Complementary Raman and IR spectroscopies for Rapid Diagnosis of Neonatal Respiratory Distress Syndrome

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Abstract: Aqueous lipid solutions were analyzed by mid-IR and Raman spectroscopies to establish a diagnosis approach for neonatal respiratory distress syndrome. We show the ability to predict lipid ratios around the diagnostic cutoff of 2.2.

1. Introduction

Neonatal respiratory distress syndrome (nRDS) is a leading cause of death in preterm neonates for which exogenous surfactant replacement can increase the chance of better prognosis. There is currently no point of care test that identifies the subset of neonates that respond to such treatment, requiring clinicians to treat cautiously. Early treatment of nRDS is associated with better prognostic outcomes [1] and would benefit from a point-of-care device to assist with clinical decision-making. Diagnostic biomarkers for nRDS make use of 2 lipids found in lung surfactant, lecithin (L) and sphingomyelin (S), to give an L/S ratio, where values less than 2.2 indicates nRDS.

Vibrational spectroscopy provides clinicians with rapid, point-of-care diagnostic information to assist them in deciding appropriate treatment approaches [2]. Mid infrared (mid-IR) and Raman spectroscopies are two complementary approaches to obtain information in both the functional groups and the fingerprint region allowing at once both identification and quantification of species present in a biosample [2]. As these modalities are based on different selection rules, they provide alternative routes to access the L/S ratio. These spectroscopies can be used to make quantitative analysis of chemical species present in a given biological sample [3]. Raman, unlike mid-IR spectroscopy, is not affected by water, and therefore can be used to analyze aqueous samples and in-vivo analysis. We have prepared aqueous lipid vescicles as a model of extracted lung surfactant from preterm neonates, unlike previous studies using solvent extracted/dried lipids, and employed Raman and mid-IR vibrational spectroscopy and machine learning techniques to analyze the L/S ratio. Our studies show that complementary vibrational spectroscopy can be used as rapid diagnosis tool for nRDS.

3. Materials and methods

Synthetic S and L (1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC) were purchased form Avanti Polar Lipids and Merck respectively. To prepare the vescicles, appropriate volumes of 1 mM DPPC and S solutions in dichloromethane were aliquoted and dried under nitrogen. 1 ml of deionized water was added to the dried lipid films and then warmed to 60° C and vigorously mixed using a vortex mixture. The resulting turbid solution was centrifuged at 1600 g for 25 minutes and the precipitates were used for the measurements. mid-IR spectra were collected using 10-bounce Pike® zinc selenide (ZnSe) horizontal ATR accessory on a Cary 670 FTIR using 0.5ml of sample under nitrogen purging. 32 co-added scans were taken at a resolution of 4cm^{-1}. Raman spectra were collected using a Renishaw inVia micro Raman spectrometer (532 nm excitation) using 50 µL of sample on silicon wafer.

3. Results and discussion

FTIR measurements of vesicles in water were performed in triplicate for each L/S ratio. The spectral region in vicinity of 1700 cm^{-1} was affected by the presence of water vapor in the sample chamber at a different concentration to when the background scan was taken, making the analysis of these regions difficult. To remove the impact of this on the partial least squares (PLS) model, the spectra were cut so that the regions between 946-1182 cm^{-1} and 2833 – 2934 cm^{-1} remained (Fig. 1(a)). These were then subjected to vector normalization for the region 1063 – 1197 cm^{-1} before splitting into a training and test set (70%:30% respectively). Fig. 1(b) shows the ability to establish the L/S ratio of samples in aqueous form of test set spectra (L/S 2.1 and 3.1).

