J Forensic Sci, September 2003, Vol. 48, No. 5
Paper ID JFS2003084_485
Available online at: www.astm.org

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Characterization of Condom Lubricant Components Using Raman Spectroscopy and Raman Chemical Imaging*

ABSTRACT: A condom can be described as a protective sheath used as a contraceptive or to protect against sexually transmitted diseases. However, individuals also use condoms during the commission of sexual assaults to prevent identification through deposited biological material. Raman spectroscopy offers a novel approach to identifying the presence of condom lubricant components. Furthermore, Raman chemical imaging expands on conventional Raman spectroscopy to characterize multiple condom lubricant components simultaneously in a manner that effectively demonstrates heterogeneous sample mixtures both spectrally and spatially. Known reference materials, liquid and solid lubricant components of common condom brands were successfully characterized using Raman dispersive spectroscopy and Raman chemical imaging without extensive sample preparation inherent to other analytical methods. The characterization of these materials demonstrates the potential of this technique to become a routine screening method for condom lubricants. This preliminary investigation provides a basis for future studies to determine the feasibility of Raman spectroscopy and Raman chemical imaging for condom lubricant trace detection in case type samples.

KEYWORDS: forensic science, criminalistics, condom lubricant, Raman, chemical imaging

Although the use of a condom prevents exploitation of biological evidence, important evidence pertaining to sexual assault cases can be provided by the identification of condom lubricant components. Presence or absence of these materials can be used to corroborate the scenario of a victim or that of the accused while providing associative evidence (1–2).

Commercially available condoms are primarily manufactured of latex and to a lesser degree of polyurethane and sheep ceacum. All three types can contain fine powders, lubricants, and/or spermicides in various combinations. Lubricants are generally divided into two categories. Wet lubricants are water based, commonly polyethylene glycol (PEG) or propylene glycol (PG). Dry lubricants are typically silicone oils, the most common being polydimethylsiloxane (PDMS). Nonoxynol-9 (N9) is by far the most widely used spermicide. It can be found in conjunction with both wet and dry lubricants and can compose 5–15% of the lubricant mixture (2). Condoms may also contain fine particulate components that are used to prevent the sheath from sticking to itself when unrolled. These may include talc, cornstarch (or other starches), $CaCO_3$, powdered silica, $MgCO_3$, or lycopodium.

The recent accessibility of Raman instrumentation to the forensic scientist may provide an alternative method of characterizing condom lubricant traces through the use of Raman spectroscopy and Raman chemical imaging. Chemical imaging possesses the ability to identify condom lubricant components in the presence of one another, providing they possess unique Raman spectra. Raman analysis requires less sample preparation than is needed with current methods, namely Fourier transform-infrared (FTIR) analysis. This study concentrates on applying Raman spectroscopy and Raman chemical imaging to commercially available samples and demonstrating the use of Raman analysis as a primary characterization technique.

Current Methods

A number of methods including FTIR, high performance liquid chromatography (HPLC), gel permeation chromatography (GPC), matrix assisted laser desorption/ionization mass spectrometry (MALDI/MS), and desorption chemical ionization mass spectrometry (DCI/MS) have been explored for lubricant, spermicide, and particulate detection, each having inherent advantages and disadvantages. FTIR analysis is the standard method for routine screening of swabs recovered following sexual assault with respect to PDMS identification (1,2). The presence of PEG and PG from vaginal swabs can also be determined using FTIR. However, it is often not sensitive enough because PEG and PG are readily absorbed in the vaginal mucosa, making their persistence much less than PDMS. HPLC (2) and GPC (2) have been used as alternative methods to FTIR when greater sensitivity is required for PEG or PG analysis. FTIR alone is not specific enough for N9 identification since many common detergent products have similar infrared spectra. MALDI/MS (3) and DCI/MS (1) are established tests for N9 identification. The major disadvantages of these methods are the instrumentation cost and lack of accessibility to the forensic scientist. Most recently, nuclear magnetic resonance (NMR) has been used to successfully detect condom lubricant traces (6,7).

Raman Spectroscopy

In Raman spectroscopy, energy levels of molecules and solids are probed by monitoring the frequency shifts present in scattered

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* Presented at the 55th AAFS annual meeting; February 20, 2003; Chicago, IL.

Received 8 Mar. 2003; and in revised form 26 April 2003; accepted 26 April 2003; published 4 Aug. 2003.

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light. A typical experiment consists of a laser light source that is directed at a sample resulting in several phenomena including Raman scattering. The scattered light is monitored using instrumentation comprised primarily of a spectrometer and a charge-coupled device (CCD). Similar to an infrared spectrum, a Raman spectrum reveals the molecular composition of materials including the specific functional groups present in organic and inorganic molecules. Raman spectroscopy is useful because each resonance exhibits a characteristic "fingerprint" spectrum, subject to various selection rules. Raman spectroscopy has been well documented in the examination of synthetic fibers, explosives, narcotics, paints and pigments and other areas of forensic science (8–15). Unlike infrared spectroscopy, Raman systems can be used to analyze aqueous phase materials and requires little or no sample preparation.

