PROCEEDINGS OF SPIE

SPIEDigitalLibrary.org/conference-proceedings-of-spie

Improving tuberculosis treatment using mid-infrared spectroscopy for bedside therapeutic drug monitoring

David Rowe, Daniel Owens, Milos Nedeljkovic, Callum Stirling, Katrina Cathie, et al.

David J. Rowe, Daniel R. Owens, Milos Nedeljkovic, Callum J. Stirling, Katrina Cathie, Suzanne L. Parker, Saul N. Faust, Goran Z. Mashanovich, James S. Wilkinson, "Improving tuberculosis treatment using mid-infrared spectroscopy for bedside therapeutic drug monitoring," Proc. SPIE 12426, Silicon Photonics XVIII, 124260G (13 March 2023); doi: 10.1117/12.2649135

SPIE.

Event: SPIE OPTO, 2023, San Francisco, California, United States

Improving tuberculosis treatment using mid-infrared spectroscopy for bedside therapeutic drug monitoring

David J. Rowe*[a], Daniel R. Owens[b,c], Milos Nedeljkovic[a], Callum J. Stirling[a], Katrina Cathie[b], Suzanne L. Parker[d], Saul N. Faust[b,c], Goran Z. Mashanovich[a], James S. Wilkinson[a]
[a]Zepler Institute, University of Southampton, Southampton SO17 1BJ, UK; [b]NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK; [c]Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton SO16 6YD, UK; [d]UQ Centre for Clinical Research, The University of Queensland, Herston QLD 4029, Australia

ABSTRACT

Rifampicin is an antimicrobial drug used to treat tuberculosis. The deterioration of a tuberculosis patient on rifampicin is a serious event with several possible causes. Rapid bedside measurement of rifampicin would enable clinicians to determine if patient deterioration was due to subtherapeutic levels and quickly correct the dosing. It would also support personalised dosing to maximise antimicrobial effectiveness whilst minimising side effects. The optimum therapeutic concentration range is 8 – 24 mg/L.

We report ATR-FTIR spectroscopy data for the detection of rifampicin for bedside therapeutic drug monitoring (TDM). We demonstrate a limit of detection of 0.46 mg/L from 20 µL spiked whole blood samples. Using whole blood directly enables bedside measurements because it does not require centrifugation and pipetting to extract plasma, which are generally performed in a central laboratory. The absorption-concentration response had good linearity ($R^2 = 0.998$) up to the highest measured concentration of 100 mg/L.

We apply this data to the design of a miniaturised mid-infrared sensor for TDM using silicon photonics. We present an analysis of the optimum interaction length for an evanescent waveguide sensor using the absorption of rifampicin and a numerical model to quantify the contributions of different system and device noise sources. These sensors can be made more sensitive than their benchtop equivalent because of the enhanced evanescent electric field strength and the increased power spectral density of tunable quantum cascade lasers.

Keywords: mid-infrared applications, ATR-FTIR spectroscopy, waveguide sensors, biosensors, therapeutic drug monitoring, tuberculosis, point-of-care

1. INTRODUCTION

1.1 FTIR spectroscopy

Fast and minimally invasive methods are required for drug concentration analysis to optimise dosing in clinical practice. Fourier transform infrared (FTIR) spectroscopy can provide point-of-care drug levels from microsamples of whole blood for personalised dosing and pharmacokinetic research. FTIR spectroscopy uses mid-infrared (MIR) radiation to interrogate a sample. MIR radiation can excite molecular bonds where frequency corresponds to the vibration of the bond, typically in the fingerprint region of 1500 - 600 cm^{-1} or 6.7 - 10.2 µm. This means that infrared absorption spectroscopy, as measured by FTIR or an evanescent waveguide, can be used to identify compounds [1]. The Beer-Lambert law shows absorption strength is proportional to the number of bonds present so infrared spectroscopy can be used to determine both molecular composition and the concentration of a particular molecule in solution [2].

