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Review

# Applications of ATR-FTIR spectroscopic imaging to biomedical samples

S.G. Kazarian *, K.L.A. Chan

*Department of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, UK*

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**Abstract**

FTIR spectroscopic imaging in ATR (Attenuated Total Reflection) mode is a powerful tool for studying biomedical samples. This paper summarises recent advances in the applications of ATR-FTIR imaging to dissolution of pharmaceutical formulations and drug release. The use of two different ATR accessories to obtain chemical images of formulations in contact with water as a function of time is demonstrated. The innovative use of the diamond ATR accessory allowed in situ imaging of tablet compaction and dissolution. ATR-FTIR imaging was also applied to obtain images of the surface of skin and the spatial distribution of protein and lipid rich domains was obtained. Chemical images of cross-section of rabbit aorta were obtained using a diamond ATR accessory and the possibility of in situ imaging of arterial samples in contact with aqueous solution was demonstrated for the first time. This experiment opens an opportunity to image arterial samples in contact with solutions containing drug molecules. This approach may help in understanding the mechanisms of treatment of atherosclerosis.


*Keywords:* FT-IR spectroscopic imaging; Infrared spectroscopy; Drug release; Dissolution; Skin; Aorta

**Contents**



## 1. Introduction

FTIR spectroscopic imaging has significant advantages compared to many other imaging methods for the characterisation of biomedical materials because it relies on the characteristic absorbance of corresponding molecular vibrations in the sample. Therefore, FTIR imaging does not require the use of added dyes or labelling methods for visualisation of different chemical components in the sample. The application of FTIR imaging to medical samples has been presented in a number of publications, [1–6] with most recent examples in this special

* Corresponding author. Tel.: +44 207594 5574; fax: +44 207594 1989.
*E-mail address:* s.kazarian@imperial.ac.uk (S.G. Kazarian).

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issue. Some other examples are: the study of the compositional changes in genetically modified bovine chondrocytes; [7] the protein and lipid ratio in cerebella and skin; [8,9] and the imaging of bone [10,11]. Lasch et al. [3] have successfully applied FTIR imaging to study human colorectal adenocarcinoma. Choo et al. [12] have measured the β-amyloid protein deposit in a slice of human brain tissue that contains the Alzheimer's diseases with FTIR spectroscopic imaging. All these recent applications have demonstrated the power and the applicability of this imaging method with the focal plane array (FPA) detector in the biomedical field.

FTIR imaging using ATR mode represents a complementary approach to the use of FTIR imaging in transmission or reflection modes. This approach has been previously demonstrated in combination with an infrared microscope [13] and is patented by Varian [14]. We have pioneered many applications of FTIR imaging in ATR mode [15–22]. In this paper, we review applications of this method to study dissolution and drug release and present some new results on applications of this methodology to study medical samples.

One of the key advantages of ATR-FTIR imaging is that it requires minimal or no sample preparation prior to spectral measurements. This is due to the fact that the penetration depth of IR light in the sample for ATR measurements is independent of sample thickness. Consequently, this approach is particularly suitable to measure substances with strong infrared absorption such as water. Furthermore, contrary to the general belief that a pressure must be applied to study solid samples with ATR spectroscopy in order to achieve good contact with the ATR crystal, we have shown that good contact and corresponding reliable FTIR images can be obtained by simply placing the tissue of skin directly on the diamond ATR crystal [15] or by applying very light force to the cross-section of aorta [23].

The use of synchrotron source infrared radiation provides significant improvement in signal-to-noise ratio but it is suitable for the measurements of rather small sample areas and requires mapping approach for the analysis of larger areas. Also, the spatial resolution achievable with a synchrotron source using FTIR imaging in transmission is inherently worse than that achieved by micro ATR-FTIR imaging with a Ge crystal. This is because the numerical aperture of the objectives is significantly greater for ATR objectives than for the objectives used for transmission measurements. Significantly enhanced spatial resolution (3–4 μm) was achieved [15] with micro ATR-FTIR imaging with the use of a conventional and inexpensive Globar infrared source. This is particularly important for the chemical imaging of biomedical samples. Thus, the enhanced spatial resolution allowed us to detect heterogeneity of the distribution of cholesterol esters in the cross-section of aortic plaques [23]. This has also allowed us to obtain clear chemical images of the cross-section of the human hair and extract information from the medulla of the hair without spectral "cross-contamination" from the nearby cortex domain of the hair [19]. The application of ATR-FTIR imaging also provided information about the size of the hydroxyapatite domains in polylactide/bioglass materials upon immersion to phosphate-buffer saline [24]. This example highlights the great potential of ATR-FTIR for the characterisation of biomedical materials in tissue engineering.