In the past several years, a number of key technologies have been introduced into wide use that has enabled the utility and growth of Raman spectroscopy. These technologies include high efficiency solid state lasers (16), holographic notch rejection filters (17,18), and silicon CCD detectors (19).

Raman Chemical Imaging

Raman chemical imaging (RCI) combines microscopy, digital imaging and Raman spectroscopy to elicit molecular based information from materials. By combining these methods, comparative and qualitative/quantitative chemical information not attainable by simple Raman microprobe analysis is added.

RCI methods utilize efficient electro-optic imaging spectrometers (20–23). Spectra are generated corresponding to hundreds of thousands of unique spatial locations at the sample surface by tuning the imaging spectrometer over a range of wavelengths and collecting images sequentially. A finite optical band pass of light is transmitted through the spectrometer and the reflected, emitted, or scattered light is recorded for each image pixel over a designated spectral range. Figure 1 shows the typical data structure that is collected in a chemical imaging experiment, in which images represent an X, Y spatial arrangement of absorption, visible reflectance, fluorescence or Raman scatter. Contrast is generated in the images based on the relative amounts of Raman scatter or other optical phenomena created by the different species located throughout the sample. Since a spectrum is generated for each image pixel, chemometric analysis tools such as principle component analysis (PCA) (24,25), multivariate curve resolution (MCR) (26,27), or cosine correlation analysis (CCA) (28) can be applied to the image data to extract pertinent information otherwise missed by ordinary univariate measures. RCI combines the chemical/structural analysis capabilities of Raman spectroscopy with the visualization power of an optical microscope. RCI provides a potential solution for obtaining image information about chemical composition, structural quality and uniformity.

Materials and Methods

A FALCON™ Raman Chemical Imaging System (ChemImage Corporation, Pittsburgh, PA) equipped with 532 nm laser excitation and a 100 W quartz tungsten halogen (QTH) broad band source was used to collect brightfield microscopic images, Raman chemical images and dispersive Raman spectra. ChemAcquire 6.0 (ChemImage Corporation) and ChemAnalyze 6.0 (ChemImage Corporation) software packages were used for data acquisition, analysis, and visualization. RCI data were acquired by tuning the electro-optic imaging spectrometer over the fingerprint and/or CH stretching regions of the Raman spectrum to generate contrast based on spectral differences.

Small amounts of components commonly present in condom lubricants were provided by the Naval Criminal Investigative Service (NCIS) forensic laboratory and placed onto aluminum-coated glass microscope slides for analysis. Pure component

