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A Raman spectroscopic investigation of bioadhesive tetracaine local anaesthetic formulations

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Abstract

Raman spectroscopy at 785 nm has been employed to characterise the properties of tetracaine in bioadhesive gel and patch formulations. In the first study, interactions between the drug and excipients in novel bioadhesive patch systems were characterised. It was determined that the drug did not interact with any of its formulation components, and that this was an important factor in its clinical performance, particularly the rapid onset of anaesthesia. Investigations of drug uptake in the stratum corneum from a gel formulation suggested that tetracaine rapidly undergoes a phase-change upon application to the skin. The intensity of the tetracaine Raman bands at approximately 1600 cm^{-1} suggests that the local anaesthetic is rapidly absorbed into the skin. Decreases in Raman tetracaine band intensities, along with an absence in the concomitant alteration in the internal standard spectra, indicates an decrease in the tetracaine concentration present in the gel. Further, a baseline indicating complete tetracaine absorption appears to be reached after approximately 40 min of exposure. After this time little further absorption was observed, suggesting that the stratum corneum "reservoir" was saturated with tetracaine at this time. This is consistent with the optimum application time required for tetracaine gels to attain maximum clinical efficacy. This study has indicated the effectiveness of Raman spectroscopy in the analysis of gel-based pharmaceutical preparations, showing it to be a simple, rapid, virtually non-invasive technique for determination of tetracaine.

Keywords: Percuatneous local anaesthesia; Raman spectroscopy; Skin

1. Introduction

Raman spectroscopy has previously been applied to the successful study of various therapeutic and narcotic agents and polymer based drug delivery devices (Davies et al., 1990; Hodges and Akhavan, 1990; Pelletier, 1999). Historically, visible Raman spectroscopy has not found wide acceptance in pharmaceutical analysis, due to sample fluorescence and photodegradation of some molecules upon visible laser illumination. Because of these shortcomings, FT-Raman, using 1064 nm excitation and yielding Raman spectra outside the fluorescent region, has found broader acceptance in this field. However, this

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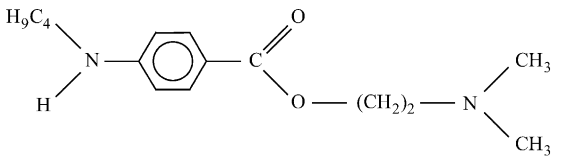

Fig. 1. Tetracaine (amethocaine) (2-dimethylaminoethyl 4-butylaminobenzoate).

technique does require high laser powers (typically 1 W) and may often cause sample decomposition which can go unnoticed (Bennett et al., 1990). Dispersive Raman spectroscopy using 785 nm excitation is an excellent compromise, involving the use of low laser power (20 mW) and typically resulting in fluorescent-free spectra (Wang and McCreery, 1989).

There has been a substantial increase in the clinical use of local anaesthetic preparations in recent years. These preparations are employed ostensibly for the provision of pain relief prior to venepuncture in adults and children (McCafferty et al., 1989, 2000) but increasingly find use in various minor topical surgical procedures, for example, the treatment of port wine stains (McCafferty et al., 1997).

Eutectic mixture of local anaesthetics (EMLA) cream has been widely used for the provision of topical local anaesthesia to healthy, intact skin. It consists of 2.5% (w/w) of both lidocaine and prilocaine presented in an emulsified base. Typically, a 1 h occluded application of EMLA cream produces approximately 30 min cutaneous anaesthesia (Juhlin et al., 1980). Formulations based on tetracaine (Fig. 1) have shown a shorter onset time and a greater clinical duration of anaesthesia than EMLA cream (Woolfson and McCafferty, 1993a; Khorshid and Cerio, 1997). This is due to the tetracaine phase-change system (Woolfson and McCafferty, 1993b). Tetracaine forms a meta-stable hydrate in the presence of water, and this results in a depression in its melting point from 42 to 29 °C. Thus, the drug melts at skin temperature and forms an oil, which is rapidly absorbed into the skin and provides a superior clinical profile to local anaesthetic preparations based on drugs other than tetracaine (Khorshid and Cerio, 1997; van Kan et al., 1997).

