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A Methodology Based on NMR Spectroscopy for the Forensic Analysis of Condoms*

REFERENCE: Lee GSH, Brinch KM, Kannangara K, Dawson M, Wilson MA. A methodology based on NMR spectroscopy for the forensic analysis of condoms. J Forensic Sci 2001;46(4): 808–821.

ABSTRACT: Both solution and solid state Nuclear Magnetic Resonance (NMR) spectroscopic techniques have been used to determine differences in commercially available condoms. Whilst solid state NMR is useful for determining the polymer backbone, it is not useful for forensic analysis due to the commonality of the latex condom. However solution NMR spectra obtained following a simple extraction procedure using hexane, provides a fingerprint of the additives in the lubricants. Following the development of a flow chart, basing decisions on the presence of particular peaks present in the solution spectra, 33 of 38 condoms could be individualized. Samples were also analyzed after having the lubricant manually removed and soaking the condom in water for 3 to 24 h. These experiments were performed to simulate a case of the sample having been used and disposed of by flushing down the toilet, as may be experienced in a case of a sexual assault. The results indicated that the only significant water soluble component was polyethylene glycol. The overall results suggest that the method developed may be a quick and useful technique in characterizing condoms. The information obtained can be used to provide associative evidence between suspect and crime, and so be useful in sexual assault cases.

KEYWORDS: forensic science, condom, polydimethylsiloxane, PDMS, polyethylene glycol, PEG, nonoxynol-9, nuclear magnetic resonance, NMR, identification systems

The recovery and identification of lubricant and spermicide traces associated with condom use can provide important evidence in sexual assault cases. As the number of cases involving condoms is on the rise, researchers have begun to explore different analytical techniques for their ability to detect the presence of lubricants and spermicides. Previous work has focused on the lubricant polydimethylsiloxane (PDMS) extracted from samples taken from victims to confirm the use of condoms (1–5). Infrared (IR) spectroscopy was the main analytical technique used in the majority of these studies, however chromatographic, mass spectrometric (2) and NMR spectroscopic (4) methods have also been used. There has also been some infrared and mass spectrometric work on the identification of traces of the nonoxynol-9 spermicide (2,6). These methods have been successful in providing evidence that a condom was used in the crime, however they do not provide associative evidence, which can link a suspect to that particular crime. Hence a method which is capable of differentiating between lubricants originating from different condom manufacturers or even different individual brands is still in demand. At present it appears that no such technique exists, however Blackledge and Vincenti (2) have suggested that gel permeation chromatography may be able to distinguish between PDMS used in different condom brands. Blackledge has also employed two other methods, ^{29}Si NMR and Fourier transform infrared (FTIR) spectroscopy with Fourier self-deconvolution (FSD), to investigate the siloxane chain length of different PDMS samples. While ^{29}Si NMR was found not to be sensitive enough, FTIR with FSD showed some ability to discriminate (2). Some degree of discrimination has been achieved by the identification of a number of insoluble particles such as talc, silica, and cornstarch by light microscopy and scanning electron microscopy/energy dispersive X-ray spectroscopy (7,8).

In an attempt to find a technique that could distinguish between different brands of condoms, it was decided to study all the soluble organic components that make up the lubricant of the condom, by ^{1}H NMR as opposed to just the PDMS base (9). The lubricant formulation from different manufacturers should differ due to proprietary product information and thus should afford different ^{1}H NMR spectra. Moreover, a study of the actual condom sheaths by solid state ^{13}C NMR was also performed. NMR was chosen as it has been shown to be one of the most suitable methods for the analysis of mixtures of general unknown content. Besides being quantitative and highly sensitive (10,11), with just one experiment NMR provides a complete "picture" or fingerprint of all the soluble organic components present in the sample. As these organic molecules are likely to be the lubricant trace evidence left after a sexual assault, the NMR spectrum provides the examiner with a "fingerprint" of the condom used in the assault.

Components of Condoms

The vast majority of condoms are made from thin latex rubber. Latex is a natural elastomer with the chemical structure *cis*-1,4-polyisoprene (see insert, Fig. 2). During manufacture the liquid latex is vulcanized. This process crosslinks the chain-like rubber molecules to form an elastic network, with sulfur being the predominant vulcanizing agent (12). Condoms are also manufactured from other materials, primarily synthetic polymers and sheep caecum (13). At the time of this investigation, polyurethane was the only synthetic material used in commercially available condoms (13).

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* This work was presented at the 15th International Symposium of Forensic Science, Gold Coast, Australia, 2000, 5–10 Mar. 2000.

Received 20 March 2000; and in revised form 1 August 2000; accepted 1 August 2000.

Apart from the actual sheath itself, many condoms are available with added lubrication, spermicides, and fine particles. There are two types of lubricants used on condoms—those based on PDMS and those using polyethylene glycol 400 (PEG). Both are clear, odorless liquids. The majority of condoms have a PDMS based lubricant. The only spermicide found on condoms available in Australia is nonoxynol-9, a polyoxylated nonylphenol. It is a mixture of ethoximers, the "9" representing the average number of glycol monomer units (see insert, Fig. 8 for the chemical structure). Finally, manufacturers often add fine powders to the condoms in the "finishing process" to prevent the rolled up condom sticking to itself (14–16). The most commonly used powder is cornstarch although talc, mica, calcium carbonate, silicon dioxide, magnesium carbonate, lycopodium, and dry silicone have also been used.

Experimental

Materials

Thirty-eight representative samples of condoms from twelve different manufacturers (Table 1) were purchased from various sources: supermarkets, pharmacies, and specialty shops around Sydney, Australia. The Durex Avanti and Trojan Naturalamb samples were purchased in the United States, as they are not yet available for retail sale in Australia. All analyses were carried out on unused condoms.

Polyethylene Glycol 300 oil (100%, Aldrich) was used as a reference material for polyethylene glycol 400 used in condom lubricants. An extract of the vaginal cream "Orthocreme" (Janssen-Cilag) containing 20 mg/g nonoxynol-9 was the reference sample for the spermicide.

Sample Preparation

All sample preparation was carried out using two pairs of clean plastic tweezers to hold the samples. The samples were unrolled using the tweezers, one pair to hold the tip, the other used to pull down the rolled portion of the condom. The tweezers were thoroughly cleaned between samples to avoid contamination. Gloves were also avoided so that there was no sample contamination. It was thought that this might occur as the gloves are also manufactured from natural latex and are coated with a fine powder. The condoms were cut to the required size using clean surgical scissors and placed directly into clean containers for further treatment.

Extraction of the Hexane Soluble Organic Component of the Lubricant

All extractions were carried out at room temperature to avoid any alteration of the sample due to heat. A section of condom (approximately one third) was taken and swirled in 7 to 10 mL n-hexane (98.8% pure GLC grade HiperSolv, Merck) in a 21 mL glass scintillation vial for ten min. It was found that when the condom was left in contact with hexane for periods greater than twelve minutes, the latex began to absorb the solvent causing appreciable loss of solvent and hence sample. The condom was then removed and the hexane was allowed to evaporate. In some cases the removal of solvent was accelerated by using a stream of high purity nitrogen. A yellow or colorless liquid or oil remained.

Effect of Water on the Analysis

A section of condom (approximately one third) was taken. The lubricant was removed by wiping with clean tissues and the condom was then left to soak in a beaker of tap water for between 3 and 24 h. The sample was then removed and allowed to dry at room temperature. The dried sample was then extracted with hexane as described above.

NMR Spectroscopy

All NMR spectra were obtained using a Bruker DRX 300 MHz narrow bore magnet instrument operating at 300 MHz for protons and 75 MHz for carbon. Solid state magic angle spinning (MAS) spectra were recorded at ambient temperature. Solution spectra were recorded at 300 K.

^{13}C Solid State NMR Spectroscopy

The reservoir at the tip of the condom was cut off and packed into a 4 mm zirconia rotor with a Kel-F cap. This small section was approximately 4 mm^2. A Bloch Decay experiment was employed in conjunction with high power proton decoupling. The decoupling was turned on only during acquisition. The spectra were obtained in 2 K data points, a 90° pulse of 8 μs, 30 000 Hz sweep width, and a pulse repetition rate of 2 to 10 s. 256 to 1000 scans were required. All chemical shifts are expressed relative to tetramethylsilane (TMS) using adamantane as an external reference (the CH_2 peak of adamantane was found to be 38.3 ppm downfield from the 0.00 ppm TMS peak). No interference from spinning sidebands was observed, hence all experiments were spun at 3 kHz. Blanks were run of the rotors to ensure there would be no artifacts in the spectra.

Low power solid state experiments were performed by inserting a strip of the condom of approximately 2 by 6 cm into a 5 mm o.d. NMR tube. The spectrometer was set up as one would for a one pulse solution ^{13}C experiment. Single pulse decoupled experiments were performed at a spin rate of 20 Hz. The experimental parameters were; 90° pulse of 8 μs, spectral width of 240 ppm and a time domain of 64 K. 256 scans were collected.