## 2. FTIR imaging for the study of dynamic systems

As mentioned before, FTIR images can be obtained by mapping with an aperture and the process is often automated with a computer controlled motorising stage. A consecutive measurement on an array of points on the sample allows one to obtain a map of spectra which can be used to generate chemical images. For the imaging of a dynamic system such as tablet dissolution, diffusion and crystallisation, all spectra measured for the construction of the image should be obtained at the same time. The development of multi-channel detectors which contain an array of small detector elements provides opportunity to measure thousands of spectra simultaneously. The infrared focal plane array (FPA) detector, which was originally designed for military purpose, has recently become available for civilian research. Lewis et al. [25] reported one of the first results of FTIR imaging using the FPA detector.

Some of the recent studies of dynamic systems using the FPA detector include the application of transmission FTIR imaging to study the diffusion of liquid crystals into polymers and the process of producing polymer dispersed liquid crystals, [26–28] polymer diffusion or dissolution in organic solvents, [29–35] drug release and tablet dissolution, [20,36–41] water sorption in pharmaceutical substances under controlled humidity [42,43] and high throughput applications [17,44–46]. The image acquisition only takes a few minutes with a step scan spectrometer. A step-scan spectrometer was used because a relatively long time was needed for the data to be read from the FPA detector and stored in the computer which required the moving mirror in the interferometer to stop for each data point acquired. Usually, several frames of readings are obtained and averaged at each mirror position to improve the signal to noise of the resultant spectra. However, in 1999 Snively et al. [47] demonstrated the use of a rapid scan (continuous scan) spectrometer for FTIR imaging. The main modification to the spectrometer was to have only one frame captured for each mirror position and the mirror moved in a continuous motion. They have argued that the displacement of the mirror during the snapshot of each frame was comparable to the vibration of the mirror in a step-scan spectrometer. In this way, the total acquisition time was reduced as fewer frames are being captured (no co-addition) and no stabilising time for the mirror was needed. They have demonstrated an FTIR image with 4 $cm^{-1}$ spectral resolution and a spectral range of 1360 $cm^{-1}$ can be collected in 34 s. New FPA detectors with faster data readout rates (ca. 10 times faster) are now available. This type of detector also runs in continuous scan mode and reduces the acquisition time significantly (down to a fraction of a second per scan) which allows more co-additions of data to be collected to improve the

SNR of spectra. The improvement in temporal resolution is important for the study of relatively fast dynamic systems.

## 3. Diamond ATR-FTIR imaging

ATR imaging with a diamond ATR accessory has shown to be a valuable tool with a wide range of applications, including pharmaceutical applications, [16,18,39–41,48] polymer systems under high pressure $CO_2$, [21] and biological systems, [15,23] thanks to the desirable properties of the diamond, reasonable spatial resolution and field of view [15]. Lenses are employed in the accessory to focus IR light to the diamond, which has a surface area of ca. 4 $mm^2$, giving a ca. 4× magnification to the sample imaged.

## 4. Applications of ATR-FTIR imaging to drug release

Application of FTIR spectroscopic imaging to study dissolution and drug release is an emerging area of pharmaceutical applications of vibrational spectroscopy. The quantitative information about the spatial distribution of chemical components in pharmaceutical tablets in contact with water as a function of time provides an important basis for building new mathematical models for the optimisation of controlled drug delivery. Better understanding of the mechanism of drug release from pharmaceutical formulations is needed to facilitate the design of such formulations. Unfortunately, despite numerous studies of dissolution of solid formulations there is still lack of understanding of processes within the formulation (or tablet) upon its contact with dissolution media. Currently, the pharmaceutical industry uses cumulative drug release data, which is a crude measure of drug dissolution, to infer a mechanism from this. Analysis of the drug concentration in dissolution media as a function of time provides little insight into the complex processes within the formulation. The chemical specificity of FTIR imaging provides new valuable information for the analysis of dissolution and for the development of new mathematical models. Below, we review and summarise recent developments in this field which were primarily performed in our laboratory. Formulations of poorly water-soluble drugs in water-soluble polymers are usually used to enhance drug dissolution rates. Significant questions still remain unanswered with regard to polymer dissolution and drug release. For example, how does drug release happen, primarily via diffusion or via polymer degradation? Does the molecular state of a drug change during the dissolution process? We attempted to answer some of these questions by utilising the opportunities offered by FTIR spectroscopic imaging to investigate the dynamic properties of specific polymer/drug systems. Our group has pioneered applications of ATR-FTIR spectroscopic imaging to study in situ dissolution of polymer/drug formulations in water. The ability of recording spatially resolved chemical images as a function of time has allowed us to view a dynamic process via simultaneous measurement of the distribution of polymer, drug and water. This spectroscopic imaging method is substantially superior to many of the other imaging methods due to the inherent chemical specificity of infrared spectroscopy and fast acquisition times of this technique. Koenig and co-workers [36] used FTIR imaging in transmission to study dissolution and drug release. This introduced a number of restrictions, most notably the very stringent restrictions on tablet size—only very thin samples can be studied in transmission when aqueous solutions are used. Furthermore, their transmission imaging studies necessitated the use of $D_2O$. The ATR-FTIR imaging is suitable for imaging of realistic tablets in contact with aqueous solution because of the shallow penetration of the evanescent wave into the sample. The resultant image represents the distribution of different components in a thin surface layer of the sample. This can be advantageous over the transmission imaging because imaging of the relatively thick samples in transmission represents a danger of obtaining spurious images if the thickness of the sample is greater than the size of studied domains; the image would represent the averaging information through the thickness of the sample [22].