Further, Woolfson et al. (1998) described the formulation and development of a novel integrated bioadhesive patch device for percutaneous local anaesthesia. This device offered a similar clinical profile to tetracaine gels (McCafferty et al., 2000), but contained substantially less drug.

The aims of the present study are two-fold; firstly, to establish non-invasively whether the tetracaine patch delivery system was stable, and secondly to study the diffusion of tetracaine into living skin as a function of time. Raman spectroscopy was chosen as a suitable tool to achieve these aims, partly on account of the largely non-invasive nature of the technique, the weak Raman scattering cross-section of water, but primarily because spectra could be recorded without prior sample preparation, enabling direct investigation of formulations in their clinically applicable form.

2. Methods

2.1. Materials

Bioadhesive patches were prepared from tetracaine base USP (Orgamol Ltd., Switzerland), Natrosol (hydroxyethylcellulose (HEC), grade 250 HHX-Pharm) (Hercules Ltd., UK) and a co-polymer of poly(methylvinyl ether/maleic anhydride) (PMVE/MA) (Gantrez® grade AN-139, ISP Corp., UK) as described previously (Woolfson et al., 1998).

2.2. Methods

For in vivo studies, tetracaine gels were prepared by a standard technique (Woolfson and McCafferty, 1993b) but with the addition of 2% (w/w) rutile TiO_2. Rutile TiO_2 was prepared by placing a portion of anatase TiO_2 in an oven at 700 °C for 1 h. The completion of this polymorph transition was monitored by powder X-ray diffraction and Raman spectroscopy. An analogous gel containing 2% (w/w) rutile TiO_2 but without tetracaine was prepared as a control. In order to remove any potential errors due to inhomogeniety within samples, all samples were rotated under the laser beam during acquisition. Raman spectra of the completed patch preparation, and of individual patch components, were all recorded.

Seven areas of skin, each approximately 1 $in.^2$, on a human forearm were coated with 0.5 g of gel and covered with a polythene film to prevent the gel drying out. The time of each application was noted

and at varying points after sample application (0, 10, 20, 30, 40, 50 and 60 min) the covering film was removed from one application region and the remaining gel removed, mixed thoroughly, and placed in a sample container. This was repeated for all areas of application at the appropriate time intervals. This entire procedure was repeated twice, leaving 2 weeks between application sessions. Peak areas were measured in order to generate peak intensity ratios.