^{1}H Solution NMR Spectroscopy

^{1}H solution NMR spectra were obtained for all extracted samples in a solvent of deuterated chloroform, $CDCl_3$ (99.8 Atom % D, Aldrich). The spectra were obtained with a 90° pulse of 7.4 μs, 4000 Hz sweep width, and a pulse repetition rate of 2 s. 200 scans were required. All chemical shifts are referenced to a solution of neat TMS (0 ppm).

Results and Discussion

Market Share

Information from the Therapeutic Goods Administration (TGA) shows that there are 21 different brands of condoms available for retail sale in Australia. This list also details the supplier of the latex, whether spermicide is used, type of lubricant, and the Australian distributors. It should be noted that this is not an exhaustive list of all brands that may be encountered in a sexual assault investigation, as many others are obtainable by mail order through specialty shops and over the Internet.

In selecting samples to investigate, it was necessary to ensure that the most popular and commonly available condom types were represented. These would be the samples most likely to be encountered at a sexual assault scene. It was also necessary to investigate the characteristics of the more uncommon brands to determine if any of these would produce the same result as the common brands.

If this were the case the evidence gleaned from the technique would not be without value, but would carry less evidentiary weight. For example, a situation in which a condom from a crime scene could only be categorized as being one of two brands and the suspect had an empty condom packet on his person or among his possessions of one of these brands. This evidence is not as strong as if the brand had been individualized, but much stronger than if the suspect's packet had been of a totally different brand. If, however, the condom found at the scene could be identified as an uncommon sample the strength of the evidence is increased.

Figure 1 illustrates the percent market share based on volume of particular condom brands in the grocery (17) and pharmaceutical markets (18) in Australia for the 12 months ending October 1999 and February 1999 respectively.

It must also be remembered that the commonality of particular brands of condom is not solely influenced by their retail availability. Approximately 40% of all condom sales in Australia are derived from wholesale and bulk sales, i.e., government services, family planning, and sexual health clinics that distribute condoms free of charge, and brothels. Of this market, an approximate breakdown of figures is: Ansell 50%; Glyde 30%; others 20%. For certain manufacturers, such as Four Seasons, this is their largest market [Mrs. J. Woodworth, managing director, Kia-Ora Pacific (distributors of Glyde Products), 15. 11. 1999, personal communication].

The 38 individual condoms selected for investigation come from 12 different manufacturers and are listed in Table 1. This cross section of samples contains all the popular and most easily obtainable brands. In addition we have included two condoms which are yet unavailable for retail sale in Australia, the Durex Avanti made from polyurethane and Trojan Naturalamb, made from sheep caecum. These brands were included to investigate the validity of the technique on samples known to be fundamentally different. Their inclusion yields a more comprehensive data set so that when approval by the TGA is given, these samples will already have been analyzed. Moreover, we have also included two novelty condoms from Ria and Nite Glow. These condoms have not been tested according to the International Standards Organisation (ISO) (ISO 157: Mechanical Contraceptives, 1997; ISO 4074 (Parts 1–9): Rubber Condoms, 1996) but were included in the sample set in order to determine if any difference in quality could be detected. It is also possible that these condoms may be found at the scene of a sexual assault as the perpetrator may not realize the difference in quality, or be concerned of the risk of sexually transmitted disease (STDs). Although not ISO or FDA (Food and Drug Administration) approved, these condoms

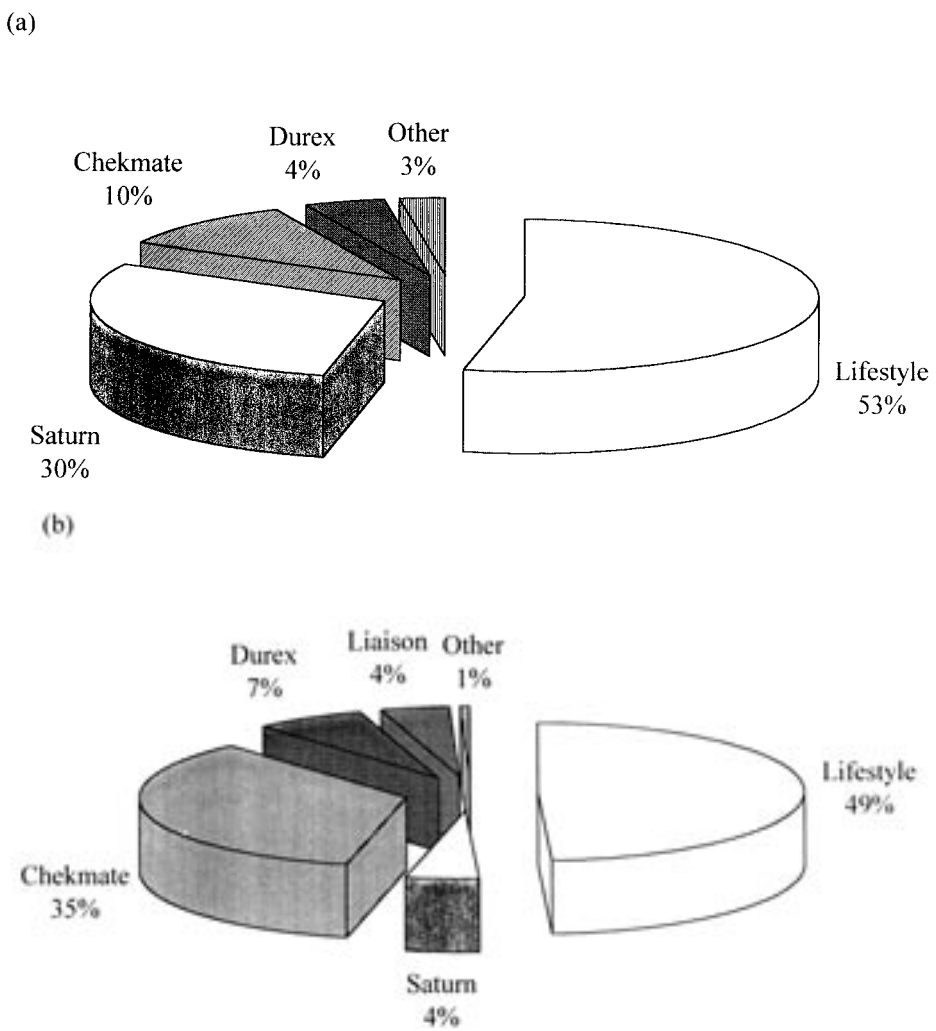

FIG. 1—*Brand market share of condom sales based on volume.* a) *grocery sales,* b) *pharmaceutical sales. Data from A.C. Nielsen, references 17 and 18.*

TABLE 1—*Details of condom samples used in this study.*

CONDOM	
Manufacturer	Type
Ansell	Chekmate Nonlubricated Lifestyle: Flared Studded Green Super Power Play Nonoxynol-9
Saturn	Regular Ribbed Silicon-Free Nonoxynol-9 Blue Pink Black
Liaison	Revel Liberte
Durex	Fetherlite Ribbed Tangerine Strawberry Banana Ice Mint Avanti
Trojan	Naturalamb
4 Seasons	Extra Blue Orange Orange Flavoured
Passion	Regular Nonoxynol-9
Glyde	Strawberry Wildberry Blueberry Black
Sure	
Duo	Regular
Ria	Midnite Magic—Chocolate
Nite-Glow	

are still effective in greatly reducing the possibility of detecting biological fluids and thus should be considered.

Solid State Experiments

Solid state ^{13}C MAS NMR allowed discrimination between condoms with different polymer backbones without interfering with the composition of the sample. A Bloch decay experiment was used to achieve the best resolution. Cross polarization yielded a spectrum of poor quality, but this was to be expected with an elastomeric substance. No discrimination could be made between condoms made from natural latex, as there were no peaks present due to cross-linkages with sulfur vulcanisates. As no peaks except those attributable to *cis*-1,4-polyisoprene were present in the spectra, it can be concluded that all the lattices are subjected to very similar, mild vulcanization processes. Figure 2 shows a representative ^{13}C solid state NMR spectrum of a latex condom (Lifestyle Flared). The peak assignments due to the latex are as follows, C5 23.8 ppm, C4 27 ppm, C1 32.7 ppm, C3 125.6 ppm, and C2 135.1 ppm. The peak at 1.2 ppm is due to the presence of PDMS. In cases where PEG was used as the lubricant, three peaks at 61.3, 70.5, and 72.9 ppm were present and attributed to the PEG. The major peak at 70.5 ppm is attributable to the -(CH_2-O)- unit, however the other two peaks are unassigned. A standard sample of PEG was run confirming the presence of these three resonances. Nonoxynol-9 was not detected in the solid state experiments.