## 5. Development of a macro-ATR-IR imaging approach for dissolution of polymer/drug formulations

A heated single-reflection (incident angle of 45°) ATR accessory with a ZnSe crystal has been used for imaging [20]. The film of polymer/drug formulation was attached to the ZnSe crystal in such a way to ensure good contact of the polymer/drug sample with the ATR crystal so that water would not leak into the space between the polymer and crystal. The reproducible contact between sample and ATR crystal was verified by measuring the absorbance of the polymer spectral bands for several prepared samples. The absorbance of the polymer bands for different films of the same polymer remained almost the same in these measurements. The sample was sandwiched between the crystal and a cover glass with a relatively thin spacer (few hundreds micrometers) which defined the thickness of the sample. Under this arrangement, water enters the sample film only from the side. Half of the area on the ATR crystal to be measured via the FPA detector was left uncovered by the sample for imaging the materials leaving the sample after the addition of water (Fig. 1). The first

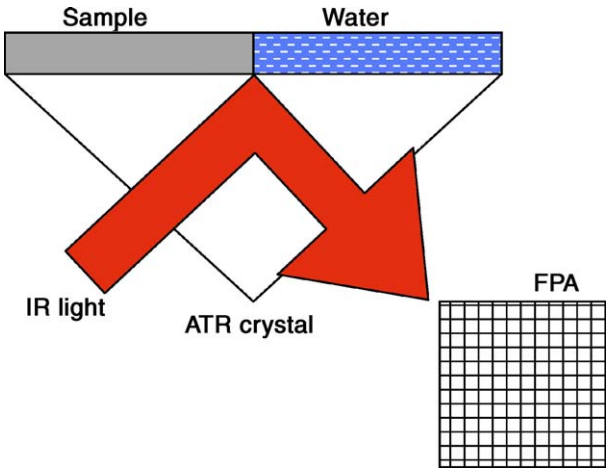


Fig. 1. Schematic presentation for studying the polymer–water interface using macro-ATR-imaging approach with inverted pyramid ZnSe crystal.

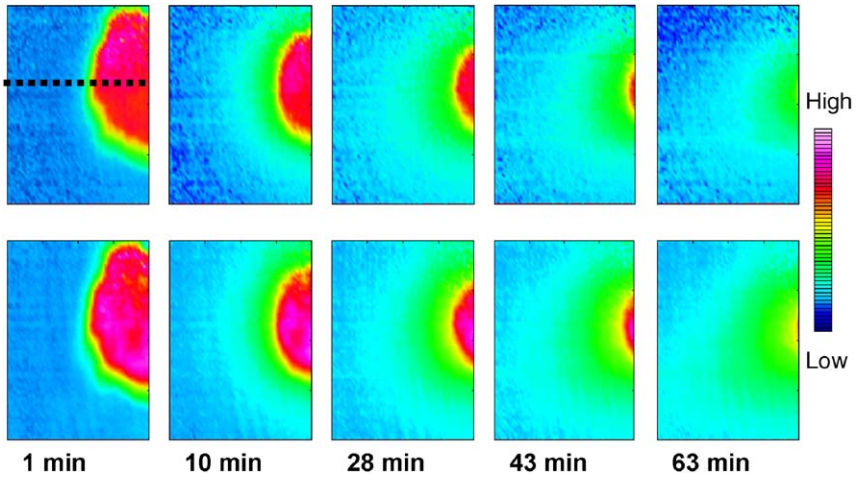


Fig. 2. Macro ATR-IR images of PEG/sodium benzoate showing distribution of sodium benzoate (top row) and PEG (bottom row) as a function of time. (Reprinted with permission from Macromolecules, 2003, 37, 9866–9872, Copyright (2003) American Chemical Society.)

polymer matrix that we used in our ATR-FTIR imaging was poly ethylene glycol (PEG) and some of our first experimental results involving drug formulations with PEG are summarised below.