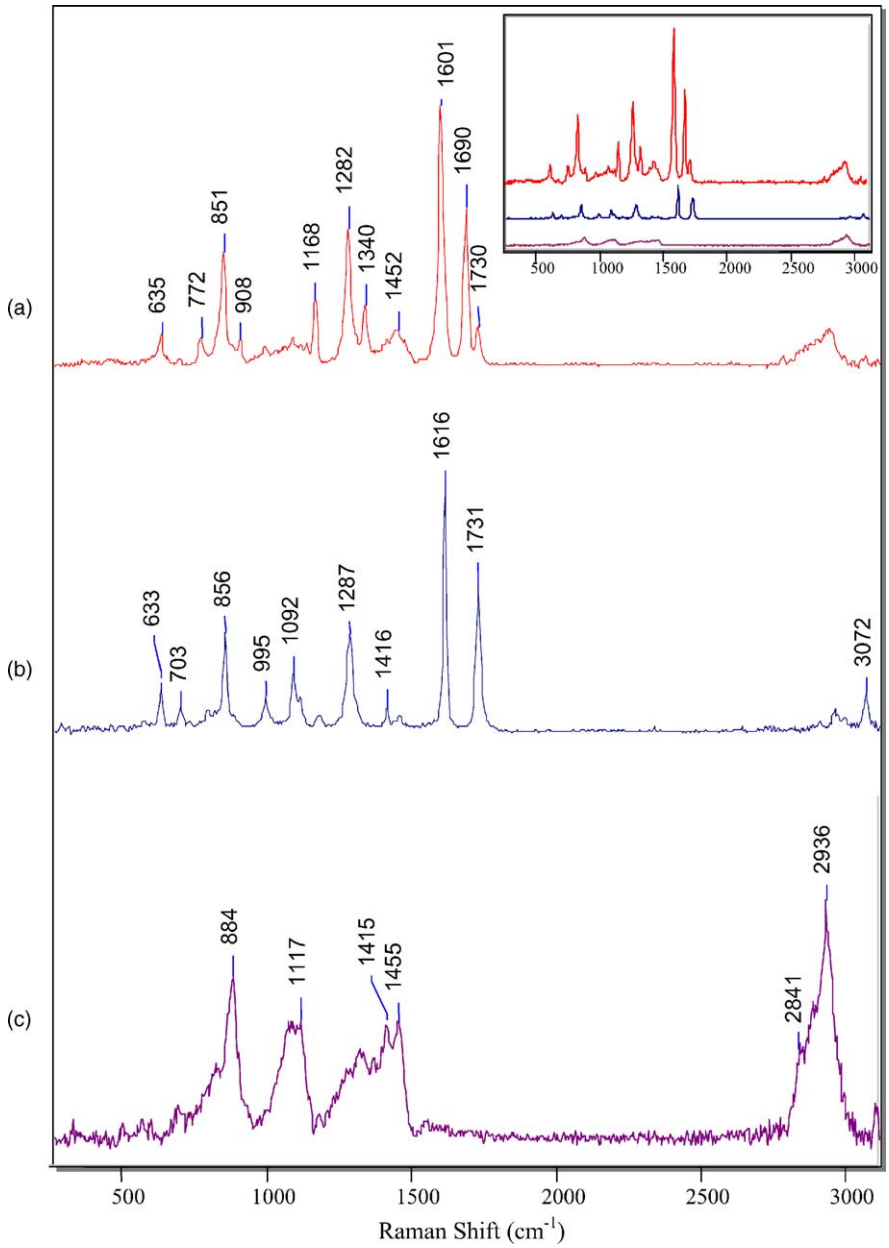

Fig. 2. Raman spectra of: (a) entire patch in clinically applicable form, (b) aluminium-coated backing film, (c) HEC-PMVE/MA. Note: Inset shows all spectra to scale.

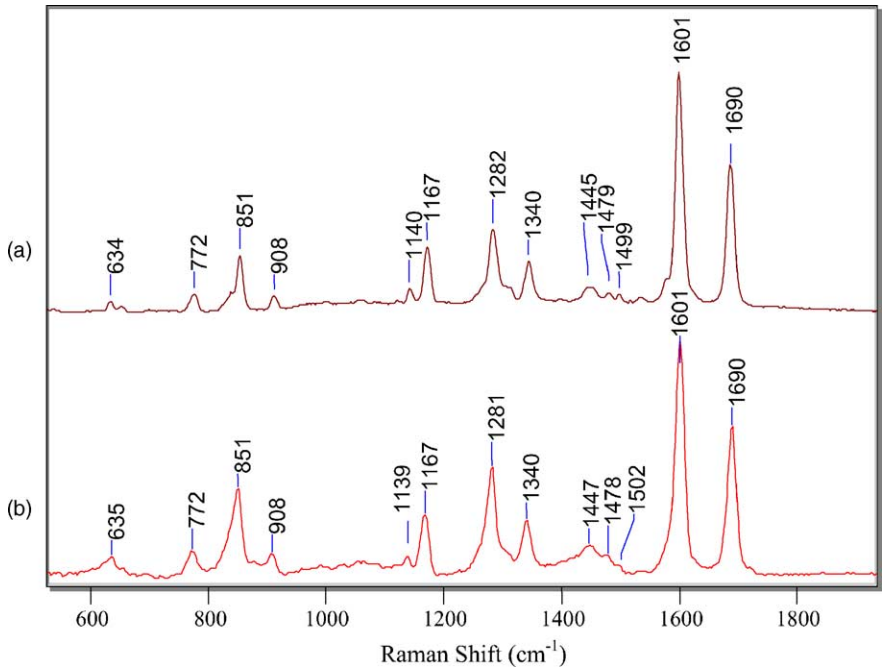

Fig. 3. Raman spectra of: (a) pure tetracaine, (b) subtraction of Raman spectra of HEC PMVA/MA and backing film from the Raman spectrum of entire patch system.