The two samples that could be distinguished were the Durex Avanti and the Trojan Naturalamb. Their spectra are shown in Figs. 3*a* and *b* respectively. The Durex Avanti condom is made from polyurethane, and the chemical shifts can be assigned as follows. The two peaks at 25 and 30 ppm are attributable to alkyl groups. The former may be either a methyl or methylene group, the latter is a methylene group. The peak at 65 ppm is due to an ether linkage, the two small peaks between 100 and 150 ppm are due to aromatic compounds. The final peak, at 175 ppm, may be due to either a urethane (NC = O), or ester (OC = O) linkage. The Trojan Naturalamb condom is made from a sheep's caecum, which is a part of the digestive tract. Its chemical structure is unknown and thus the peaks can only be generically assigned, i.e., aliphatics between 15 and 35 ppm; an aromatic compound at 130 ppm; and an ester linkage at 175 ppm. A peak at 1.2 ppm shows the presence of PDMS.

As NMR spectrometers equipped with solid state facilities are usually not available in forensic laboratories, low power experiments were performed in order to determine whether the same information might be extracted on machines without MAS capabilities. It was found that the spectra obtained using this method gave the same details of polymer backbone and lubricant base as the high power MAS experiments. However, the quality of the spectra was significantly reduced. The peaks were much broader with typical line widths of 3.5 ppm compared to those obtained by the high power MAS experiments (typically 1.5 ppm). Nevertheless, the same information can be extracted from the low power NMR spectra. Hence solid state ^{13}C NMR is a useful technique for the discrimination of the condom material and as a method for providing information on whether the condom was lubricated or not and what type of lubrication was used (PDMS as opposed to PEG).

^1H Solution NMR Experiments

In order to measure the ^1H solution NMR spectrum of the lubricants, they must first be separated from the condom itself. To minimize the chances of contamination and alteration of the sample, the choice of solvent was important and determined by a number of factors. It was necessary that the solvent be: (a) sufficiently volatile, to allow rapid and complete evaporation from the sample; (b) nonpolar, so that the components analyzed following extraction would not be those that would be washed off in the instance that the condom comes into contact with water or absorbed in the vaginal vault; and (c) inexpensive and easily available. Preliminary experiments showed hexane to fill the above criteria. For the measurement of ^1H NMR, the solubility of the extract and the number and location of solvent peaks in the resulting spectra were taken into account. Deuterated chloroform, $CDCl_3$, was shown to be the ideal solvent. ^1H NMR spectra of the soluble organic components of the condoms revealed that in addition to the lubricant base and spermicide, there are numerous minor components pre-

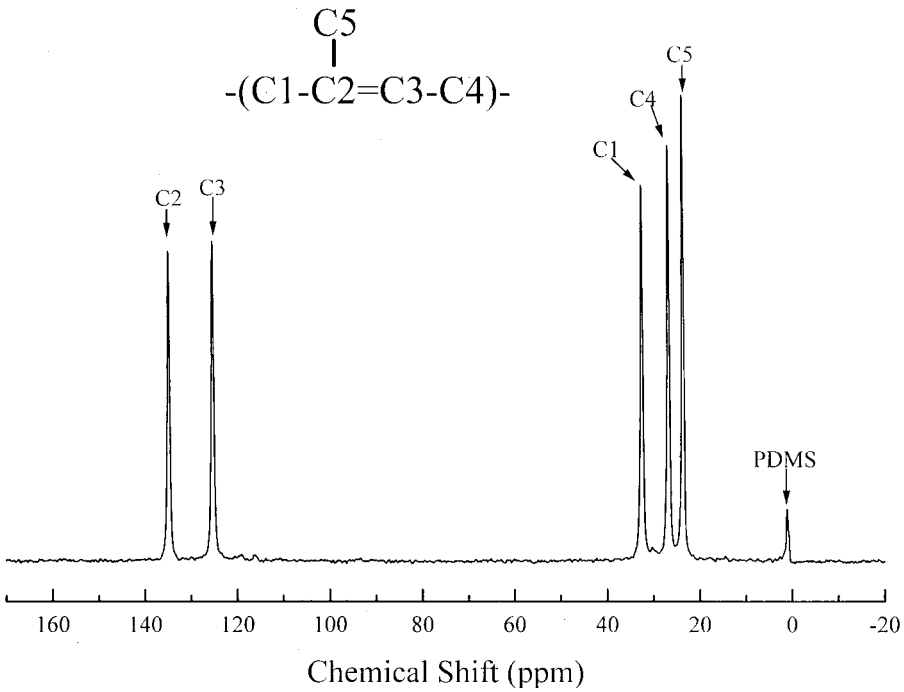

FIG. 2—*^{13}C solid state NMR spectrum of a Lifestyle Flared latex condom. Insert: Carbon backbone and numbering of latex, cis-1,4-polyisoprene.*

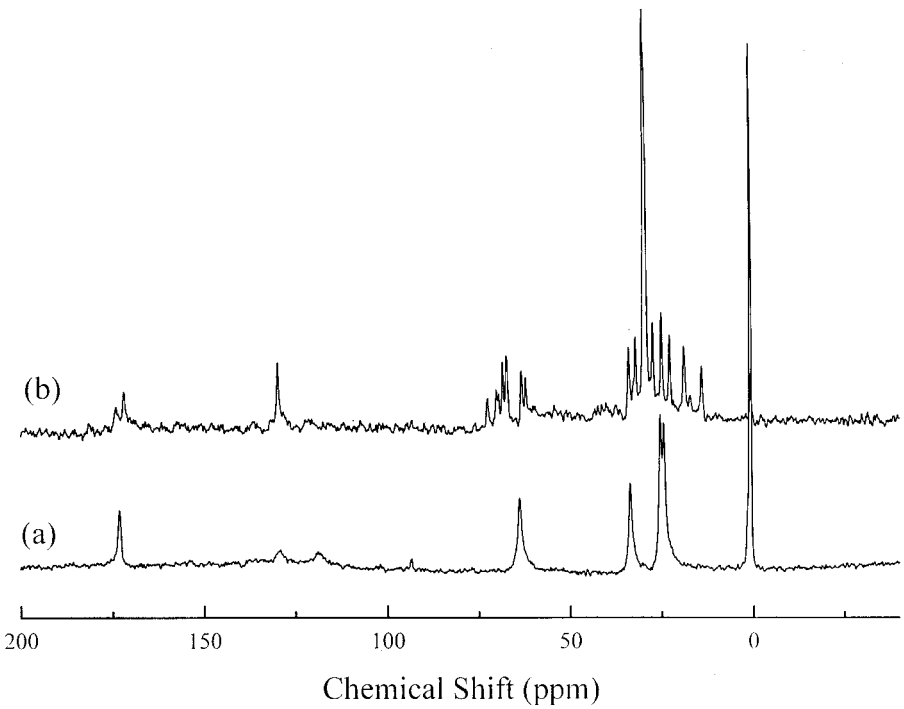

FIG. 3—*^{13}C solid state MAS spectrum of* (a) *Durex Avanti condom made of polyurethane and,* (b) *Trojan Naturalamb, made from sheep caecum.*

sent. A typical spectrum is shown in Fig. 4. Moreover, it was found that most of the soluble organic components extracted from the condoms afforded different ^{1}H NMR spectra although the majority of peaks were common to all samples. Thus the condom extracts could be separated merely by comparisons of their ^{1}H NMR spectra. As ^{1}H NMR spectra of mixtures of this kind are generally very complex (for example see Fig. 4), a flow chart (Fig. 5) was developed to support the technique and allow the information to be used by a nonspectroscopist. The flow chart was developed by systematically analyzing each spectrum for the presence and/or absence of particular peaks and patterns. Table 2 provides a summary of the flow chart.