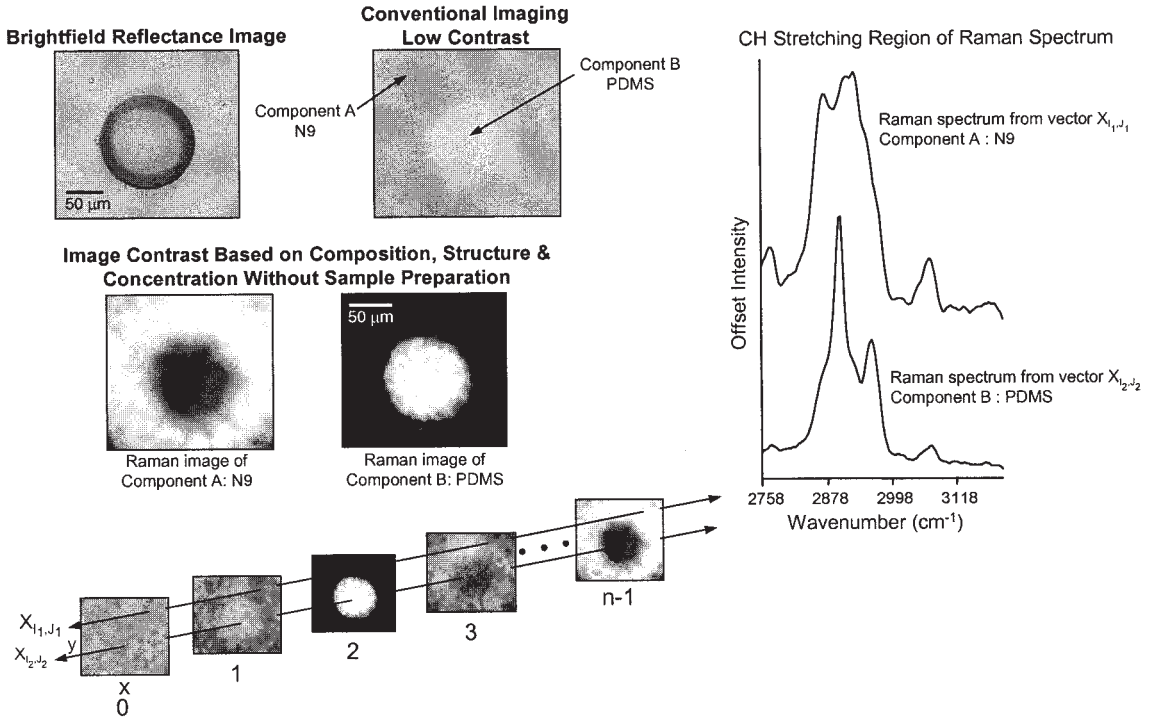

FIG. 1—*Raman chemical imaging concept (PDMS/N9 reference standard mixture).*

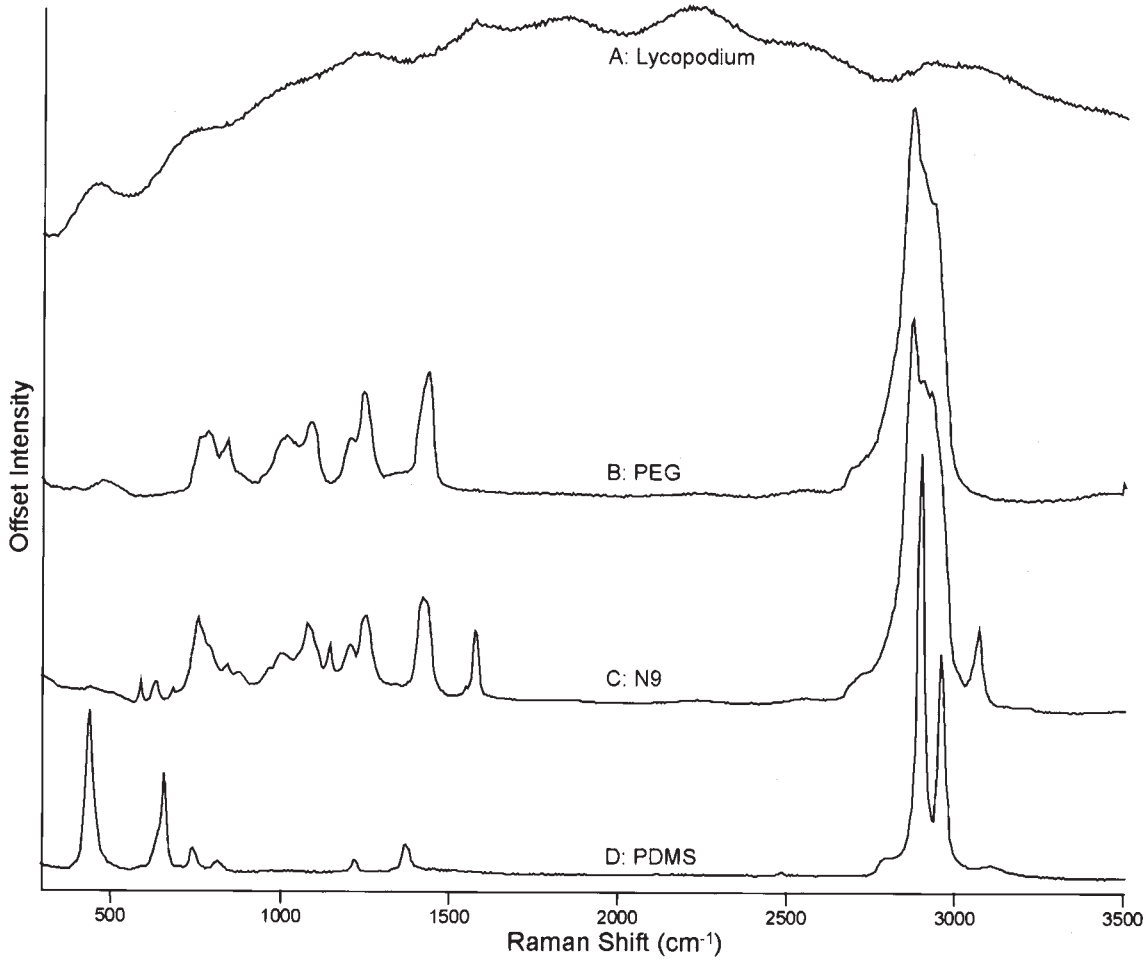

FIG. 2—*Dispersive Raman spectra of reference samples;* (A) *20× magnification, 2.5 s acquisition, 333 W/cm^2 (laser power density at sample);* (B) *50× magnification, 25 s acquisition 2065 W/cm^2;* (C) *10× magnification, 30 s acquisition, 86 W/cm^2;* (D) *50× magnification, 17 s acquisition, 2065 W/cm^2.*

dispersive spectra were acquired of lycopodium spores, polyethylene glycol, nonoxynol-9, and PDMS (Fig. 2). Trojan® Ultra Thin Spermicidal Lubricant (Carter Wallace, Inc., Carter Products Division) and Plus Beyond Seven® (Okamoto Industries, Inc.) condoms were analyzed in this study. The Trojan® condom is described on its packaging as a lubricated latex condom containing the spermicide N9. The Beyond Seven® condom is described as a natural rubber latex Sheerlon® condom with spermicidal-N9 lubricant.

Sterile cotton swabs were rolled on the interior portions of the commercially available condoms, and small amounts of raw material were spread onto aluminum coated microscope slides for analysis. Dichloromethane and water extracts were also performed on the Trojan condom sample according to established methods (2). Portions of the extracts were placed on aluminum coated microscope slides and allowed to evaporate for analysis.