3. Results and discussion

Representative spectra of tetracaine patch formulations and its constituents are shown in Fig. 2. Fig. 3 shows the spectrum of pure tetracaine and a spectrum generated by subtracting HEC, PMVA/MA and the backing film spectra from a spectrum of the entire patch system. This 'generated' spectrum compares closely with that of the pure tetracaine (Fig. 3a), with detailed inspection showing no significant band shifts or relative intensity changes. From this it can be reasonably inferred that there are no interactions between the drug and the formulation excipients (HEC and PMVA/MA).

This is significant in terms of drug release, indicating that the drug has not reacted with the polymer matrix and that it is apparently unbound within this formulation. Although this conclusion has also been previously suggested by HPLC (Woolfson et al., 1998) the chromatographic technique required significant sample preparation and lengthy subsequent analysis compared to the Raman method used in this study. The present study also suggests that the drug is readily compatible with its formulation and that this particular vehicle does not inhibit or retard drug release. This is of particular importance in the provision of clinically effective percutaneous local anaesthesia, where onset time is of key importance (McCafferty et al., 2000; Woolfson and McCafferty, 1993b).

Raman spectroscopy is not an absolute technique, in that spectral peak intensities are not indicators of sample concentration. This is the result of factors such as intensity-dependent focus, instrument alignment and degree of sample homogeneity. In order to determine the absolute concentration of a component within a mixture it is necessary to measure the spectrum of one component to another, where the concentration of one component remains constant while the other increases or decreases. As tetracaine concentration in the gel should decrease with the length of time the gel has been in contact with the skin and the other gel components (HEC/PMVE-MA) should not diffuse into the skin to any appreciable extent, the spectral peaks associated with both HEC and PMVE/MA could theoretically be used as internal standards. However, as the Raman spectra of HEC

and PVMA/MA are quite weak (see Fig. 2 inset) in comparison with the tetracaine spectrum there is considerable potential for error in adopting this approach. Therefore, an internal standard was added to the tetracaine gel. Such an internal standard must satisfy a number of criteria. Specifically, it must not diffuse into the skin, nor migrate to the gel–skin interface and it must be clinically and chemically inert.

Due to issues associated with the use of $CaCO_3$ (spectral overlap with tetracaine) and anatase TiO_2 (photodegradation), they were not employed in this study, and rutile TiO_2 was chosen as the standard as it did not interfere with the spectra of tetracaine and was photostable. Further, TiO_2 is commonly added to many topical cosmetic formulations and its topical safety is well established.

Following application of the TiO_2-doped tetracaine gel, partial local anaesthesia, self-assessed, as described previously (Cooper et al., 1987) was experienced after approximately 10 min, with complete local anaesthesia achieved after approximately 20 min. Cooper et al. (1987) employed four methods of pain