First, we applied this approach to the system with a water-soluble model drug incorporated into PEG [20]. Sodium benzoate (SB) was chosen as a model substance because of its high solubility in water and because it is frequently used as antimicrobial preservative and tablet lubricant. The spatial distribution of SB and PEG was followed as a function of time using an ATR-IR imaging approach and the corresponding images presented in Fig. 2. These images show that both polymer and drug dissolve almost simultaneously and imply that drug release proceeds via polymer dissolution rather than diffusion of the drug through the polymer. It is important to note that the images shown in Fig. 2 represent concentrations profiles as a function of time due to the interrelation between spectral absorbance and concentration.

The imaging data can be presented in a different way, such as in Fig. 3 which shows the corresponding absorbance profiles measured along the dotted black line shown in Fig. 2, as a function of time for three components: PEG, SB and water. These quantitative data are particularly important for diffusion analysis and modelling.

These results with a water-soluble substance were very encouraging; however, many drugs are actually poorly soluble in water. It is generally believed that molecular dispersion of a drug in a polymer matrix would ensure enhanced dissolution of the drug [49]. Fortunately, we have recently shown that supercritical fluid impregnation of ibuprofen in polymer matrices allows one to achieve a molecularly dispersed drug [50]. This approach is summarised in a recent publication [51]. This approach was applied to prepare molecular dispersion of ibuprofen in PEG for subsequent dissolution studies via ATR-FTIR imaging [20]. The FTIR spectra extracted from the bottom left image shown in Fig. 4 of such formulation clearly showed that all ibuprofen is molecularly dispersed without presence of crystallites. However, the evolution of these images as a function of time has revealed first accumulation of drug in the outer layer of circular formulation and then the presence of dimerized drug followed by the formation of crystallites of ibuprofen [20]. This was an important observation of crystallization of an initially molecularly dispersed drug which occurred upon contact with the dissolution medium because crystallization slows overall drug dissolution. This phenomenon would not be detected by the conventional dissolution tests (when only the total amount of dissolved drug is measured in the dissolution media as a function of time) and demonstrated that ATR-FTIR imaging can provide important insight into mechanism of drug release.

Our next steps have been to explore the effect of different factors on the detected phenomenon of drug crystallization. For example, change of molecular weight of polymer, ratio of drug loading, change in pH of aqueous solution and addition of surfactant. However, these factors did not have significant effects on crystallization of ibuprofen upon dissolution in water. Finally, we used the known approach of drug entrapment in cyclodextrines and applied ATR-FTIR imaging to dissolution of

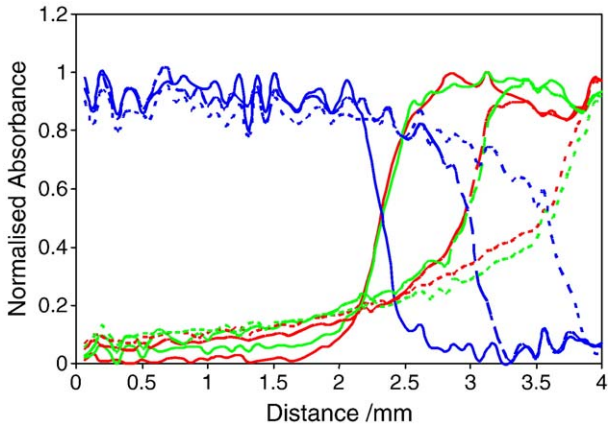


Fig. 3. The normalised absorption profile of water (blue), SB (green) and PEG (red) along the line indicated on the image in Fig. 2 at different times: 1 min (solid line), 10 min (dash line) and 43 min (dotted line).

