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# Quantifying low levels (<0.5% w/w) of warfarin sodium salts in oral solid dose forms using Transmission Raman spectroscopy

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ABSTRACT

In this feasibility study Transmission Raman spectroscopy (TRS) has been used to build quantitative models for warfarin sodium and warfarin sodium clathrate. The type of warfarin present in manufactured tablets may affect product quality. Models were used to predict warfarin sodium in commercially available tablets at extremely low dosage levels (0.5% w/w). The laboratory made calibration samples used in the modelling varied in amorphous sodium, crystalline clathrate warfarin forms, excipients and dye. This application was highly challenging due to the low level of API and high level of a Raman-active colourant which varied significantly between production batches. A photon recycling optic, known as a Beam Enhancer, was utilised to improve the signal to noise of the Raman spectra to attain a low limit of quantification of 0.19% w/w.



## 1. Introduction

### 1.1. Introduction to warfarin

Warfarin, a coumarin derivative, is an anticoagulant that reduces the formation of blood clots and is used to treat patients to reduce the risk of strokes, heart attacks and other thromboembolic conditions. Warfarin is administered orally and is available in either a liquid solution or in a solid dose form. It is the most prescribed oral anticoagulant and one of the most prescribed medicines in the US [1]. Warfarin is available in 9 dose strengths ranging from 1 mg up to 10 mg. This large range is needed as the treatment has a narrow therapeutic range, the dose response is genetically determined and varies significantly from patient to patient [2].

Pure warfarin is most readily available as a salt, either in its amorphous form of warfarin sodium (WS) or a crystalline form as warfarin sodium clathrate (WSC). WSC is a dimer of two warfarin molecules bridged by an isopropyl alcohol (IPA) moiety. WSC is used in commercial formulations. WSC decomposes with the loss of IPA to WS at high (>68% RH) humidity [3]. Traditional chromographic assay methods, *e.g.* high-performance liquid chromatography (HPLC) cannot be used to differentiate between WSC and WS as in solution the species are identical.

Previous work has shown that the presence of WS polymorphic forms affect product quality [4]. Further studies suggest that manufacturing variables can influence crystallinity of WSC and hence affect critical quality attributes such as dissolution time [5]. It is therefore critically important to control and measure the crystalline WSC content of the drug form in the final solid dose product and throughout the products lifetime from manufacture to patient.

### 1.2. Transmission Raman spectroscopy (TRS)

TRS was pioneered by Schrader in early days [6]. The technique remained relatively unutilised, until ~2006, from which point it has seen several applications towards pharmaceutical analysis [7,8]. Initial work made comparisons with conventional backscatter geometry Raman techniques [9,10]. Pharmaceutical samples including powders, tablets and capsules were analysed demonstrating the improved accuracy of whole sample analysis and how the reduction in subsampling improves quantitative model performance [11,12]. As with any other Raman based technology TRS tends to be non-destructive. The transmission geometry adds favourable characteristics of bulk sampling [13,14] and the ability to measure the whole intact final dosage form avoids sample preparation steps.

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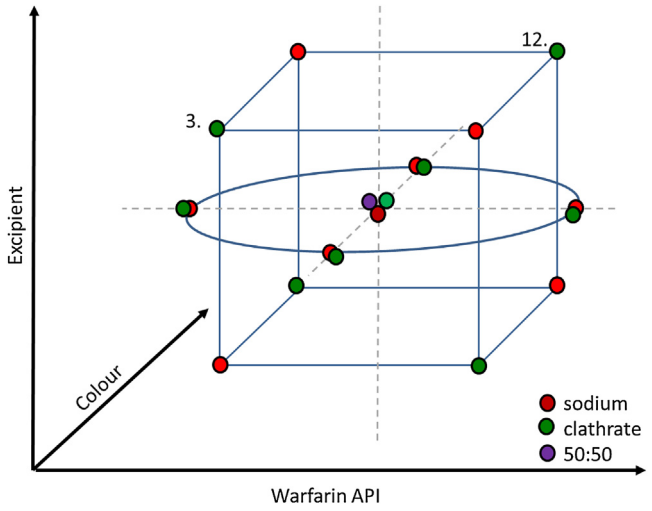


**Fig. 1.** Design of Experiment (DoE) sample space, centric cubic design consisting of 19 samples.

Solvation states and polymorphic forms have been extensively studied using Raman based technologies [15,16]. There are a few notable examples that utilise TRS; initial work showed the expected benefit of superior quantification compared to backscatter geometries [17,18]. Further work compared TRS to NIR, with TRS achieving marginally better quantitative model performance statistics [19]. TRS has also been used to quantify the amount of amorphized material in a polymer tablets after microwave irradiation, al be it a high >10% w/w drug loading [20]. A brief study compared TRS to XRD and ssNMR in spray dried solid dispersions, critically evaluating both performance and suitability for routine testing. TRS demonstrated a limit of detection (LOD) comparable with the ssNMR method (0.9% w/w) al be it with a measurement time of seconds and without the need to alter the sample in any way [21]. The most recent work demonstrated the capability of quantifying low (0.62–1.32% w/w) levels of polymorph content, and additionally the sole decomposition and transformation from one form to another while the excipient content remained constant [22].