Attenuated total reflection (ATR)-FTIR uses an optical beam internally reflected within a prism or crystal. A sample placed upon the internally reflecting surface will interact with the evanescent field at each reflection of the beam. In the MIR, the evanescent field penetrates approximately 1 µm into the sample. This means the beam can measure absorptions in the

*d.rowe@soton.ac.uk, +44(0)23 80597029, www.siliconphotonics.co.uk.

Silicon Photonics XVIII, edited by Graham T. Reed, Andrew P. Knights,
Proc. of SPIE Vol. 12426, 124260G · © 2023 SPIE
0277-786X · doi: 10.1117/12.2649135

presence of highly absorbing solvents such as water in aqueous biological samples [3].

FTIR spectroscopy has been applied to diverse applications in biomedicine, including the analysis of blood [3], neural tissue [4] and renal stones [5]. Its main limitation for clinical medicine is its ability to detect low concentrations of molecules in complex biological media such as blood. The plasma concentrations of clinical interest for pharmaceutical compounds are often 1 – 100 mg/L.

1.2 Therapeutic drug monitoring for rifampicin

Tuberculosis (TB) is a serious mycobacterial infection that is the 13th leading cause of death globally. Prior to 2019, it was the leading cause of death from a single infectious agent and estimated to be second only to COVID-19 in 2020 and 2021 [6]. Rifampicin is an antimicrobial drug used as a first line treatment for TB and as such is listed as an essential medicine for both adults and children by the World Health Organisation [7, 8].

Rapid measurement of rifampicin at the bedside would enable clinicians to quickly determine whether any clinical deterioration is due to subtherapeutic levels. These can be caused by suboptimal adherence to therapy or intestinal absorption of rifampicin, or intercurrent bacterial causes [9]. Performing drug level measurements at the point-of-care, known as therapeutic drug monitoring (TDM), would remove the logistical burden of transporting samples to a central laboratory and communicating the result back to the clinician. This can improve clinical efficacy because maintaining the rifampicin dose at its optimum level will maximise antimicrobial effectiveness and minimise side effects. This is particularly important for resource-poor settings.

1.3 Evanescent waveguide sensing

A planar waveguide can perform the same function as the ATR attachment for an FTIR spectrometer. A sample placed on top of an unclad waveguide will interact with the evanescent field of the mode confined within the waveguide. By tuning the wavelength of the source to the waveguide and measuring the optical power output, infrared absorption spectra can be taken. In the case of rifampicin TDM, the concentration of rifampicin can be determined by subtracting the measurement of whole blood from the measurement of a rifampicin-blood solution.

Waveguide cladding can be used to set an interaction length where light can interact evanescently with the sample by removing a section of the cladding from a defined length of waveguide. A longer waveguide will cause a stronger target signal, which in this case would be absorption peaks from rifampicin. However, the highly absorbing aqueous background of blood means that too long an interaction length will result in so much light being absorbed by the water that no signal can be measured. Therefore, the optimum LoD of an evanescent waveguide sensor can be estimated using a noise model together with the complex refractive index data for rifampicin and blood.

The three primary sources of noise are Johnson/thermal noise, shot noise and relative intensity noise (RIN). The total r.m.s. noise current $i_{n(rms)}$ can be calculated by adding the noise currents from these three sources:

$$i_{n(rms)} = \sqrt{{i_{TN}}^2 + {i_{SN}}^2 + {i_{RIN}}^2} \tag{1}$$

The noise-equivalent power *NEP* ($Hz^{1/2}$) is calculated by dividing the total noise current by the photodetector responsivity R_{det}:

$$NEP = \frac{i_{n(rms)}}{R_{det}} \tag{2}$$

The sensitivity *S* of output power to analyte concentration is defined using power incident on the photodetector *I*, specific absorption coefficient ε, fraction of evanescent power overlapping with the sample η and waveguide interaction length *l*:

$$S = -\varepsilon \eta l I \tag{3}$$

The limit of detection LoD is taken as three times the smallest measureable change in concentration, calculated using the *NEP*, the sensitivity and the receiver bandwidth *B:*

$$LoD = 3\frac{NEP\sqrt{B}}{|S|} \tag{4}$$

2. METHODOLOGY

Whole blood was collected from a healthy volunteer donor according to the Declaration of Helsinki 2008 and guidelines for Good Clinical Practice. NHS research ethical approval was provided via the Southampton Research Biorepository (REC reference 17/NW/0632). Written informed consent was obtained from the participant and the samples were labelled anonymously. Blood samples were taken using EDTA vacutainer tubes (Becton, Dickinson and Company, USA). Solutions of rifampicin (Sanofi, Reading, UK) in whole blood were prepared at concentrations of 1 – 100 mg/L by serial dilution.