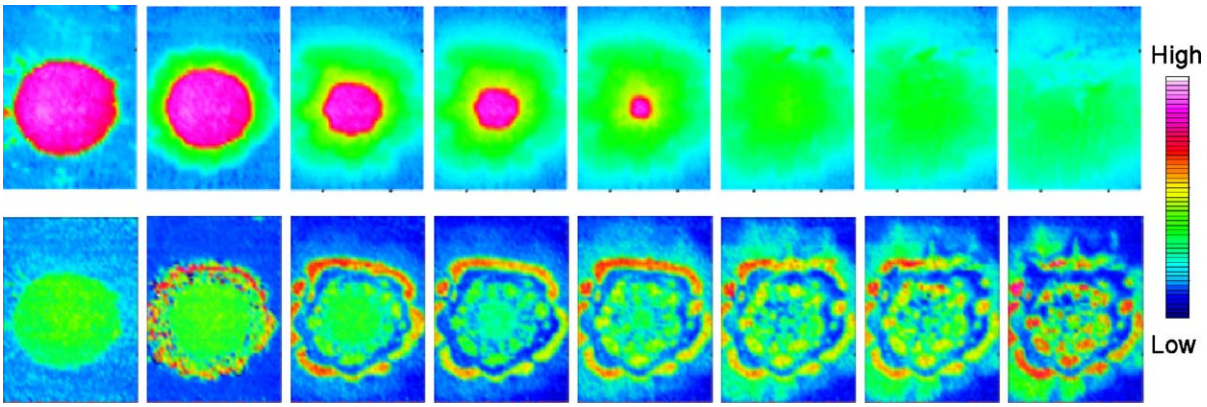


Fig. 4. Macro ATR-FTIR images of PEG/ibuprofen formulation show distribution of PEG and ibuprofen as a function of time (from left to right) during contact with water. The images (size 3.8 × 5.3 $mm^2$) are based on the distribution of the integral absorbance of the IR band of PEG-8000 (top row) and based on the distribution of the integral absorbance of the $\nu$(C=O) band of ibuprofen (bottom row) acquired sequentially as a function of time (time when last images on the right were acquired was 40 min after addition of water). (Reprinted in part with permission from Macromolecules, 2003, 37, 9866–9872, Copyright (2003) American Chemical Society.)

ibuprofen/cyclodextrine formulations (Fig. 5). In these studies [20] convincing evidence was provided that no ibuprofen crystallisation occurs in such inclusion complexes until the ratio exceeds 1:1. These our first results have revealed the origin of enhanced dissolution rates of ibuprofen in certain formulations and opened new opportunities for studying drug release [20].

## 6. Innovative design of cell for imaging of compaction and dissolution of pharmaceutical tablets

The use of ZnSe crystal for macro ATR imaging was promising but the following step was our discovery that some modification and alignment of a diamond ATR cell (Golden Gate), which was not designed for imaging applications, can actually be used for imaging applications with enhanced (for macro ATR) spatial resolution up to 15 μm without the use of a microscope [15]. Imaging with this accessory has been proven to be successful for the first time and images from the area of ca. 1 $mm^2$ have been obtained. The suitability of this single reflection diamond ATR accessory for imaging opened up exciting applications for pharmaceutical samples [16] including compaction and drug release. We have developed a novel compaction cell based on this diamond ATR accessory [40]. This approach allowed us to compact a tablet directly on a diamond crystal with subsequent imaging of the tablet. This approach allowed us to prepare tablets from HPMC, a common excipient of pharmaceutical tablets, and also ensure good contact between the ATR crystal and the tablet. A model tablet consisting of HPMC and caffeine has been studied using this approach and effects of water intake were related to the swelling of HPMC and dissolution of caffeine; translocation of a larger caffeine particle was also observed [40]. The important feature of the cell was the introduction of flow around the tablet in the cell which helped to prevent saturation of the solution (Fig. 6).

In further development, we have combined imaging of a compacted tablet with a conventional dissolution test [52]. This was a useful development of applications of ATR-FTIR imaging to study the dissolution of a compacted tablet. The combined approach was achieved by linking a flow-through cell to a UV detector to measure and quantify the amount of dissolved drug (Fig. 6). In this way, changes in drug concentration in the aqueous solution have been measured similarly to the conventional dissolution test [52].

The demonstrated possibility of ATR-FTIR imaging using a diamond ATR opened tremendous opportunities for studying tablets prepared via compaction [40,41,52]. The special compaction cell was designed and built in such a way that introduction of water flow was possible once the tablet was compacted. This has allowed us to combine our imaging approach with measurement of concentration of dissolved drug in water as a function of time, and thus interrelated imaging data with more conventional dissolution data. Nicotinamide was chosen as one of the model drugs used for these studies [52]. An important development in this work was the application of a partial least squares (PLS) calibration for accurate quantitative analysis of the concentrations of the different components [52]. This analysis, using all available spectral region, has allowed us to obtain images of the real concentration of water, HPMC and nicotinamide as a function of time rather then images based on absorbance of one particular spectral band for each component. The total amount of dissolved drug obtained from these imaging data has been compared with the amount of dissolved drug in the effluent of the cell by the UV detector [52]. It has been shown that the results of the FTIR imaging can be related to the results of the conventional dissolution test. Good correlation was obtained in the case of the water-soluble drug nicotinamide [52]. The correlation proved the validity of the technique, and


Fig. 5. Schematic diagram showing a drug molecule being encapsulated in a cyclodextrines molecule.

