Investigation of self-forming lipids and nanovesicles using vibrational spectroscopy

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The United States demand for drug delivery systems using phospholipids is projected to increase more than 10% annually reaching about US$ 130 billion in 2012. Accurate targeting advantages in the treatment of cancer and other debilitating diseases stimulate applications of less toxic, more cost effective drug delivery systems. Such reduced toxicity benefits would broaden the usage of liposomes encapsulated drugs, especially in the areas of cancer, neurological disorder and anti-infective therapy. Therefore, we have applied improved vibrational spectroscopic methods such as Near-Infrared (NIR), Fourier Transform Infrared (FTIR) and Raman scattering methods to elucidate the structure, dynamic and enhanced analytical techniques for quality control of such systems. In this work, three different vibrational spectroscopic techniques, namely NIR, FTIR and optical tweezers Raman spectroscopy have been employed to characterize the newly developed self-forming synthetic PEGylated lipids trademarked as QuSomes™ and its nanovesicles.

In contrast to conventional used egg or lecithin based phospholipids,1,2 these QuSomes™ spontaneously form liposomes upon hydration, without the supply of external activation energy. The amphiphiles considered in this study contain long hydrophobic acyl chain and various units of polyethylene glycol (PEG) hydrophilic head groups.3 These lipids are composed of 1,2-dimyristoyl-rac-glycerol-3-dodecaethylene glycol (GDM-12), 1,2-dioleoyl-rac-glycerol-3-dodecaethylene glycol (GDO-12) and 1,2-distearoyl-rac-glycerol-3-tricosaehtylene glycol (GDS-23). Such systems may enhance substance and drug absorption and could have significant impact on delivery efficiency of numerous materials.

NIR spectroscopy is most useful for measuring bonds involving hydrogen such as O−H, C=H, N−H etc. Thus, the technique appears most suitable for the identification of compounds having hydrated and hydroxyl groups. NIR absorption spectra of these new artificial lipids have been recorded in the spectral range of 4800-9000 cm$^{-1}$ by using a new miniaturized spectrometer based on micro-optical-electro-mechanical systems (MOEMS) technology. Three NIR spectral regions are identified, (a) the high wavenumber region between 6500 and 9000 cm$^{-1}$ attributed to the second overtone of the C=H stretching mode; (b) the 5350–5900 cm$^{-1}$ region attributed to first overtone of the C=H stretching mode; and (c) the 4800–5300 cm$^{-1}$ region attributed to the second overtone of the C=O stretching mode. For each of these regions, the lipids show distinctive spectra which allow their identification and characterization (see Fig. 1).

Furthermore, thin layered FTIR spectroscopy can be applied for in situ and vivo measurements of biological samples and has been used here as a powerful tool to characterize QuSomes™ samples. The infrared spectra of such lipids in the spectral range of 500 to 3100 cm$^{-1}$ have been obtained by means of FTIR spectrometer in conjunction with a demountable liquid cell having a path length of 15-µm. A characteristic FTIR spectrum of QuSomes™ sample is demonstrated in Fig. 2. Similarly, Raman spectra of optically trapped single lipid particle and nanovesicle have been recorded in the spectral range of 500-3100 cm$^{-1}$ by utilizing a laser tweezers Raman microscopy system (see Fig. 3).

As an application of these vibration spectroscopic techniques, thermotropic FTIR and Raman spectroscopic approaches have been employed in this study to detect and analyze the phase behaviour and conformational order in PEGylated lipids and its nanovesicles suspended in buffer solution. These investigations probe the phase behaviour and conformational order of these artificial lipids as they undergo phase transitions. The gel to liquid phase transitions of the sample lipids have been detected by examining the changes in the FTIR and Raman spectra of the lipids and nanovesicles caused by temperature variation particularly in the C=H stretching vibrational modes (see Fig. 4). The phase changes have been detected by investigating the various spectral indicators such as the plot of peak intensity ratios in the C=H stretching region ($I(\nu_{a}(\text{CH}_3))/I(\nu_{a}(\text{CH}_2))$ as a function of temperature.1 Furthermore, the
observed changes in the Raman spectra allowed to calculate various spectral indicators which are correlated to the $I_{\nu(CH_2)}$ to $I_{\nu(CH_3)}$ ratio observed in the C–H stretching region. This ratio is considered to be the primary indicator of rotational and conformational order in lipids. To confirm the observations, we have also applied differential scanning calorimetry (DSC) and are in a good agreement with the Raman spectroscopy results. The information obtained from this investigation may find various applications including the development of lipid based novel substances and drug delivery systems.

![Figure 1. NIR spectra for three samples of QuSomes™.](image1)

![Figure 2. Characteristic thin layered FTIR spectrum of QuSomes™.](image2)

![Figure 3. Characteristic Raman spectrum originating from an optically trapped single nanovesicle of QuSomes™ suspended in phosphate buffered saline (PBS). The most pronounced vibrational bands as indicated in this figure are dominated by CH$_2$ deformation modes.](image3)

![Figure 4. Representative temperature-induced Raman spectra of QuSomes™ illustrating the change in peak intensities in the C–C stretching region (~1000-1200 cm$^{-1}$) and the C–H stretching region (~2800-3000 cm$^{-1}$) near the lipid main phase transition temperature, $T_m$ (~5.2 °C).](image4)

References:

1. Egg lecithin for pharmaceutical applications, Phospholipid Research Center, Heidelberg, Germany.