are feasible. Lower detection limits may be obtained by increasing the ratio of sample to hexamethyldisiloxane, although this may result in decreased recoveries. It was noted that microwave digestion did lead to a small amount of methyl cleavage, which precludes the possibility of absolute quantitation of D, T, and Q units originally present in the sample. It is possible that methyl cleavage could be further reduced by changing the digestion conditions. Possible modifications might include using enzymatic digestion to break down the matrix prior to the acid digestion, using milder digestion conditions (at the cost of extending the analysis), or changing the digesting acid.

When using the test, it is absolutely critical that detection limits be properly established and that replicates are run to establish precision. In those cases in which precision is poor, both recovery and precision can be improved by diluting the sample, although this comes at the cost of raising the detection limit of the technique.

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