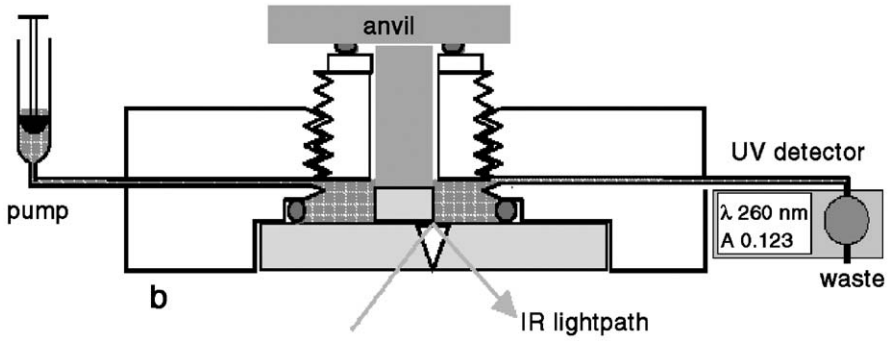


Fig. 6. Schematic presentation of compaction flow-through cell used in ATR-IR imaging linked to a UV detector shown on the right for quantitative measurement of dissolved drug. (Reprinted from Ref. [52], with permission from Elsevier).

the obtained drug concentration profiles during dissolution can be used to fit the data to current models for drug release. Direct observation and quantification of this phenomenon is unattainable by dissolution techniques, but FTIR imaging in ATR mode provides a means to achieve this. In a more recent development, we applied this combined approach to study dissolution of tablet containing poorly water-soluble drug, diclofenac sodium [37]. In this work, the potential of this combined approach was fully realized. Specifically, precipitation of the drug was observed in a number of cases (depending on pH) via ATR-FTIR imaging [37]. The relative amounts of precipitate were correlated to the release profiles calculated from FTIR imaging measurements and compared to the results obtained by the cumulative analysis of dissolved drug using UV-Vis detection. The effect of precipitation could clearly be visualised due to the high chemical specificity of FTIR imaging. FTIR imaging combined with the presented flow-through dissolution test show significant potential for studying dissolution and drug release from pharmaceutical tablets [37].

## 7. Validation of ATR-IR imaging approach and multivariate analysis

As part of our studies we have also clarified the issue of possible leakage of water into interface between the ATR crystal and a tablet comprising swellable polymer, HPMC [41]. This was required for validation of our macro ATR approach for studying compacted tablets. It has been shown that the speed of

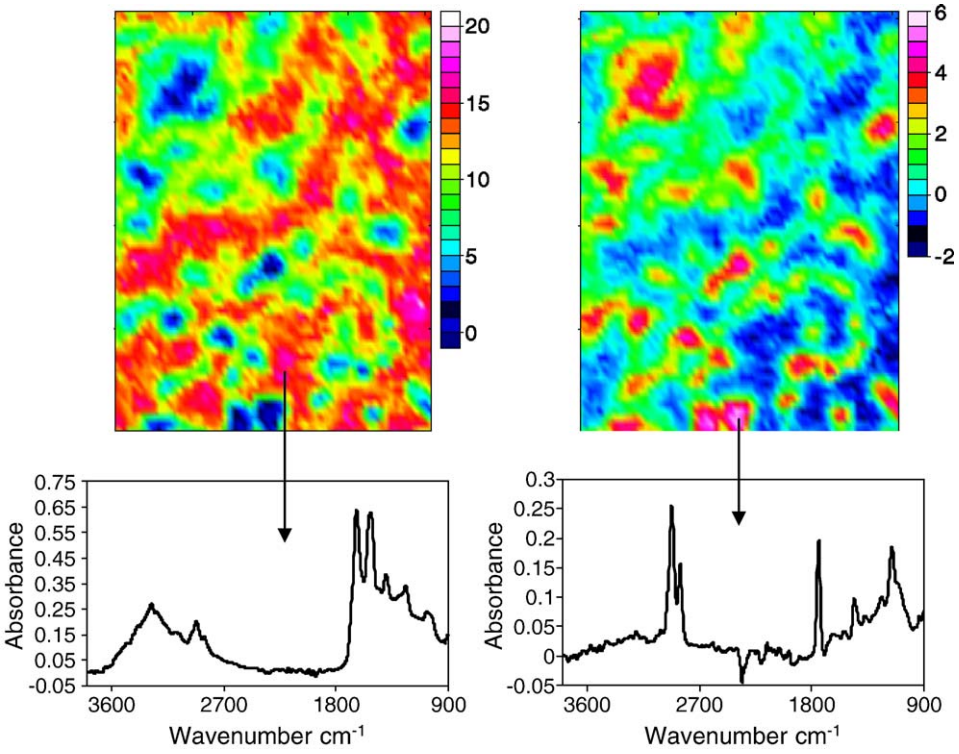


Fig. 7. FTIR images of skin with distribution of protein rich (amide I band, 1680–1660 $cm^{-1}$) and lipid rich (carbonyl band, 1780–1720 $cm^{-1}$) domain shown on the left and right respectively.

