Spectroscopic Quantification of Synthetic Neonatal Respiratory Distress Syndrome Biomarkers in Lipid Mixtures

Zixing (Hings) Luo[1,3,#], Waseem Ahmed[1,#], Aneesh Vincent Veluthandath[1], Anthony Postle[3,4], Michael Grocott[2,3,4], Ahilanandan Dushianthan[2,3,4] and Ganapathy Senthil Murugan[1,*]

[1]Optoelectronics Research Centre, University of Southampton, Southampton SO17 1BJ, United Kingdom
[2]General Intensive Care Unit, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, United Kingdom
[3]Perioperative and Critical Care Theme, NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, United Kingdom
[4]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom
**smg@orc.soton.ac.uk*
[#]These authors contributed equally

Abstract: Vibrational spectroscopy is an important tool to quantify lipid biomarkers in lung surfactant, aiding rapid diagnosis of neonatal respiratory distress syndrome. We demonstrate a method, using a small sample volume, to predict lecithin, sphingomyelin and phosphatidylglycerol concentrations in complex synthetic lipid mixtures. © 2024 The Authors

Introduction

Neonatal respiratory distress syndrome (nRDS) is a serious condition affecting pre-term neonates due to a deficiency in lung surfactant, either from insufficient surfactant production or surfactant inactivation in immature lungs. While treatment and management, through oxygenation and ventilation support, surfactant replacement therapy, antenatal steroids, and supportive care, have improved the outcomes of nRDS patients, challenges in diagnosis can lead to delays in receiving appropriate treatment and consequent poor prognosis. Mid-infrared spectroscopy can be used to measure the concentrations of species in lung surfactants, such as two diagnostic biomarkers of nRDS, lecithin (L) and sphingomyelin (S), with the potential to be employed as a point-of-care diagnostics tool. We previously demonstrated quantification of lipids using attenuated total reflectance Fourier transform infrared spectroscopy (ATR–FTIR) combined with machine learning of synthetic lipid mixture models of lung surfactant [1-3]. This study utilizes a lung surfactant model of five lipids to predict lipid concentrations with partial least squares regression (PLSR) approach using a diamond-based ATR platform that requires a smaller sample volume.

Methods

Measurements were performed with an Agilent Cary 670 FTIR instrument equipped with a potassium bromide (KBr) beam splitter and a deuterated triglycine sulphate (DTGS) detector. A MIRacle® 9-reflection diamond/zinc selenide (ZnSe) ATR accessory with a high-pressure clamp and a solvent cover was used as the sample platform. Nitrogen purging was set to 9 L/min. Scan settings were set to 32 scans at a 4 cm^{-1} resolution. The mixtures, including dipalmitoylphosphatidylcholine (DPPC, 1.035-1.308 mM), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, 0.508–0.632 mM), S (0.273–0.853 mM), phosphatidylglycerol (PG, 0.008–0.333 mM), and cholesterol (Chol, 0.207–0.310 mM), were prepared by adding measured masses of each component along with the required amounts of dichloromethane (DCM). Sample lipid compositions were developed using an extreme vertices experimental design using Minitab®. These mixtures reflect a range of physiological concentrations of these lipids in human lung surfactant as recovered in bronchoalveolar lavage or gastric aspirate samples. The solutions were heated, if required, for 2 min at 63 °C followed by vortex mixing for another 2 min. This was repeated until the solutions were visibly homogenous. For each liquid sample (30 µL) dispensed onto the ATR crystal, 9 successive spectra were recorded, and this was repeated twice more. To introduce the variability associated with a clinical-use scenario, a background spectrum was recorded prior to each set of spectra.