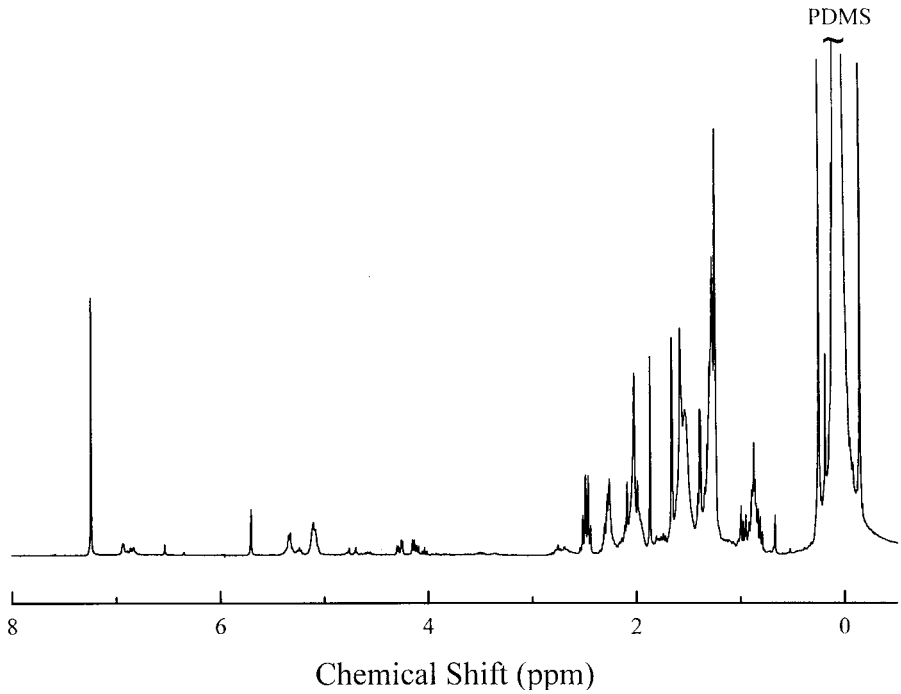

FIG. 4—*The ^{1}H solution NMR spectrum of a hexane extracted condom. (Lifestyle Studded).*

The bulk of the discussion will take the reader stepwise through the flow chart (Fig. 5). This chart should be consulted when reading the text. It should be noted that certain classes may have more than one individual feature, but those chosen for the purpose of analysis are those that are the most easily distinguished and least likely to cause indecision over what constitutes a positive match.

Step 1: Determination of Polymer Backbone—This step is based upon the results of the solid state experiments, as it is the simplest way to identify the nonlatex condoms. From this step, the Durex Avanti and Trojan Naturalamb condoms can be identified. A striking feature of the ^{1}H spectra of these two samples is their simplicity when compared to spectra obtained from latex condoms.

Step 2: Determination of Lubricant Base—Interestingly, our analysis shows that all the condoms, even the ones marketed as silicon-free (PEG based lubricants) or nonlubricated, contained PDMS. Therefore the PDMS peak (0.1 ppm) could not be used for initial identification purposes. The only nonlubricated condom, Chekmate Non-Lubricated, could be distinguished from all the others by a number of sharp resonances in the region 6.8 to 7.3 ppm as shown in Fig. 6 (Path 2.1, Fig. 5). This is the only condom that gave rise to this unique pattern of resonances. Furthermore, there are also peaks individual to this condom in the region from 4.0 to 4.2 ppm. As the rest of the condoms were found to contain varying amounts of PDMS in their lubricant formulation, the distinction between PEG and PDMS based lubricants required the identification of the PEG peaks first. The presence of resonances at 3.2 to 3.8 ppm (Fig. 7) corresponds to the lubricant PEG and results in the analysis traveling down Path 2.2. The PEG resonance is usually the most intense peak in the spectrum. It is composed of three sets of three peaks. The middle set of signals is the strongest and is centered at 3.6 ppm. The other two sets of peaks are located either side of the 3.6 ppm peak at approximately 3.4 and 3.7 ppm. There is also a peak at 3.1 ppm. The condoms which have the PEG peak in the ^{1}H NMR spectra of their extracted lubricants are Lifestyle Super, Saturn Silicon-free, and Saturn Nonoxynol-9. Further differentiation of these condoms can be achieved by the presence of a peak at 1.32 ppm (Path 2.2.1). This peak is unique to Saturn condoms, thus distinguishing them from the Lifestyle Super condom. The remaining two condoms in this pathway are both from Saturn. They can be individualized by the nonoxynol-9 peak at 4.1 ppm, which is present in the Saturn Nonoxynol-9 condom and absent in the Saturn Silicon-Free condom (Step 3a). All other condoms contain a PDMS (0.1 ppm, see Fig. 4) lubricant base and thus follow Path 2.3 onto Step 3.

The detection of sizeable amounts of PDMS in PEG based lubricant and condoms that were supposedly nonlubricated poses some question over the validity of published techniques that detect the presence of traces of PDMS. That is, the presence of PDMS may not necessarily mean a PDMS-lubricated condom was used. Thus caution must be exercised to ensure that the results are not misinterpreted.

Step 3: Determination of Spermicide—Step 3 separates the remaining condoms into two categories depending on the presence or absence of the spermicide, nonoxynol-9. This compound gives rise to peaks at 1 to 2 ppm (aliphatic protons), 3.63 ppm (other glycol monomer units), 3.70 ppm (H_7), 3.83 ppm (H_6), 4.10 ppm (H_5), 6.81 ppm (H_3 and H_4), and 7.15 ppm (H_1 and H_2). The proton numbering refers to Fig. 8. Figure 8 shows the ^{1}H NMR spectrum of the organic extracts from a Liaison Liberte condom. The peaks due to nonoxynol-9 are indicated with an arrow.

In some samples, particularly those with a PEG based lubricant, some of the nonoxynol-9 peaks may be partially obscured. However the presence of nonoxynol-9 can be determined by the triplet at 4.10 ppm (Fig. 8, insert). This resonance, despite the fact that it overlaps other less intense peaks, is located in an area where dif-