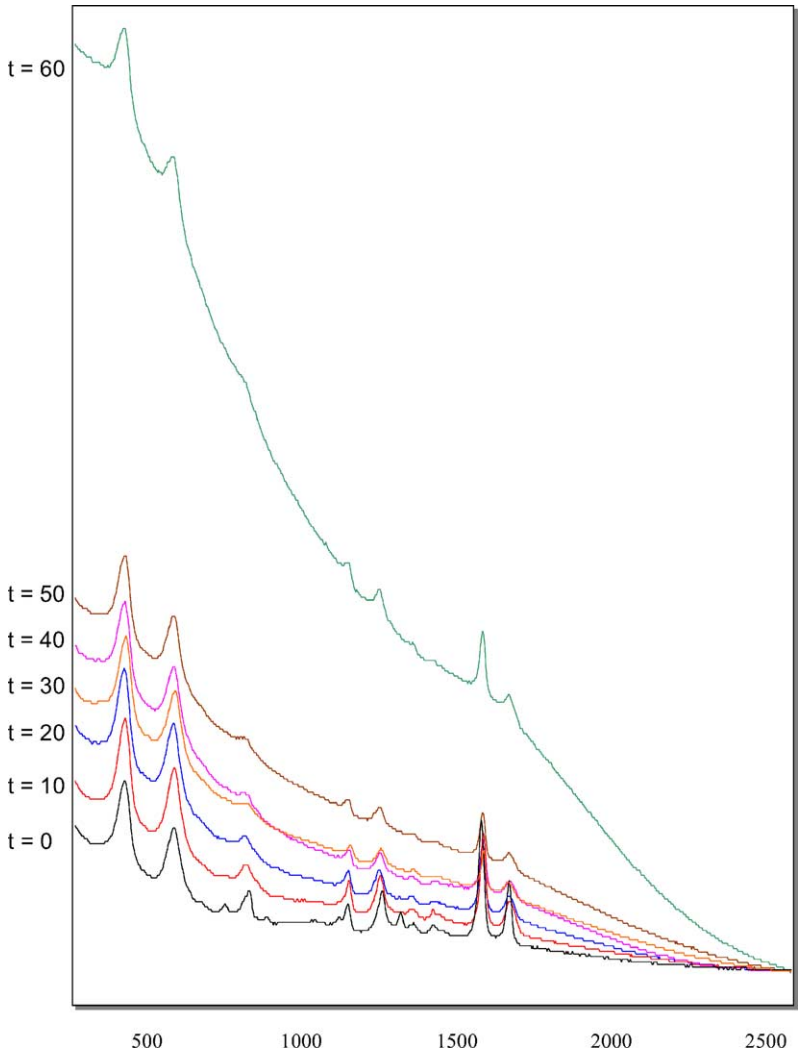

Fig. 4. Raman spectra of the rutile TiO_2-tetracaine gel, indicating changes in baseline over the recorded time range, 10–60 min (*t* is the exposure time of gel preparation to human skin).

assessment. These were a verbal rating scale, a visual analogue scale, and visual analogue and four-point observation scales employed by the practitioner. All methods employ the use of a pin-prick with which to provide pain. Significant differences were found between each pain scale, with the lowest pain scores recorded by the practitioners. Where pain has been assessed by both the patient and an observer, the patient usually records a higher pain score. With this in mind, the method of self-assessment of pain used