Spectra were measured using an Alpha II FTIR spectrometer with a diamond ATR attachment (Bruker Optik, Germany) between 400 – 4000 cm^{-1} with 4 cm^{-1} resolution. A 20 µL sample volume was used for each measurement. Unspiked whole blood was used as the reference for each sample measurement. Post-processing was performed using MATLAB (The Mathworks Inc, USA).

The LoD noise model requires refractive index spectra for blood and rifampicin-blood solutions. The refractive index spectra were calculated according to a previously-reported empirical method [3]. Briefly, a model of the effective penetration depth of the ATR-FTIR spectrometer was used to calculate the imaginary component of refractive index from the absorbance measurement data. The Kramers-Kronig relations were then used to calculate the real component of refractive index from the imaginary component.

3. RESULTS

3.1 FTIR LoD for rifampicin

Figure 1a shows the absorbance spectra for solutions of rifampicin in whole blood with rifampicin concentrations between 1 – 100 mg/L. Figure 1b shows the corresponding concentration-absorbance response to be linear (R^2 = 0.998) at the centre frequency of the largest peak at 1545 cm^{-1}.

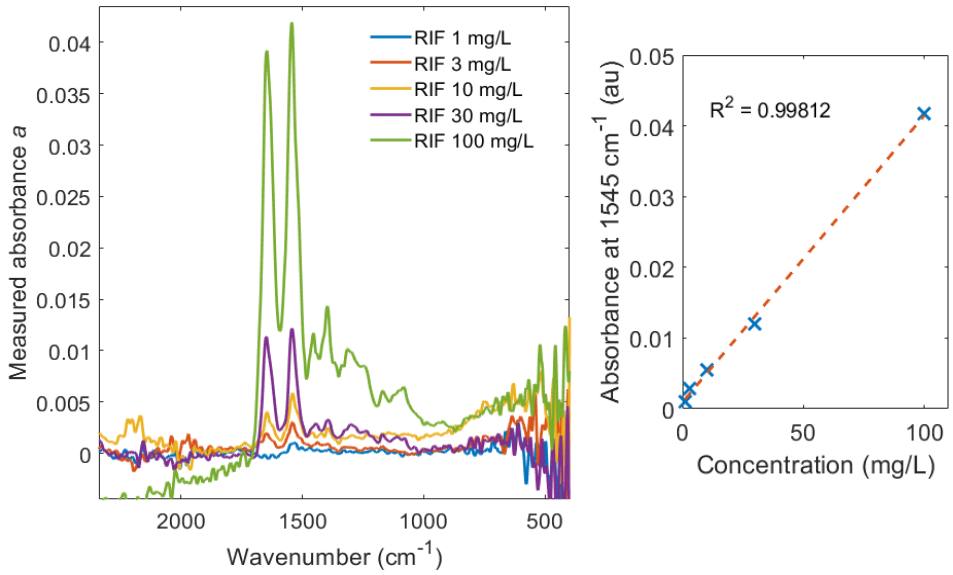

Figure 1. (a) absorbance spectra for dilutions of rifampicin in whole blood and (b) absorbance at 1545 cm^{-1} with respect to rifampicin concentration.

The LoD is defined as the concentration equivalent to three times the standard deviation on the blank measurement. The LoD can be read from Figure 1b as the concentration corresponding to three times the standard deviation in absorbance at the selected wavenumber. The measurement noise spectrum was calculated by taking the standard deviation of ten measurements of whole blood. The mean absorbance is given with plus/minus one standard deviation shaded in Figure 2.