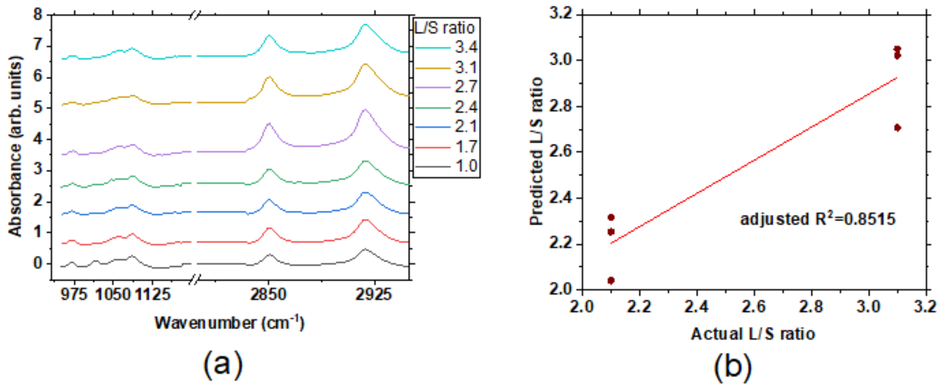

Fig. 1. Mid-IR spectra of DPPC and S vesicles (a). Mid-IR spectra of vesicles with different LS ratio recorded between 3000 cm $^{-1}$ - 800 cm $^{-1}$ region (b). L/S ratio predicted by the PLS model of the processed test set spectra

Fig. 2 (a) shows the Raman spectra of L and S vesicles at 800 cm $^{-1}$ - 1800 cm $^{-1}$ region. Both lipids show similar spectra at 800 cm $^{-1}$ - 1600 cm $^{-1}$ region. The uniqueness of L and S spectra were observed at 1600 cm^{-1} – 1800 cm^{-1} region where S show a C=C vibration at 1672 cm^{-1} and L shows ester carbonyl stretching vibration at 1742 cm^{-1}. Fig. 1 (b) shows the Raman spectra of vescicles with different L/S ratio at 1600 cm^{-1} – 1800 cm^{-1} region. The ester carbonyl bond strength increases with increase in concentration of L. The ratio of characteristic vibrations of L and S is shown in Fig. 1(c). The ratio of strength of C=C in S and C=O in L vibrations can be used as a calibration curve for ratiometric analysis of L and S.

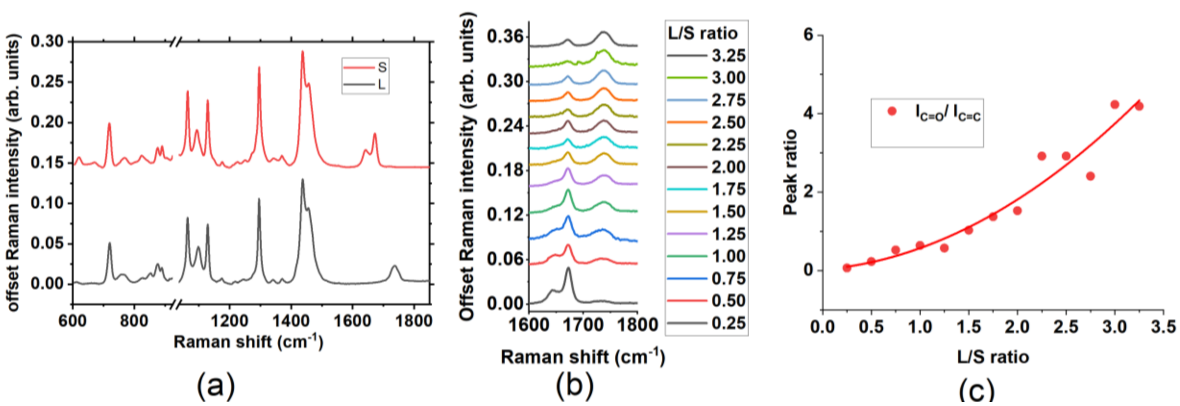

Fig. 2. (a). Raman spectra of L and S vesicles. (b). Raman spectra of vesicles with different L/S ratio recorded at 1600 cm $^{-1}$ - 1800 cm $^{-1}$ region. (c). Ratio of L C=O vibration and S C=C vibration

4. Conclusions

Vibrational spectroscopy is a simple, rapid and label-free, diagnostic technique. mid-IR and Raman spectroscopies provide complementary information. Here we used Raman and mid-IR spectroscopies to analyze aqueous lipid samples in each modality and were able to predict the L/S ratio. This could open the opportunity to rapidly diagnose nRDS in a manner consistent with the point-of-care paradigm.

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5. References

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