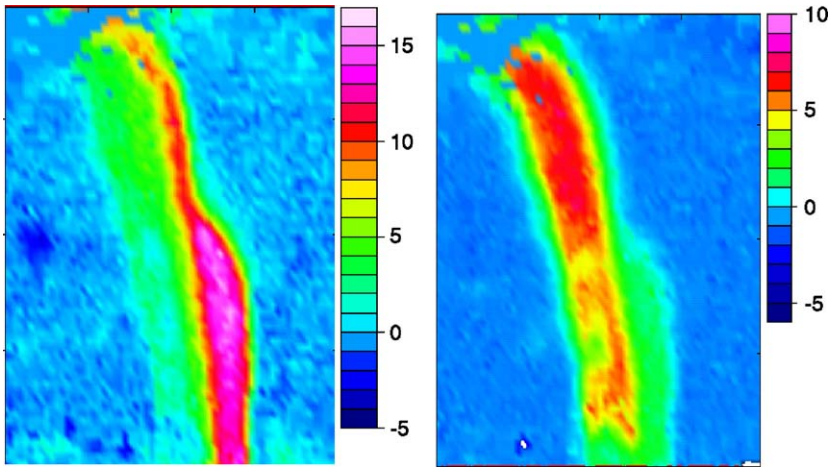


Fig. 8. FTIR images representing the distribution of aortic plaque (left) and protein (right).

water intake appeared independent of the contact (which was function of compaction pressure), indicative that the leakage of water was negligible [41]. It has also been shown that hydration of HPMC with formation of the corresponding gel layer effectively "seals" the gap between tablet and crystal thus preventing the leakage of water into the possible void. The swelling of HPMC upon wetting establishes a barrier, which effectively increases the contact between tablet and crystal. The quantitative treatment of these imaging data involved the use of the principal component analysis (PCA) which is one of the multivariate methods of analysis of imaging data [41]. This analysis also provided evidence for a feature that has not been found in the previous mathematical models of dissolution of HMPC tablets, namely a fast initial water uptake which we explained was due to initial porosity of the polymer. Our imaging data demonstrated that only after the HMPC was exposed to water for a while, its surface layer forms a gel, thereby slowing down the further water intake.

The most recent development of applications of ATR-FTIR to pharmaceutical tablets includes in situ imaging of compaction to study the effect of compaction pressure on the distribution of different components in a tablet [48]. The power of the multivariate approach was also demonstrated in that work to distinguish components with spectral bands that were not well resolved and overlap. The effect of additives on the density distribution in the thin layer of the tablet was also studied with in situ ATR-FTIR imaging. Another very promising application of ATR-FTIR imaging is its use for high-throughput analysis of pharmaceutical formulations [17,44]. The approach, demonstrated in that work, may have an impact for studies of biomedical materials in aqueous environments. For example, many medical samples (e.g., tissues) may be simultaneously studied under identical conditions to ensure reliable comparison between different samples.

## 8. Application of FTIR imaging to the surface of skin

Skin is the largest organ in the human body. It is a barrier that protects the body from the surrounding environment. The uppermost layer of the skin is the stratum corneum (SC) which is the major resistance to skin permeation. Understanding the diffusion process across the skin is very important for the development of transdermal drug delivery. ATR-FTIR spectroscopy has been shown to be a useful tool for studying the diffusional behaviour in SC [53]. However, it is not easy to determine the actual route that diffusion takes place in the SC [54]. ATR imaging offers the opportunity to obtain both chemical and spatial information of the skin which provides a possible means to determine the pathway of the diffusion process in the SC. The following images in Fig. 7 demonstrated the result obtained with the ATR-FTIR imaging of the surface of skin. The heterogeneous distribution of lipid rich and protein rich domain can easily be distinguished with this technique by noting the spectral differences between each component. Representative spectra extracted from the different domains of the sample are shown under the corresponding images in the Fig. 7. These spectra demonstrate that the generated complementary images represent the true distribution of the particular component (protein or lipid) rather than presentation of the "contact quality" which is possible in ATR-FTIR in the case of imaging of samples with hard and rough surfaces. The pathway

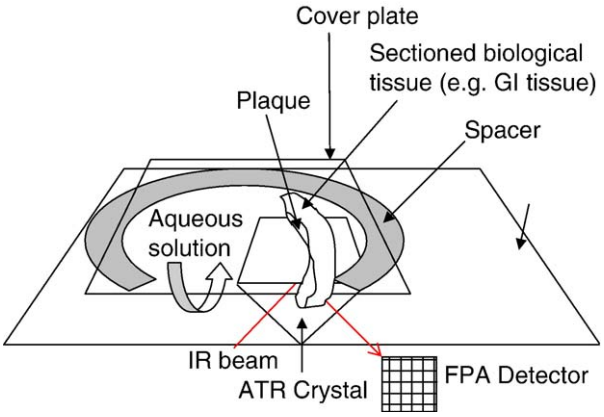


Fig. 9. Schematic diagram showing the setup of the in situ experiment.