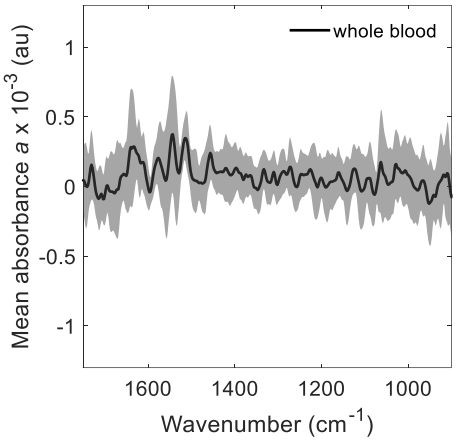

Figure 2. The mean absorbance spectra for ten repeated measurements of whole blood taken relative to a background measurement of whole blood. The shaded region represents one standard deviation of the repeated measurements.

The LoD can be compared across the entire spectrum by calculating a line of best fit similar to Figure 1b at every frequency. The measurement noise spectrum in Figure 2 can therefore be used to calculate the LoD at each measurement frequency in order to optimise for the best LoD. The minimum LoD value is 0.46 mg/L at 1595 cm^{-1}. This corresponds to a shoulder between the two largest absorbance peaks and a local minimum of measurement noise.

3.2 Modelling waveguide LoD for rifampicin

The waveguide model in Section 1.3 was used to calculate the LoD for rifampicin in whole blood. Estimates for typical values for noise, responsitivity and bandwidth were taken from existing laboratory setups. The detector noise equivalent power (NEP) was taken as 1.25×10^{-10} $W.Hz^{-1/2}$, a relative intensity noise value of 10^{-3} $Hz^{-1/2}$ (as a fraction of the optical power reaching the photodetector) was used, and a bandwidth of 1 Hz was used in the calculation. The laser intensity was assumed to be 10 mW over the whole spectrum, with 20 dB of excess insertion loss in the optical path (excluding losses in the sensing region itself). The specific absorption coefficient ε was calculated from refractive index derived from the absorbance data shown in Figure 1. The fraction of evanescent power overlapping with the sample η was estimated using numerical modelling of a standard germanium-on-silicon (GOS) rib waveguide, giving a value of 2.5%. A GOS waveguide loss of 8.5 dB/cm was assumed. The optimum interaction length l for rifampicin in blood was found by sweeping values of l and calculating the corresponding LoD. In this way, the optimum interaction length for a standard GOS waveguide was calculated as being between l = 1 mm and l = 3 mm, depending on the wavelength. It should be noted that the optimal length will depend on the relative strengths of the noise sources.

The LoD corresponding to the optimised interaction length and typical experimental parameters can therefore be calculated at each frequency of the measured spectrum, as shown in Figure 3.

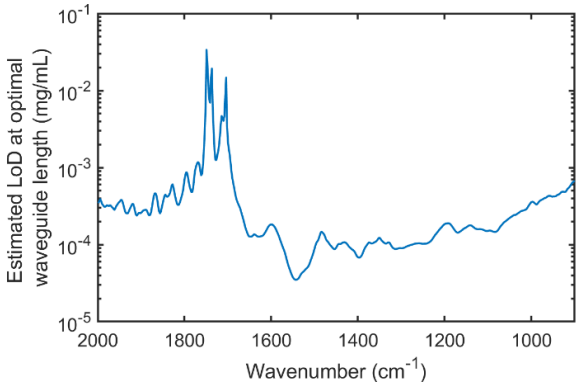

Figure 3. The estimated LoD spectrum for rifampicin in whole blood, calculated for a GOS evanescent waveguide sensor using the waveguide noise model. The calculation uses the optimal waveguide length at each wavelength.

4. DISCUSSION

The linear response of absorbance with concentration up to 100 mg/L, coupled with LoD < 0.5 mg/L, show this method can be used to characterise the clinically relevant concentrations of rifampicin. This includes concentrations that are adequate but not ideal, as well as being able to positively identify the trough level of 0.5 mg/L. It is crucial to have LoD lower than the trough level to ensure that sub-therapeutic levels are confidently identified.