Results and Discussion

As two main types of L, DPPC and POPC were combined together for the model of L prediction. The PLSR models were able to determine the concentrations of L, S, and PG within the physiological ranges tested (R^2 = 0.86, 0.84, 0.78, respectively, Fig. 1). These are important biomarkers present in lung surfactant (L/S ratio and PG in late gestation) and can be combined to give a holistic understanding of the lung maturity level. The model performances for DPPC, POPC, Chol, were less useful than that in these models (data not shown). The prediction intervals for the lipids, determined using the jackknife + -after-bootstrap method, indicate the uncertainty in the model predictions and are shown as error bars (average width of prediction intervals for L, S, PG, is 0.35, 0.247, 0.172 mM, respectively). These results demonstrate the ability to quantify lipid lung maturity biomarkers at physiological concentrations using a small sample volume by PLSR. Such an approach could be replicated in a clinical context to predict the concentration of lipid biomarkers within minutes to inform nRDS diagnosis and reduce the time required to access appropriate treatment. More widely, this approach of combining ATR-FTIR spectroscopy and machine learning methods has the potential to quantify any biomarkers which have vibrational spectroscopic fingerprints in clinical samples rapidly.

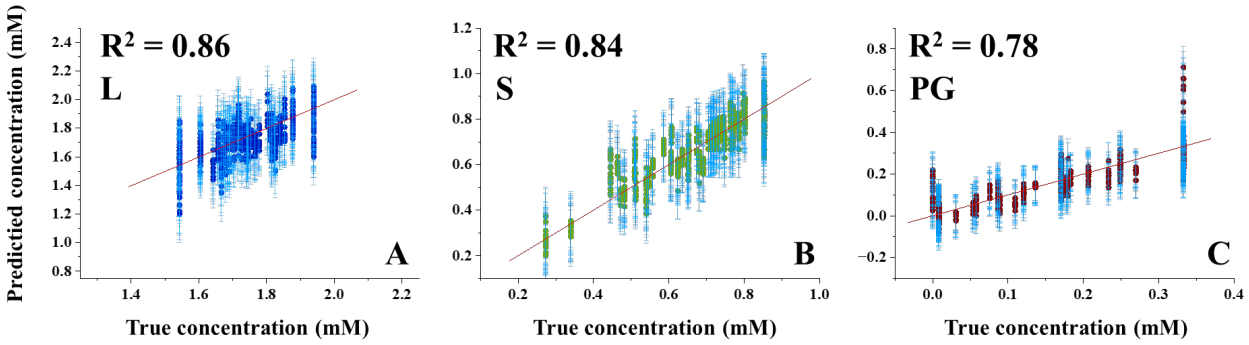

Fig. 1. Predicted vs true concentrations of lipids (A: L, B: S, C: PG) with the R^2 values for each lipid and the 95 % prediction intervals (indicated by error bars) generated by the jackknife + -after-bootstrap method. The red line in each graph corresponds to the expected prediction value.

Conclusions

In conclusion, this study utilized a physiologically informed model of lipids in lung surfactant with only 30 µL sample volume to investigate the effects of varying lipid composition on the FTIR spectra. The spectra were used to generate models to predict the concentration of each lipid in lipid mixtures matching physiological concentrations. The prediction models of L, S and PG performed the best, while models of DPPC, POPC and Chol performed less well. Prediction intervals for each model were generated using jackknife + -after-bootstrap methods. Future studies can focus on the transferability of the models to human samples.

Acknowledgements

This work was supported by the UK Engineering and Physical Sciences Research Council (EPSRC grant EP/S03109X/1).

References

[1] Ahmed, W., et al., *Towards quantifying biomarkers for respiratory distress in preterm infants: Machine learning on mid infrared spectroscopy of lipid mixtures.* Talanta, 2024. **275**: p. 126062.

[2] Ahmed, W., et al., *Prediction of Neonatal Respiratory Distress Biomarker Concentration by Application of Machine Learning to Mid-Infrared Spectra.* Sensors (Basel), 2022. **22**(5).

[3] Veluthandath, A.V., et al., *Quantification of lung surfactant lipid (dipalmitoylphosphatidylcholine/sphingomyelin) ratio in binary liposomes using Raman spectroscopy.* Journal of Raman Spectroscopy, 2024. **55**(3): p. 386-393.