Results and Discussion

Most of the pure components were Raman accessible. Characteristic bands for PDMS can be seen at 487 cm^{-1}, 707 cm^{-1}, 2903 cm^{-1}, and 2965 cm^{-1}, and for polyethylene glycol at 831 cm^{-1}, 1129 cm^{-1}, 1282 cm^{-1}, 1472 cm^{-1}, and 2876 cm^{-1}. N9 has identifying peaks at 801 cm^{-1}, 1121 cm^{-1}, 1287 cm^{-1}, 1455 cm^{-1}, 1609 cm^{-1}, 2876 cm^{-1}, and 3071 cm^{-1}. The pure component spectra reveal that chemical imaging experiments used to distinguish these three components may be performed over the CH stretching region (approx. 2750–3150 cm^{-1}) of the Raman spectrum since each pure component exhibits a unique spectral profile in this region. Lycopodium was found to be extremely fluorescent. However, the extreme fluorescence of the spores taken into account with their unique surface morphology aid in characterization. Under optical inspection, both PDMS and N9 appear transparent, while the N9 standard contained bubbles that appear as dark spheres in the liquid. Spectra between the liquid and bubbles differed only in intensity.

A mixture of the PDMS and N9 standards was analyzed in an effort to differentiate spermicide and lubricant in the presence of one another. Small amounts of PDMS and N9 were mixed on a microscope slide. Emulsions resembling the bubbles in N9 were apparent in the brightfield reflectance image (Fig. 1, top left). In this case, however, the spectrum over the emulsion was consistent with PDMS (Fig. 3*A*) and the spectrum taken in the surrounding area was consistent with N9 (Fig. 3*B*). The emulsions occurred due to the immiscibility of the two components. Raman chemical imaging was performed over one of the emulsions to investigate if imaging could differentiate the components without first having to perform an extraction. The PDMS emulsion as well as the surrounding N9 were both identified as shown in the Raman images in Fig. 1 (middle left) and their corresponding spectra (right). Bright (white) regions of the images correspond to a heightened Raman intensity at