The absorption peaks do not correspond directly to the absorption peaks of rifampicin when it is dissolved in water. This is likely due to the very high level of protein binding for rifampicin in blood [10] so that, instead, here the absorption peaks correspond to the protein-drug complex. This is supported by the fact that the absorption peaks closely resemble those of whole blood but shifted slightly in frequency. This effect has been replicated with blood from different donors and different batches of rifampicin.

Such secondary sensing of a larger molecule (the protein-drug complex) improves the limit of detection beyond that of rifampicin in water precisely because the drug is bound to a larger molecule, which is more strongly absorbing due to its greater number of molecular bonds. This does reduce the specificity of the method because other strongly protein binding drugs could interfere with the signal. However, the majority of common drugs do not exhibit such MIR behaviour and are instead identifiable by their own weaker but unique absorptions.

The capability of this method to use whole blood not serum for analysis is crucial. Unlike the majority of clinical diagnostic tests, this means that a centrifuge is not required to extract plasma or serum from whole blood, which means that the sample does not need to be processed in a laboratory but instead can be processed at the bedside using an FTIR spectrometer or miniaturized waveguide sensor. The diagnostic procedure is simpler and faster; a result can be obtained within one minute.

The noise model results show that a significantly lower LoD could be achieved using a waveguide sensor instead of an FTIR spectrometer. This is in part due to the greater control over the interaction length enabled by using a waveguide geometry as well as allowing a much more powerful source, such as a quantum cascade laser, to interrogate the sample.

5. CONCLUSION

The limit of detection of rifampicin in whole blood has been found to be below the trough dose for TB. The concentration response is linear across all clinically-relevant concentrations. This suggests that point-of-care sensors will be able to provide useful information directly to the clinician at the bedside without any laboratory processing. A noise model has been developed to calculate the limit of detection for similar measurements performed with an evanescent waveguide sensor. This model was used to find the optimum interaction length for maximum sensitivity and to show that the limit of detection is likely to be improved even with conservative estimates of the different sources of noise. The next steps are (i) a complete bioanalytical validation for the FTIR method to quantify stability and inter-patient variability and (ii) to demonstrate an equivalent waveguide sensor to verify the noise model.

REFERENCES

[1] Y. Chen, C. Zou, M. Mastalerz *et al.*, "Applications of Micro-Fourier Transform Infrared Spectroscopy (FTIR) in the Geological Sciences--A Review," International journal of molecular sciences, 16(12), 30223-30250 (2015).

[2] D. J. Rowe, D. R. Owens, S. L. Parker *et al.*, "The Effect of Haematocrit on Measurement of the Mid-Infrared Refractive Index of Plasma in Whole Blood," Biosensors, 11(11), (2021).

[3] D. J. Rowe, D. Smith, and J. S. Wilkinson, "Complex refractive index spectra of whole blood and aqueous solutions of anticoagulants, analgesics and buffers in the mid-infrared," Sci Rep, 7(1), 7356 (2017).

[4] A. Lux, R. Müller, M. Tulk *et al.*, "HHT diagnosis by Mid-infrared spectroscopy and artificial neural network analysis," Orphanet J Rare Dis, 8, 94 (2013).

[5] N. A. Türk C, Petřík A, Seitz C, Skolarikos A, Somani B, Thomas K, Gambaro G, Davis NF, Donaldson JF, Lombardo R, Tzelves L, "EAU Guidelines on Urolithiasis," (2021).

[6] World Health Organization, [Tuberculosis], (2021).

[7] World Health Organization, [World Health Organization Model List of Essential Medicines – 22nd List] World Health Organization, , Geneva(2021).

[8] World Health Organization, [World Health Organization Model List of Essential Medicines for Children – 8th List, 2021.], Geneva(2021).
[9] D. R. Owens, C. M. Medalla, K. N. Brown *et al.*, "Problem-solving in clinical practice: Persisting respiratory distress in a premature infant," Arch Dis Child Educ Pract Ed, 106(4), 239-243 (2021).
[10] P. Mishra, R. P. Pawar, D. Bose *et al.*, "Stability studies of rifampicin in plasma and urine of tuberculosis patients according to the European Medicines Agency Guidelines," Bioanalysis, 11(8), 713-726 (2019).