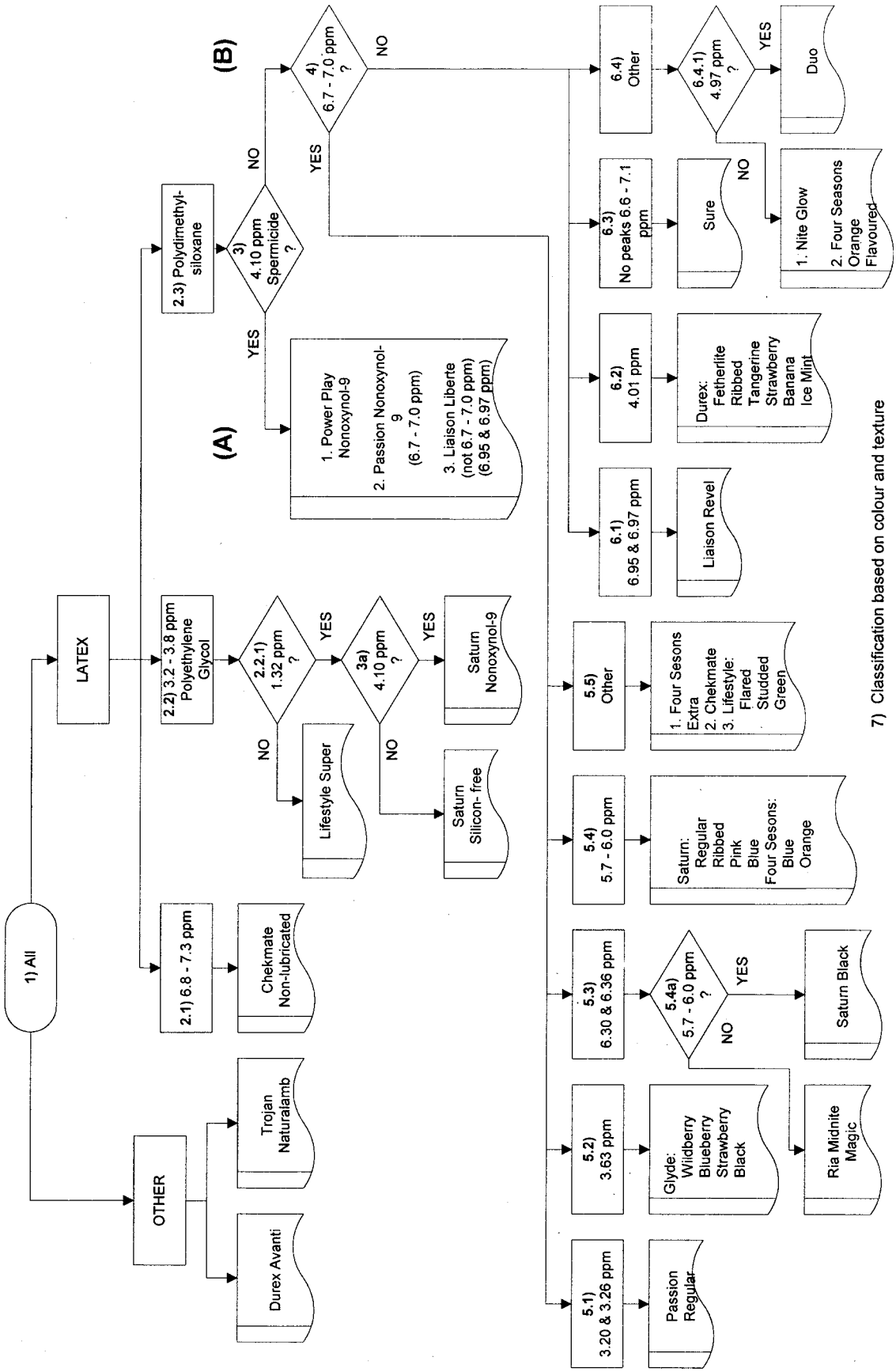

FIG. 5—*Flow chart for the differentiation of condoms using NMR spectroscopy.*

TABLE 2—*Summary of results.*

CONDOM	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6	STEP 7	INDIVIDUALIZED
Durex Avanti	✓							✓
Trojan Naturalamb	✓							✓
Chekmate Non-lubricated		6.8 - 7.3						✓
Lifestyle Super		3.2 - 3.8						✓
Saturn Silicon-free		3.2 - 3.8 1.32						✓
Saturn Nonoxynol-9		3.2 - 3.8 1.32	4.10					✓
Powerplay Nonoxynol-9			4.10					✓
Passion Nonoxynol-9			4.10	✓				✓
Liaison Liberte			4.1			6.95 & 6.97		✓
Passion Regular				✓	3.2 & 3.26			
Glyde:				✓	3.65		Purple	✓
Wildberry								
Blueberry				✓	3.65		Blue	✓
Strawberry				✓	3.65		Pink	✓
Black				✓	3.65		Black	✓
Ria Midnite Magic				✓	6.3 & 6.36			✓
Saturn Black				✓	6.3- & 6.36 5.7 - 6.0			✓
Regular				✓	5.7 - 6.0		Plain	✓
Ribbed				✓	5.7 - 6.0		Ribbed	✓
Pink				✓	5.7 - 6.0		Pink	✓
Blue				✓	5.7 - 6.0			
Four Seasons Orange				✓	5.7 - 6.0		Orange	✓
Blue				✓	5.7 - 6.0			
Lifestyle Flared				✓				
Studded				✓				✓
Green				✓				✓
Chekmate				✓				
Four Seasons Extra				✓				
Liaison Revel						6.95 & 6.97		✓
Durex Fetherlite						4.0	Coral	✓
Ribbed						4.0	Ribbed	✓
Tangerine						4.0	Orange	✓
Strawberry						4.0	Red	✓
Banana						4.0	Yellow	✓
Ice Mint						4.0	Blue	✓
Sure						No peaks 6.6 - 7.1		✓
Duo						None, 4.97		✓
Night Glow							Glows	✓
Four Seasons Orange Flavoured								✓

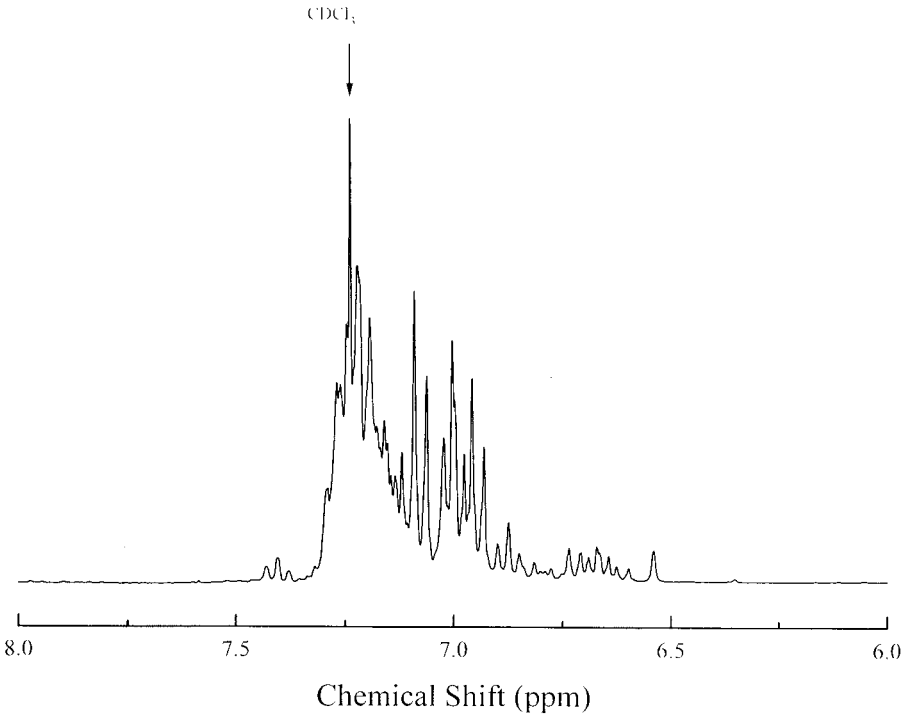

FIG. 6—*The distinguishing feature (6.8 to 7.3 ppm) of the Chekmate nonlubricated condom from Ansell.*

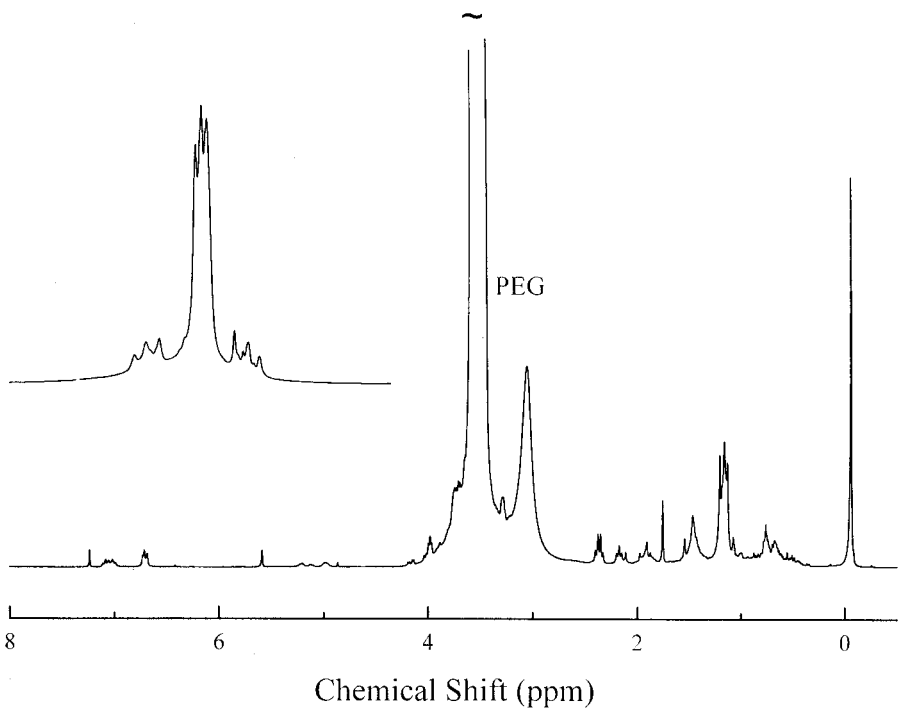

FIG. 7—*^1H NMR spectrum showing the distinguishing peak of polyethylene glycol. Insert: expanded region 3.4 to 3.85 ppm.*

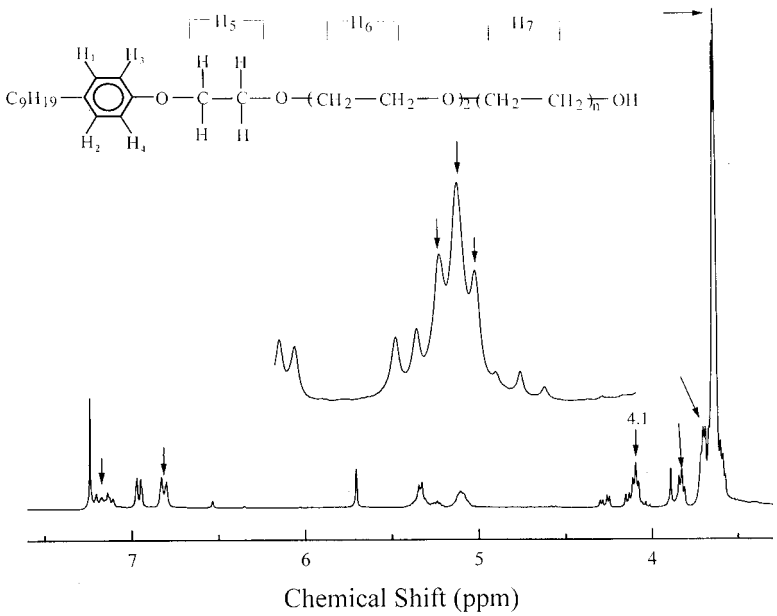

FIG. 8—*Section of a ^1H solution spectrum illustrating the peaks due to nonoxynol-9. (Liaison Liberte). The expanded section shows the 4.1 ppm tripet (marked with arrows). Insert: Chemical structure and proton numbering of nonoxynol-9.*

ferences are easily observed, and not likely to be misinterpreted. The condoms found to contain nonoxynol-9 by ^1H NMR include Power play Nonoxynol-9, Passion Nonoxynol-9, Liaison Liberte, and as mentioned in Step 2, Path 2.2 Saturn Nonoxynol-9. The first three of these are classified in group A. The remaining twenty-nine samples are classified as group B. Both groups A and B are then subjected to further analysis for classification.