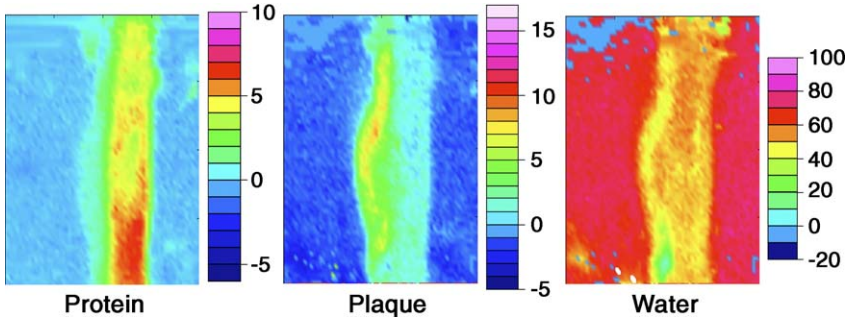


Fig. 10. FTIR images showing the distribution of different components of an aorta in contact with water.

of drug delivery, therefore, may be delineated with the ATR-FTIR imaging approach.

## 9. Application of FTIR imaging to cross-sections of aorta

The success of the in situ study of tablet dissolution inspired us to apply a similar technique to study biological tissues in contact with aqueous solution with the ATR-FTIR imaging approach. The chemistry of any interactions between the aqueous solution and the sample tissue may be understood via the analysis of spectra measured over the imaging area. Sections of rabbit aorta, which has been treated in the manner as described in a previous publication, [23] were used to demonstrate the feasibility of this in situ study. Fresh aorta containing an atherosclerotic plaque was transferred onto the surface of the diamond ATR crystal carefully. After the excess water was removed form the tissue, the aorta immediately formed a good contact with the ATR crystal. The distribution of protein (amide II band, 1565–1495 $cm^{-1}$, was used for protein characterisation) and plaque (CH stretch, 3000–2800 $cm^{-1}$, was used to show the lipid rich domain) have been revealed by the FTIR measurement and the images are shown in Fig. 8. The band assignment is only approximate which serves well for the purpose of this study. More rigorous spectral analysis such as multivariate analysis is particularly important for the analysis of biomedical substances which may extract more information form the spectral data measured [2] and will be explored in future work.

A glass slide which acted as a top plate was then placed on top of the aorta to cover the sample. A spacer with a similar thickness to the aorta section was introduced and a small pressure was applied to the top plate to ensure the sample forms good contact on both the diamond surface and the top plate. A schematic diagram of the setup of this arrangement is shown in Fig. 9.

Distilled water was used as a model aqueous solution and was introduced to the sample. FTIR image of the aorta in contact with water was measured. Images representing the distribution of protein, plaque and water (OH stretch, 3700–3100 $cm^{-1}$) are shown in Fig. 10.

From Fig. 10 we can observe that water is absorbed into the tissue, preferentially in the amide rich domain (aorta wall) rather than the lipid rich domain (the plaque). The result from this experiment implies that this imaging approach is suitable for the in situ study of biological tissue under treatment of drugs or simulated body fluid.

## 10. Conclusion

In this paper, we have shown the potential of ATR FTIR imaging in biomedical research. General principles of experimental methodology and applications of ATR FTIR imaging to study tablet dissolution and drug release have been summarised. Different fields of view in imaging with different ATR accessories are possible; thus providing versatility in this imaging method. Innovative design of compaction cell combined with flow-through dissolution test promises exciting possibilities for pharmaceutical research. Study of biological samples is another area where the ATR-FTIR imaging approach has certain advantages compared to other imaging modes. In preliminary studies, the surface of human skin was measured using ATR-FTIR imaging, demonstrating the applicability of macro-ATR imaging with a diamond accessory to skin imaging. These preliminary results also demonstrate the possibility of studying skin surface in contact with topical formulations to probe the mechanisms of transdermal delivery. It is the first time that it has been possible to obtain FTIR images of arterial tissue in contact with water. This result presents clear opportunities for further development. For example, one could add drug molecules in aqueous solution to study their partitioning and interaction with the plaque materials in a search of understanding of treatment of artherosclerosis. It is hoped that this paper has demonstrated the power of ATR FTIR imaging in applications to a range of biomedical applications, a method that provides exciting opportunities in the biomedical research.

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