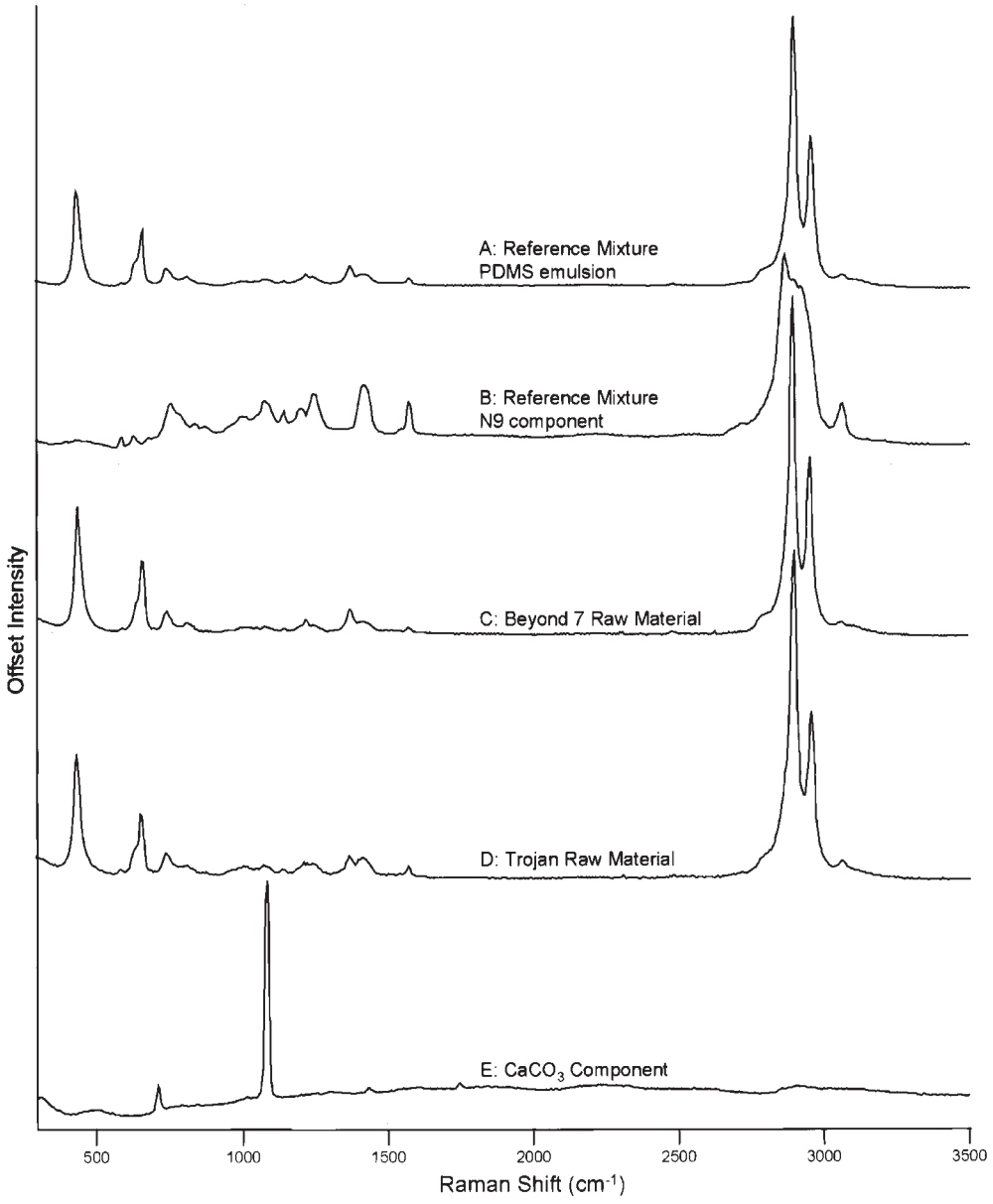

FIG. 3—*Dispersive Raman spectra of reference mixture and condom samples;* (A) *50× magnification, 10 s acquisition, 1033 W/cm^2 (laser power density at sample);* (B) *50× magnification, 10 s acquisition, 1033 W/cm^2;* (C) *20× magnification, 30 s acquisition, 184 W/cm^2;* (D) *20× magnification, 14 s acquisition, 335 W/cm^2;* (E) *100× magnification, 15 s acquisition, 4584 W/cm^2.*

that particular wavelength. The N9 extract was taken at 2930 cm^{-1} or the CH stretching peak maximum for N9. Similarly, the PDMS extract was taken at its peak maximum at 2900 cm^{-1}.

Raw material analysis for the Beyond Seven® condom showed emulsions in a transparent liquid similar to what was seen with the pure components (Figs. 4*A* and 4*B*). Weak N9 Raman bands can be seen in the dispersive spectrum but the majority of signal stems from PDMS (Fig. 3*C*). RCI was able to identify the emulsions as N9 and the surrounding material as PDMS as indicated by the imaging spectrometer spectra of the CH stretching region (Fig. 5). Figures 4*C* and 4*D* reveal the spatial location of PDMS and N9, respectively. This exemplifies how multiple lubricant components in a commercially available sample can be identified with Raman chemical imaging without prior extraction of the sample to isolate the components.

Raw material analysis from the Trojan® condom shows predominantly PDMS (Fig. 3*D*). In addition, weaker Raman bands indicative of N9 were also present. This contradicts previously published information concerning the absence of this combination in Trojan brand condoms (29). Under optical inspection the sample contains what appear to be particulate domains (Fig. 6*A*). RCI indicates a continuous layer of PDMS with domains of N9 dispersed in the sample (Figs. 6*B* and 6*C*). Raman chemical imaging was performed over the CH stretching regions and N9-specific peaks in the fingerprint region. Only the N9 domains contain the fingerprint region peak and the CH region profiles are consistent with PDMS and N9 (Figs. 7*A* and 7*B*).

Analysis of the dichloromethane and water extracts indicates that Raman spectroscopy can also be utilized to characterize extracted materials from condom lubricants. Only PDMS was found in the filtered dichloromethane extract of the Trojan condom. Also, a 14 μm by 17 μm region of N9 was identified in the C_{18} cartridge extract. Particulate matter was also identified in the sample. Figures 8*A* and 8*B* show brightfield and polarized light images of a $CaCO_3$ particle. Its corresponding spectrum is seen in Fig. 3*E*. Polarized light microscopy was utilized to identify starch particles in both samples as seen in Figs. 9*B* and 9*D*.

Conclusions

This study indicates that some of the most common materials found in condom lubricants are very Raman accessible and can be accurately characterized by Raman spectroscopy. Furthermore, the analysis can extend to Raman chemical imaging eliminating time-consuming sample preparation and multi-instrument analysis.

Further studies will investigate the use of Raman spectroscopy and Raman chemical imaging for case type samples, evaluating environmental influences and detection limits. This preliminary investigation provides a baseline of information on which to build these future studies.