Step 4: Pattern of Peaks at 6.7–7.0 ppm—This particular group of resonances (Fig. 9) is present in a number of samples, and is possibly due to some form of aromatic compound. It was chosen as it is quite distinct and serves to divide the remaining samples in both groups (A and B) roughly in half (see Fig. 5). It is important in this step to observe the shape of the feature, and not solely the presence of a peak in this region. The three largest peaks that contribute to this feature are located at 6.84, 6.86, and 6.94 ppm.

Applying this step to group A, the Passion Nonoxynol-9 sample can be identified as it is the only condom in group A that has this feature. For the condoms still to be identified in group B, the analysis shall be approached as follows: all those samples that display this feature will go on to be analyzed in Step 5; those that do not possess this feature will be analyzed in Step 6. In these two steps there will be a number of options. A number of regions will be given that can be searched simultaneously as each spectrum will contain only one (or none) of the possibilities. The one exception is the Saturn Black condom. This is easily understood by observing the flow chart (Fig. 5).

Step 5: Further Classification 1, Miscellaneous Resonances—In Step 5, there are a number of peaks that can be used to further separate the 18 condoms, which contain the 6.7 to 7.0 ppm feature in their ^1H NMR spectra (Step 4). The Passion condom can be individualized (Path 5.1) by the appearance of two small peaks at 3.20 and 3.26 ppm (Fig. 10*a*). Glyde condoms (Path 5.2) were found to be the only brand classified from Step 4 to contain a singlet at 3.6 ppm (Fig. 10*b*). Thus the condoms which fall into this category are

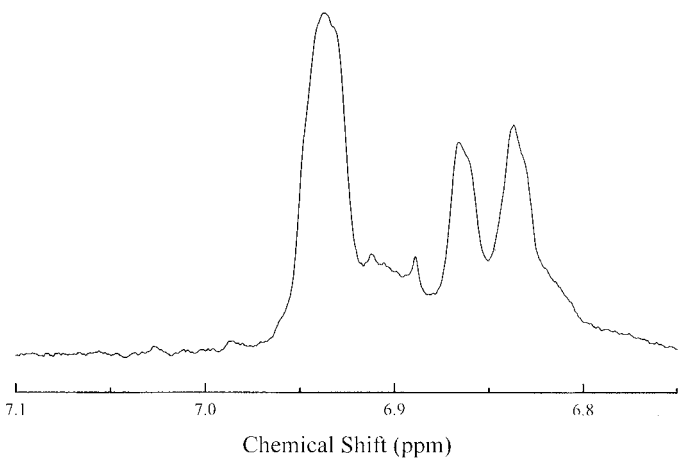

FIG. 9—*The distinguishing feature at 6.7 to 7.0 ppm at Step 4.*

Glyde: Wildberry, Blueberry, Strawberry, and Black. Path 5.3 separates the condoms whose organic component extracted ^1H NMR spectra contains two peaks at 6.30 and 6.36 ppm (Fig. 10*c*). There are two samples that exhibit both these peaks; the Midnite Magic chocolate condom manufactured by Ria and the Saturn Black condom. Note some samples contain the 6.36 ppm resonance but not the 6.30 ppm resonance. For a positive identification, both these peaks must be present. Furthermore, there are other features associated only with these two samples, most notably the sharp singlet at 3.78 ppm. These two condoms may be distinguished in the next step. Figure 10*d* shows the pattern of resonances (5.7 to 6.0 ppm) required for a positive match in Path 5.4. These peaks may be due to vinylic protons in an electron withdrawing environment. The most intense peaks are at 5.94 and 5.96 ppm. The peak marked with an X is not part of this feature as it is present in all 38 samples. There are a number of samples exhibiting this set of peaks, but only from two man-

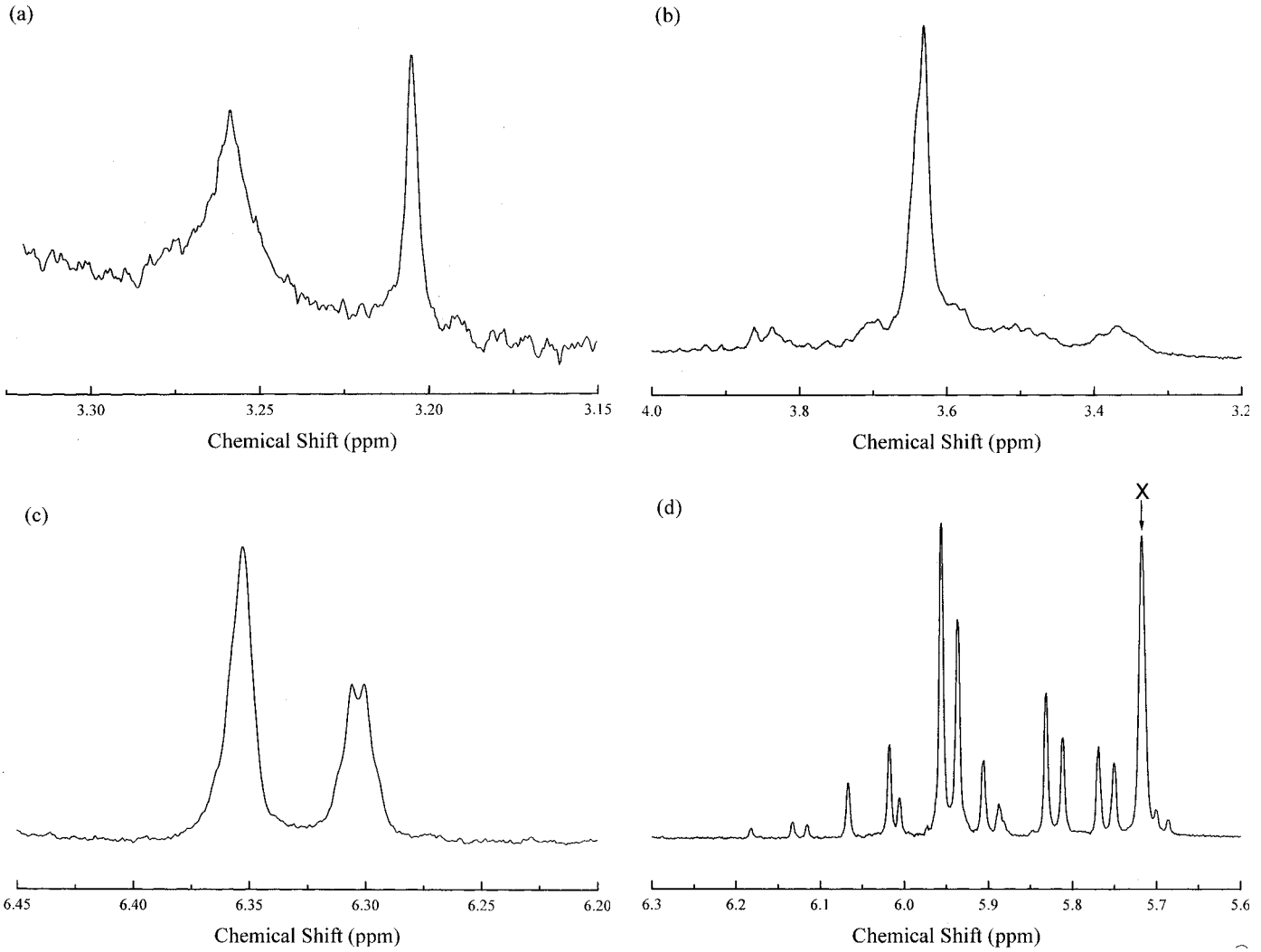

FIG. 10—*Distinguishing spectral features for* (a) *Path 5.1, Passion condoms,* (b) *Path 5.2, Glyde condoms,* (c) *Path 5.3,* (d) *Path 5.4. The peak marked with an X is not part of this feature.*

ufacturers: Four Seasons and Saturn. All Saturn samples are grouped here, namely Regular, Ribbed, Pink, Blue, and Black. However only the colored Four Seasons samples exhibit this feature (Blue and Orange). This is also the feature that allows discrimination between the Ria and Saturn Black condoms separated in Path 5.3. Care must be taken when identifying the Saturn Black condom, as peaks present in both black condoms interfere with this region.

This leaves five samples remaining that do not present any of the peaks mentioned thus far in Step 5. These are Four Seasons Extra, Chekmate, Lifestyle Flared, Lifestyle Studded, and Lifestyle Green. These samples cannot be further classified from differences in their ^{1}H NMR spectra. Note that the same manufacturer, Ansell, makes four of the five remaining condoms—Chekmate and the Lifestyle brands. Based on information from Ansell, all their condoms are produced with the same lubricant formula [5].