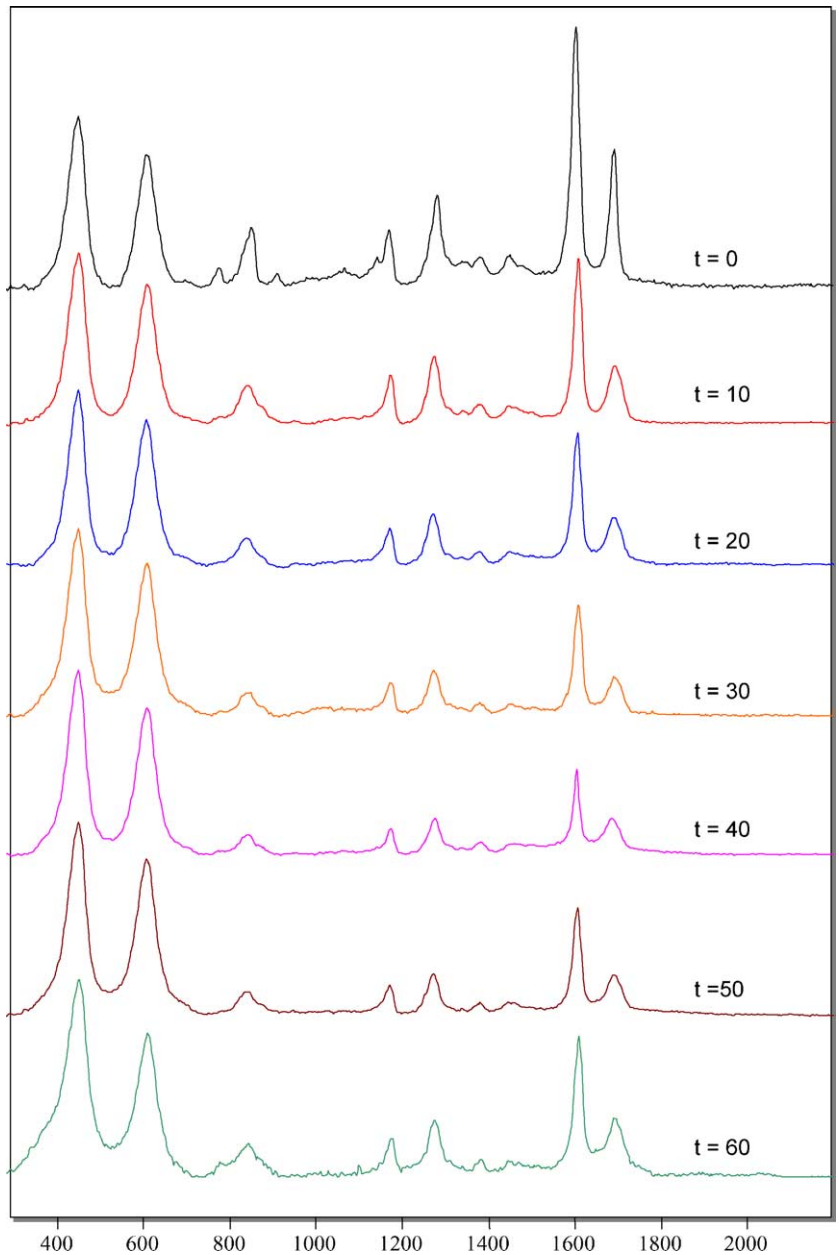

Fig. 5. Baseline-corrected Raman spectra of rutile TiO_2-tetracaine gel over recorded time range (t is the time in minutes).

in this study was chosen to reflect a potential "worst case" scenario. Although observer assessment is necessary in the study of pain in children, in adult patients self-assessed pain scores would present a more accurate reflection of the degree of anaesthesia induced by a particular application (Cooper et al., 1987; Hopkins et al., 1988; Woolfson and McCafferty, 1993a).

The observations recorded in this study are consistent with other clinical applications of tetracaine-based gels (Khorshid and Cerio, 1997; McCafferty et al., 1989, 1997). Anaesthesia lasted for approximately 1 h after removal of the gel.

Raman spectra of the gels displayed a number of trends. Firstly, a fluorescent background appeared which increased steadily with contact time to skin. The baseline increase evident in Fig. 4 may be due to dehydration of the formulation. Indeed, a deliberately dehydrated gel (allowed to dry in air under ambient conditions) and which had never been in contact with skin, exhibited a significant fluorescent background. The cause of this phenomenon in dehydrated samples is unknown, as each component—when analysed in the pure state—did not exhibit any significant fluorescence.

Secondly, after exposure to skin, changes in the Raman spectrum of tetracaine were evident, as can be seen from the comparison in Fig. 5 of the spectra recorded initially and after exposure to skin. Specifically, the changes involve a broadening of the features at 774, 848 and 907 cm^{-1} bands, all of which are attributed to the 1,4-substituted aromatic ring (cf. Fig. 1). In addition, changes involving other aromatic ring vibrational modes include an intensity decrease in the 1139 cm^{-1} band, frequency shifts in features at 1282 and 1601 cm^{-1}, and a broadening of the 1690 cm^{-1} ester band.