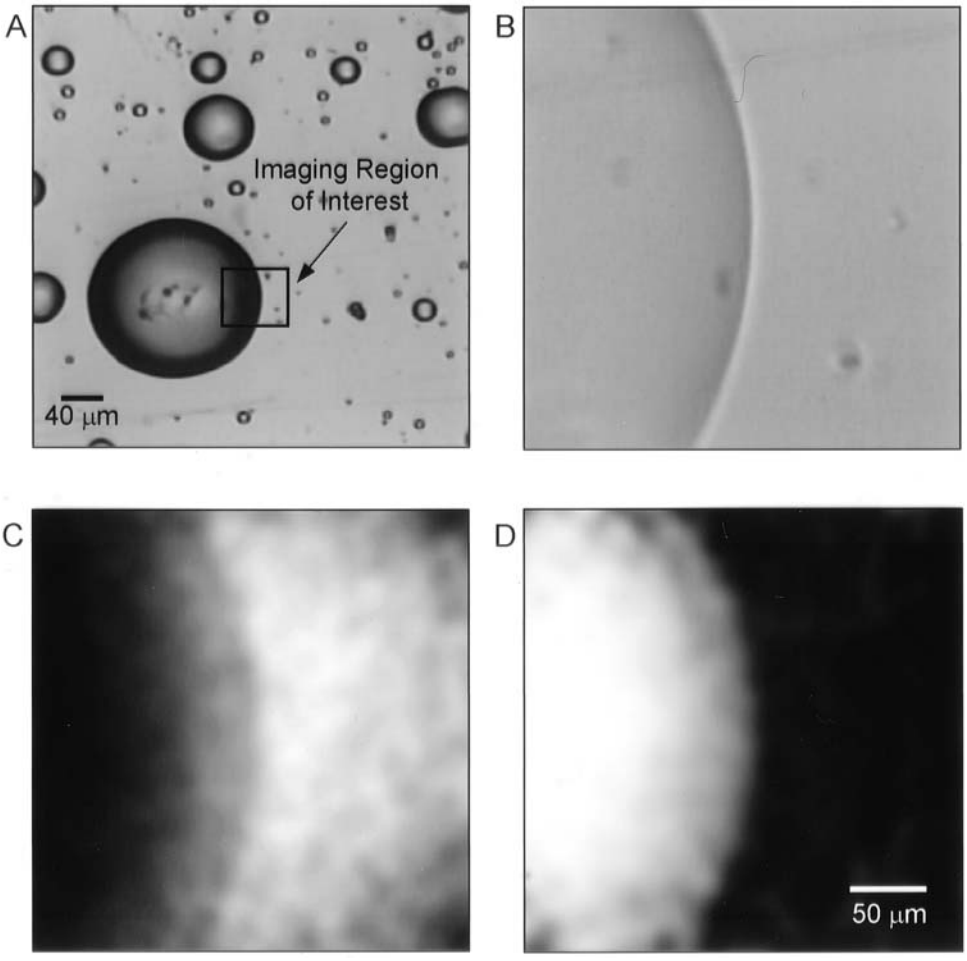

FIG. 4—*Raman chemical imaging of raw Beyond 7 condom lubricant;* (A) *Low magnification brightfield reflectance image;* (B) *High magnification brightfield reflectance image;* (C) *RCI extract at 2901 cm^{-1} of PDMS;* (D) *RCI extract at 2870 cm^{-1} of N9 (20× magnification, 184 W/cm^2 laser power density at sample, 10 s per frame, Imaging spectrometer tuning parameters: 2800–3150 cm^{-1}).*

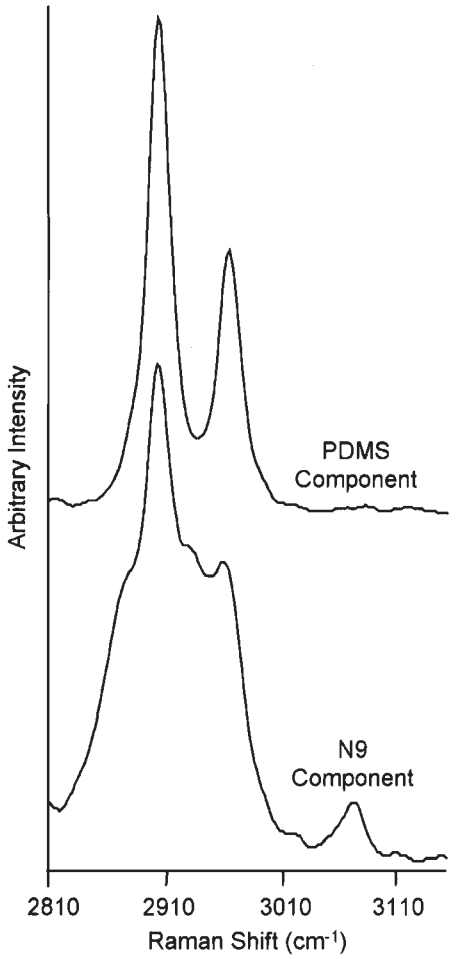

FIG. 5—*Imaging spectrometer generated Raman spectra (CH stretching region) associated with Beyond 7 condom lubricant components.*