Previous work has demonstrated the use of beam enhancer technology with TRS to improve signal to noise and/or speed of data acquisition of TRS measurements [22]. The Beam Enhancer is a dielectric bandpass filter that reflects laser radiation back towards the sample, the majority of which scatters away from the sample and would otherwise reduce the number of photons able to scatter through the sample matrix. These results in the sample being exposed to more laser radiation overall and hence leads to a higher Raman yield in the TRS geometry.

### 1.3. Previous work on warfarin

NIR reflectance has been described for the quantitative determination of WS, combining both laboratory and production tablets in a through calibration and experimental approach at dose strengths from 1 to 10 mg [23]. The same authors later demonstrated Raman spectroscopy as a complimentary method for Content uniformity of break-scored warfarin tablets between 1 and 10 mg dose strengths [24]. Warfarin crystallinity analysis work demonstrated the application of NIR chemometric methods to quantification of warfarin sodium products. Samples at 4 and 10% w/w warfarin content subsequently ranged between 0 and 100% of both WSC and WS form. This thorough study demonstrated the importance and viability of spectroscopic techniques for warfarin crystallinity quantification [25].

Another study from the FDA [26] successfully used Raman and $^{13}C$ NMR to quantify 5% warfarin (varying from 0 to 100%) in a formulation of common pharmaceutical excipients. The study concluded *'The developed chemometric models based on Raman spectroscopy provides easy and fast method for quantifying WS amorphous/crystalline fraction in the drug products.'*

However, warfarin dose strengths typically range from 1 to 10 mg and this corresponds to, in tablets that are ~200 mg in mass, 0.5–5% w/w content range. Previous studies have successfully demonstrated both NIR, Raman and ssNMR technologies but only for the higher dose strengths.

In this work we have concentrated on the lowest dose strength 1 mg (0.5% w/w). This is additionally challenging as the commercial available formulations of interest contain a red organic dye that has a very strong Raman scattering cross section and is present in approximately the same content (~0.4% w/w) as the API. Formulations from different vendors (US) contain the same dye and only distinguishable via shape [27]. The dye content may vary from batch to batch and because it is such a strong Raman active species this could affect quantitative predictions using Raman techniques.

This is the first TRS crystalline quantification study that uses lab made calibration samples to predict commercially available pharmaceutical products at such low dosage levels. This feasibility study sets TRS as a viable technology for crystallinity determination of oral solid dose forms.

**Table 1**
DoE Formulation values in% w/w. Colour = D&C red #6 barium lake, Starch = pregelatinized corn starch.

| Sample | Warfarin sodium | Warfarin clathrate | Colour | Starch | Magn. Stearate | Lactose |
|---|---|---|---|---|---|---|
| 1 | 0.35 | – | 0.41 | 7.31 | 1 | 90.9 |
| 2 | 0.39 | – | 0.32 | 8.85 | 1 | 89.4 |
| 3 | 0.39 | – | 0.32 | 5.77 | 1 | 92.5 |
| 4 | 0.50 | – | 0.29 | 7.31 | 1 | 90.9 |
| 5 | 0.50 | – | 0.53 | 7.31 | 1 | 90.7 |
| 6 | 0.61 | – | 0.50 | 8.85 | 1 | 89.1 |
| 7 | 0.61 | – | 0.32 | 5.77 | 1 | 92.3 |
| 8 | 0.65 | – | 0.41 | 7.31 | 1 | 90.6 |
| 9 | 0.50 | – | 0.41 | 7.31 | 1 | 90.8 |
| 10 | – | 0.38 | 0.41 | 7.31 | 1 | 90.9 |
| 11 | – | 0.43 | 0.50 | 8.85 | 1 | 89.2 |
| 12 | – | 0.43 | 0.50 | 5.77 | 1 | 92.3 |
| 13 | – | 0.55 | 0.29 | 7.31 | 1 | 90.9 |
| 14 | – | 0.55 | 0.53 | 7.31 | 1 | 90.6 |
| 15 | – | 0.66 | 0.32 | 8.85 | 1 | 89.2 |
| 16 | – | 0.66 | 0.50 | 5.77 | 1 | 92.1 |
| 17 | – | 0.71 | 0.41 | 7.31 | 1 | 90.6 |
| 18 | – | 0.55 | 0.41 | 7.31 | 1 | 90.7 |
| 19 | 0.25 | 0.27 | 0.41 | 7.31 | 1 | 90.8 |