Step 6: Further Classification 2, Miscellaneous Resonances—Recall that the 11 samples to be analyzed in this step are those that did not possess the feature used in Step 4 (the three specified peaks at 6.84, 6.86 and 6.94 ppm). The first feature (Path 6.1) in this step is unique to the Liaison brand and occurs in the region between 6.9 to 7.0 ppm. Close examination of this region reveals that the doublet is in fact a doublet of doublets, the coupling constant being very small, centered at 6.95 and 6.97 ppm (Fig. 11*a.*) Two samples can be individualized by this feature. Liaison Revel is the only condom from the remaining 11 samples that gave a negative result from Step 4 to contain this feature in its ^{1}H NMR spectrum. This doublet is also present in Liaison Liberte (containing nonoxynol-9 and segregated in Step 3, group A). Identification of this second condom type allows the final spermicide-containing sample (Power play Nonoxynol-9) to be identified by process of elimination, as it is the only sample in the group that does not exhibit any of these further peaks.

Just as the Liaison condoms contained a unique feature, so do the Durex manufactured latex condoms. This is a single sharp resonance at 4.01 ppm (Path 6.2), Fig. 11*b.* The remaining six as yet unclassified Durex condoms thus fall into this category: Fetherlite, Ribbed, Tangerine, Strawberry, Banana, and Ice Mint. They all show clusters of peaks in the region 3.6 to 3.8 ppm, but this region cannot be used to further differentiate samples.

The Sure brand (Path 6.3) of condoms may be individualized from the remaining condoms by the absence of any resonances in

the region 6.6 to 7.1 ppm (Fig. 11*c*). Of the 38 condoms analyzed, this was the only sample, which afforded a ^{1}H NMR spectra devoid of any resonances in this region.

Three condoms are left after all the features mentioned in prior steps have been searched for, namely; Duo, Ria Nite-Glow, and Four Seasons Orange Flavoured. The Duo brand of condoms is the only one of these three samples that has a peak at 4.97 ppm (Fig. 11*d*), and so can be distinguished from the other two (Path 6.4.1). This peak is also present in all the samples from Saturn manufactured condoms that contain PEG and all Durex condoms. Hence care must be taken in ensuring that the flow chart is followed directly to avoid misinterpretation.

Step 7: Final Classification Based on Subjective Analysis—Using the combined techniques of solid state ^{13}C and solution ^{1}H NMR spectroscopy, condoms manufactured by nine of the twelve different manufacturers could be clearly differentiated. Of the 38 specific condom brands, 15 may be individualized. The remaining 23 have been separated into five groups. The bulk of the condoms in each group belong to the same brand, the only difference lies in the appearance and texture of the product. Thus, to further identify the individual condoms, a subjective method of classification based on color and texture is used.

Hence in the final identification of the condoms, from Path 5.2, the four Glyde samples may be classified by color, i.e., Wildberry, Purple; Blueberry, Blue; Strawberry, Pink; and Black, Black. From Path 5.4, the Saturn regular is the only noncolored, nontextured sample in this subgroup, and the Saturn Ribbed is the only textured sample. The Saturn Pink and Four Seasons Orange can be identified based on color. However, the Saturn Blue and Four Seasons Blue samples cannot be distinguished. It is obvious that problems will be encountered if these manufacturers produced more samples of corresponding colors, as this scheme would not be able to identify the brand provided they exhibit the same spectral characteristics as other condoms made by the particular manufacturer.

Of the condoms grouped from Path 5.5: The Lifestyle Studded and Lifestyle Green can be distinguished on the basis of texture or color. The Lifestyle Flared, Chekmate, and Four Seasons Extra can not be further classified. The six Durex condoms from Path 6.2 are

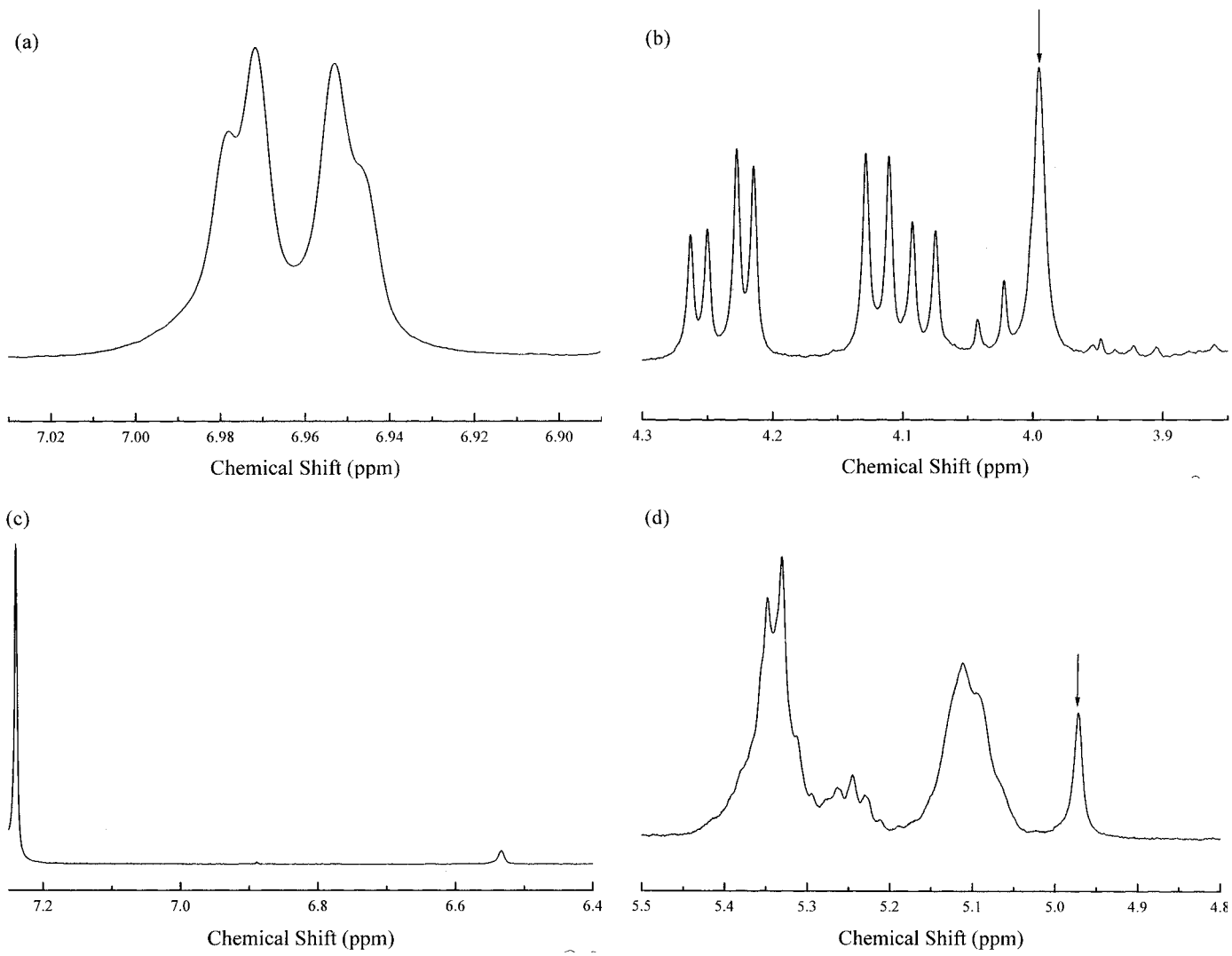

FIG. 11—*Distinguishing spectral features for* (a) *Path 6.1, Liaison condoms.* (b) *Path 6.2, Durex condoms, peak marked with an arrow* (c) *Path 6.3, Sure condoms* (d) *Path 6.4, Duo condoms, peak marked with an arrow.*

able to be identified based on color and texture: Durex Ribbed, Ribbed; Tangerine, Orange; Strawberry, Red; Banana, Yellow; Ice Mint, Blue; Fetherlite, "Coral" colored. Finally, the Nite Glow condom can be easily distinguished from the Four Seasons Orange Flavoured condom in that it glows in the dark.

Following classification using this system 33 of 38 condoms have been identified, with the remaining five in three separate groups. Table 2, a summary of the results, illustrates the peaks used to classify the condom in each step, and whether the condom could be individualized.

To ensure that the spectra were representative of the soluble organic components of the condoms, duplicate samples were performed. Some of the duplicates were from condoms of different batches while others were from the same batch. The only brand of condom to show a significant difference was the lubricated Chekmate condom manufactured by Ansell. A condom from a second batch was obtained and the soluble organic components extracted. The ^{1}H NMR spectrum of both Chekmate samples is shown in Fig. 12. The difference is a peak due to PEG. This is cause for some concern, as if this peak were to randomly appear it would mean that this sample would be grouped (following the flow chart) with those samples containing a PEG lubricant base. If this were the case, then distinction between the Chekmate and the Lifestyle Super samples will be based on intensities of the PEG peak, which is quite subjective.