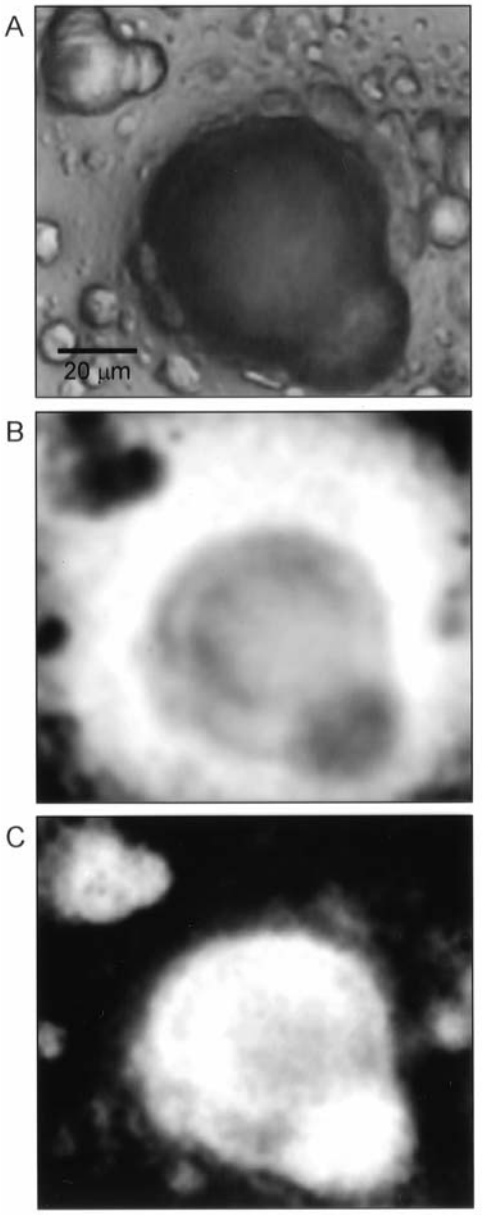

FIG. 6—*Raman chemical imaging of raw Trojan condom lubricant;* (A) *Brightfield reflectance image;* (B) *RCI CCA extract of PDMS;* (C) *RCI CCA extract of N9 (20× magnification, 335 W/cm^2 laser power density at sample, 15 s per frame, Imaging spectrometer tuning parameters: 1585–1620 cm^{-1} and 2800–3150 cm^{-1}).*

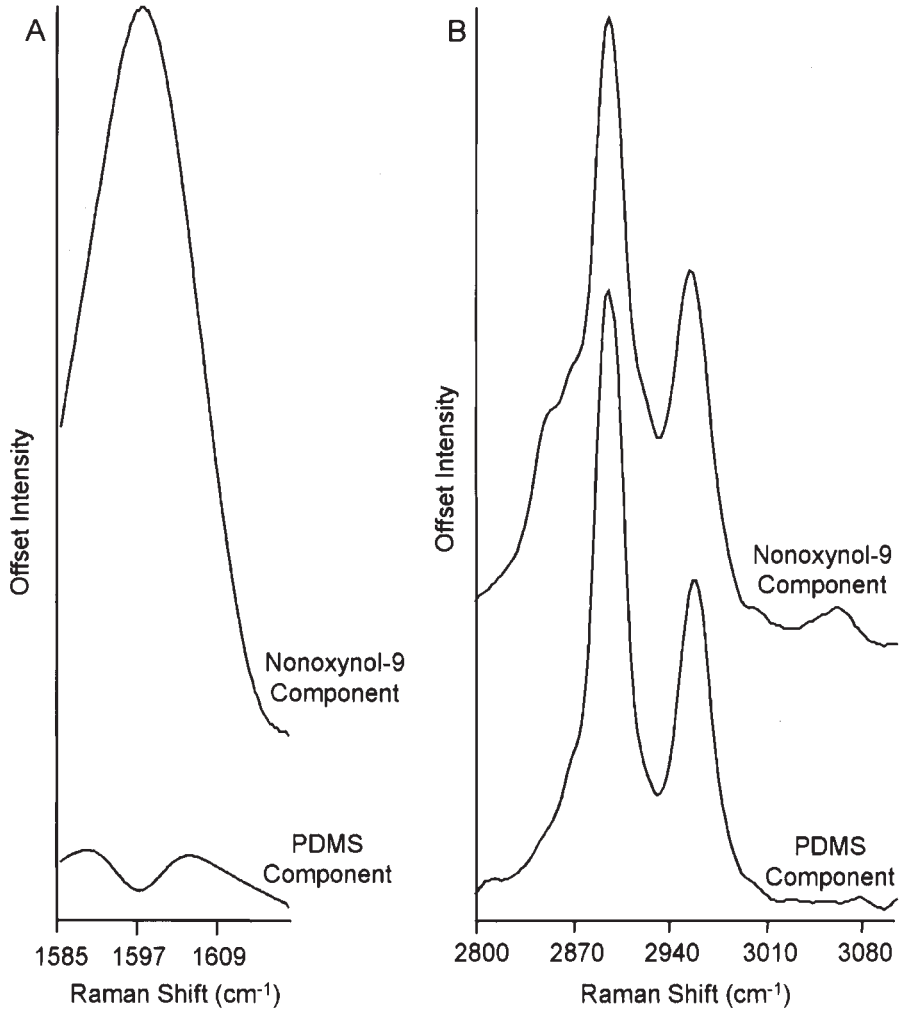

FIG. 7—*Imaging spectrometer generated Raman spectra associated with Trojan condom lubricant components;* (A) *Fingerprint region;* (B) *CH stretching region.*