The Raman spectral changes observed in this study, as noted above, suggest that tetracaine undergoes a phase-change upon application to the skin after some 10 min. The plot in Fig. 6 of the intensity of the tetracaine band (at ca. 1600 cm^{-1}) normalised to the rutile TiO_2 internal standard suggests that essentially complete absorption of the anaesthetic is achieved. The decrease in Raman tetracaine band intensities (Figs. 4–7), along with an absence in the concomitant alteration in the rutile spectra, indicates a decrease in the tetracaine concentration present in the gel. A baseline indicating complete tetracaine absorption appears to be reached after approximately 40 min of exposure. After this time little further absorption was observed. This would suggest that the stratum corneum "reservoir" was saturated with tetracaine at this time. This is consistent with the optimum application time required for tetracaine gels to attain maximum clinical efficacy.

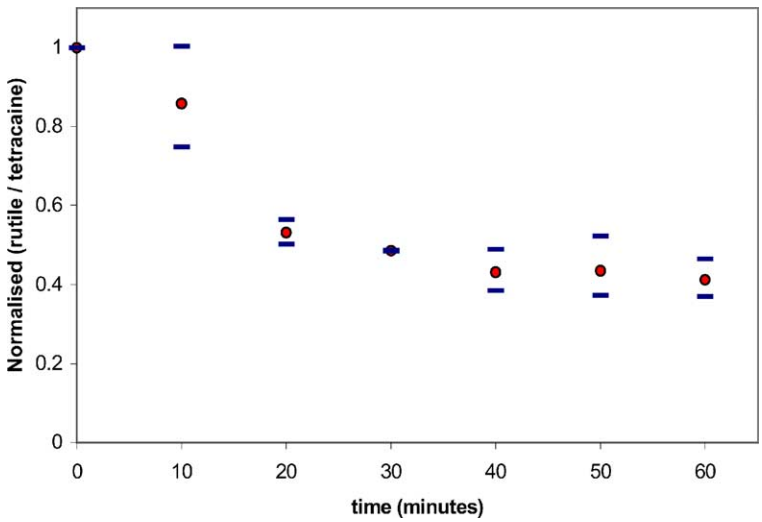

Fig. 6. Plot of peak area of normalised rutile TiO_2-tetracaine relative bands areas plotted against exposure time to human skin. Represented as the mean and standard deviation based on two scans.

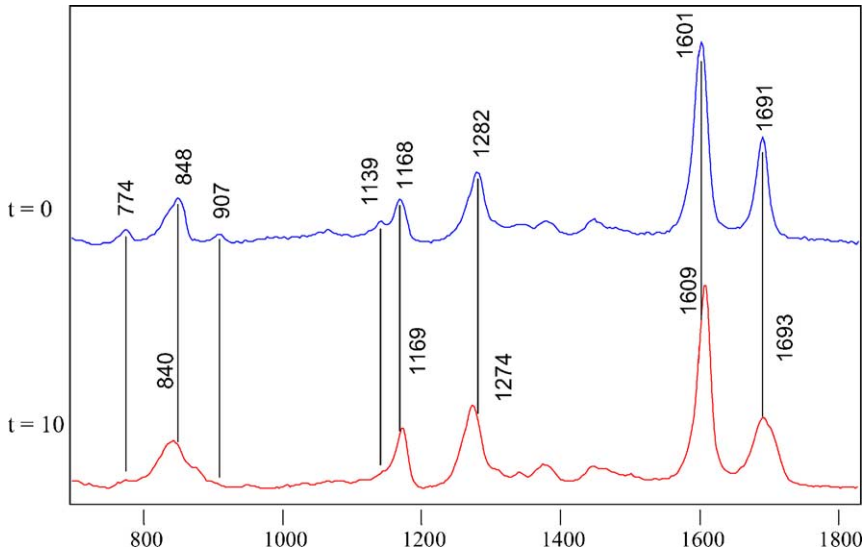

Fig. 7. Changes in tetracaine band positions observed after exposure to skin.

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