Minor differences in the region of 3.6 to 3.9 ppm were also observed in the ^{1}H NMR spectrum of duplicate Saturn samples, but as this region was not used for discriminating samples, the differences do not lead to misidentification.

With this investigation, we have started to build up a database of ^{1}H NMR spectra of condoms. However to be forensically useful, the database must be continually updated, including periodical testing of batch samples, to account for changes in lubricant formulation by manufacturers, accidental cross contamination during the manufacturing process, and other new advances in the condom manufacturing industry.

Effect of Water on the Methodology

These experiments were performed to ascertain how the methodology would be affected if the condoms were subjected to mild environmental conditions. Possible scenarios include the condom being flushed down the toilet and subsequently recovered from the sewer trap or left open to the environment after a sexual assault. In both of these cases, the discarded condom, and thus the lubricant, will come into contact with water and may even be chemically altered. This is especially significant for the PEG based condoms as PEG is water soluble. Contamination with biological fluids was also considered in this work and will be published elsewhere. To gain the desired result, as much lubricant as possible was manually removed, and then the sample allowed to soak in tap water. Following this soaking procedure it was observed that many of the samples had turned brown in patches. The hexane extracts from these samples were also a darker yellow-brown color than those obtained from fresh samples.

The only significant difference in the ^{1}H NMR spectra of these samples and those obtained using fresh condoms was that the PEG peak was much lower in intensity appearing only as a small, broad lump. Figure 13 shows representative ^{1}H NMR spectra of a sample obtained from a fresh condom and a sample obtained from one soaked in water for 24 h before the organics were extracted. In samples containing both nonoxynol-9 and PEG, the spermicide peaks also disappeared. This was not the case for those condoms that contained spermicide in conjunction with a PDMS based lubricant. A possible reason for this is that the hydrophilic glycol chain of the spermicide causes it to be soluble in the PEG, which is in turn water soluble. In samples containing PDMS the hydrophobic aliphatic chain causes the spermicide to stay dissolved in the highly insoluble PDMS, and so is not removed after soaking.

These changes in the spectra slightly limit the number of condoms that can be identified. Only twenty-nine samples (compared to thirty-three previously) can be identified. It may still be possible to use the PEG peak to classify these samples if it can be detected, however if the signal to noise is low some confusion with other peaks in this region may occur. Without the PEG peak the Lifestyle Super sample will be indistinguishable from the Four

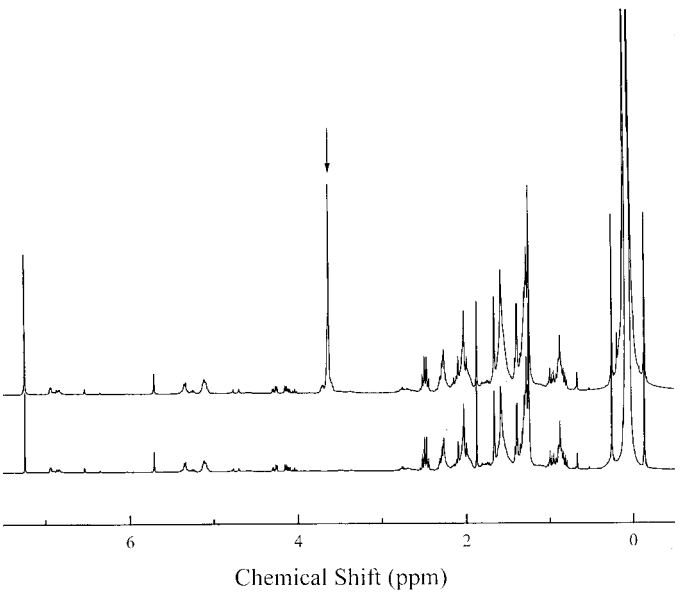

FIG. 12—*Comparison of the ^{1}H NMR spectra of two Chekmate samples. The top trace contains PEG (indicated by an arrow).*

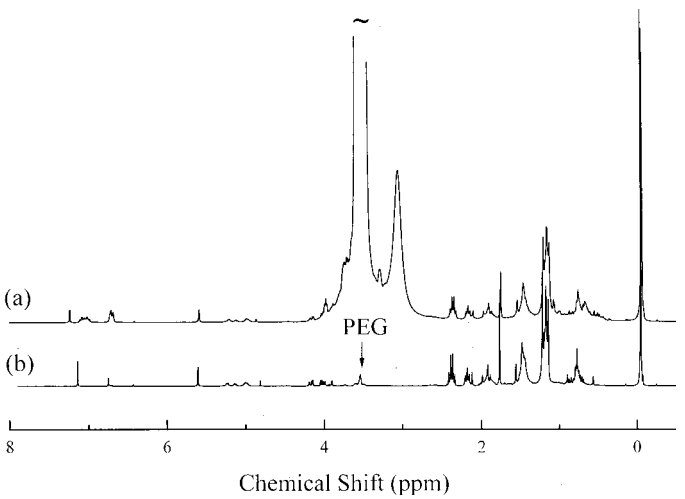

FIG. 13—*Comparison of ^{1}H NMR spectra of the hexane soluble organic extracts of Saturn Nonoxynol-9 condoms.* a) *new,* b) *after being wiped and soaked in water for 24 h. The PEG peak in spectrum* (b) *is marked with an arrow.*

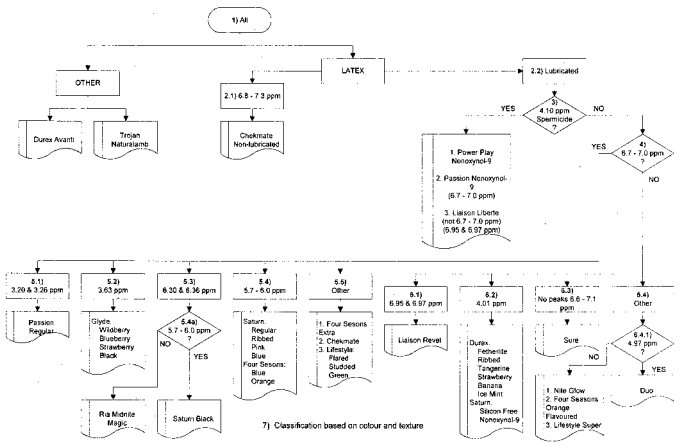

FIG. 14—*Revised flow chart for the differentiation of condoms, after treatment with water, using NMR spectroscopy.*

Seasons Orange Flavoured condom in Step 6.4. Following the dramatic loss of intensity of the PEG peak the two Saturn samples, Silicon-free and Nonoxynol-9, are now revealed to contain the same distinguishing peak as the Durex samples (the peak at 4.01 ppm). These Saturn samples cannot be differentiated from the Durex brand condoms by the presence of the peak at 1.32 ppm as this peak is also present in the Durex samples. The Saturn condoms may be differentiated from the Durex samples by color as the Saturn samples are the only two that are not colored. These two Saturn condoms cannot be differentiated from each other unless some spermicide is also detected. If this were the case it must be ensured that, to obtain a correct identification, all steps after classification of those samples with spermicide are applied to this subgroup.

Taking into account these new considerations the flow chart must be revised. This is shown in Fig. 14. The only difference in the procedure is that Step 2.2 is no longer required, as the presence of PEG is no longer a distinguishing feature.

Conclusions

NMR has been shown to be a method capable of distinguishing between sexual lubricants used in condoms by different manufacturers. Based on the ^{1}H solution NMR spectra of the extractable organic components of the lubricant, we have been able to differentiate nine of twelve brands of condoms. Of the three brands that cannot be identified outright, the Saturn and Ansell condoms can be distinguished from each other. However they cannot be distinguished from condoms manufactured by Four Seasons. Individually, 15 of the 38 condoms can be identified. The 23 condoms which cannot be individualized are manufactured by Glyde, Durex, Saturn, Ansell, and Four Seasons and are just variants of the normal condoms, i.e. they are either colored, flavored or contain an extra feature (studded, ribbed, etc.). Thus they can be separated visually. In this way, 33 of the 38 condoms could be individualized. A flow chart was devised so that the method could be easily implemented in any forensic laboratory. A slightly lower success rate was obtained when the lubricants were removed and the condom soaked in water. Eight of the twelve brands could be identified and 29 of the 38 condoms individualized. This is due mainly to the solubility of PEG. ^{13}C solid state MAS NMR yielded insufficient data for forensic analysis. However, it was found to be a quick and simple technique for the identification of the polymer backbone and lubricant base of condoms without any pretreatment of the sample. Furthermore, analysis may be carried out on any routine NMR spectrometer as it was found that the same information may be obtained by running solids experiments under solution conditions. A database of ^{1}H solution NMR spectra of condoms has been established for the samples analyzed. This database must be continually updated to be forensically useful.

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