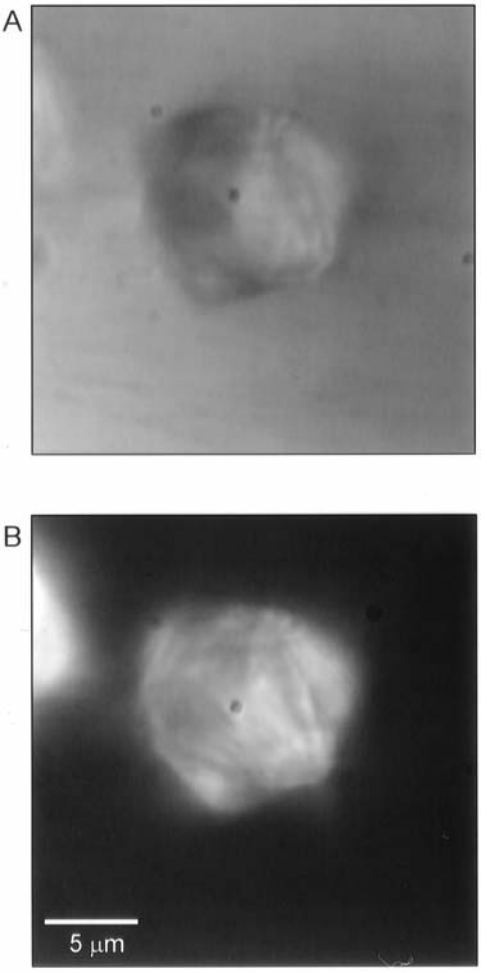

FIG. 8—*Brightfield images of $CaCO_3$;* (A) *brightfield reflectance image;* (B) *Polarized light image (100× magnification).*

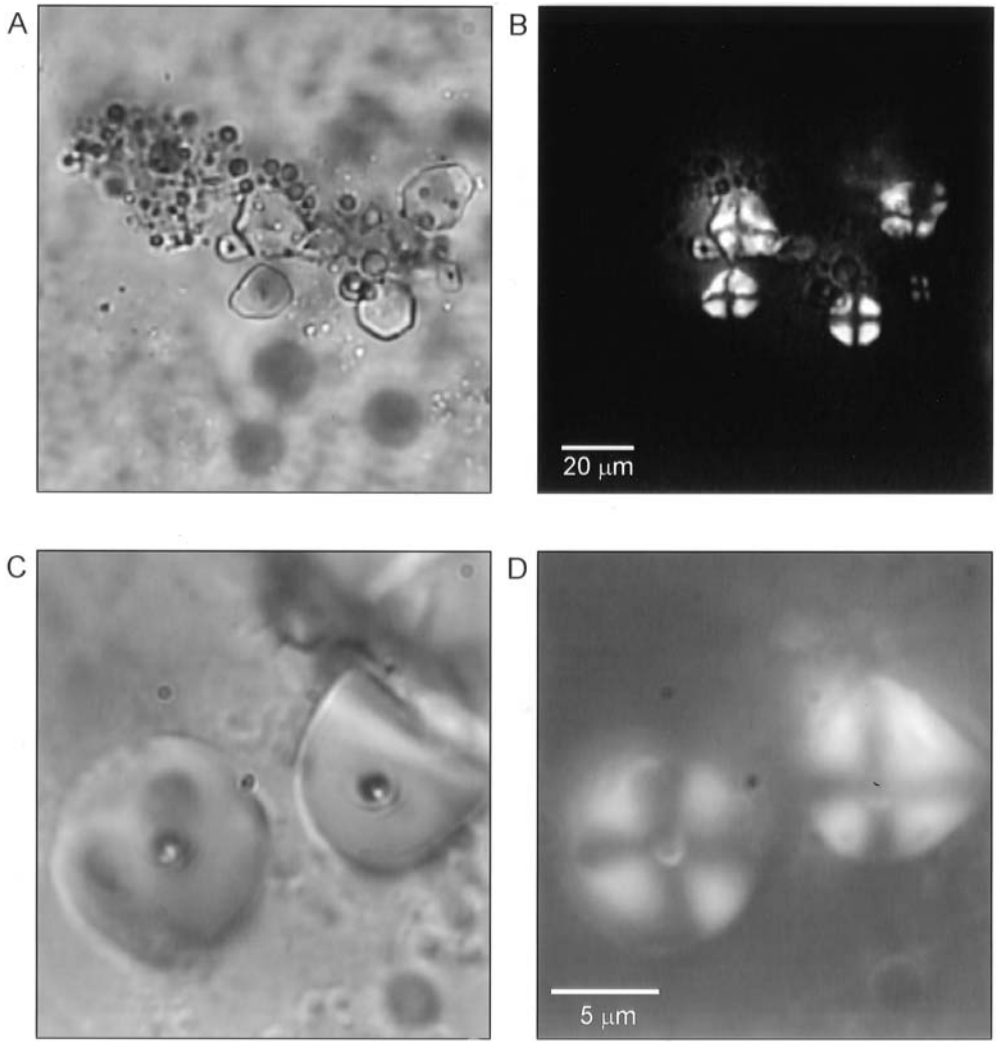

FIG. 9—*Brightfield images of condom lubricant components;* (A) *Trojan condom brightfield reflectance image;* (B) *Trojan condom polarized light image (20× magnification;* (C) *Beyond 7 condom brightfield reflectance image;* (D) *Beyond 7 condom polarized light image (100× magnification).*

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