Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 269–273

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Attenuated total reflection infrared spectroscopy (ATR-IR) as an *in situ* technique for dissolution studies

Abe Kassis, Vrushali M. Bhawtankar, John R. Sowa Jr.*

Department of Chemistry and Biochemistry, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079, USA

ARTICLE INFO

Article history:
Received 5 February 2010
Received in revised form 16 April 2010
Accepted 19 April 2010
Available online 27 April 2010

Keywords:
FT-IR
Attenuated total reflection
Tablet
Dissolution
Acetaminophen
Acetylsalicylic acid

ABSTRACT

Dissolution studies are critical tests for measuring the performance of a drug product. We have developed a novel technique using *in situ* ATR-IR spectroscopy to monitor dissolutions of pharmaceutical drug products. The accuracy of this technique is ±3% relative to HPLC using salicylic acid calibrator tablets and acetaminophen OTC tablets. This novel approach also gives the research laboratory the capability of analyzing individual ingredients in multiple tablets; for example, individual components of salicylic acid and acetaminophen tablets are easily distinguished. In addition, the individual ingredients of a multi-component tablet containing acetylsalicylic acid and acetaminophen are readily distinguished. The ATR-IR system was found to have good sensitivity and can analyze samples as low as 0.03 mg/ml. With improved sensitivity, this is a promising method for monitoring dissolution of pharmaceutical tablets with an excellent *in situ* capability for distinguishing individual components.

1. Introduction

Dissolution testing is a standardized methodology for measuring the rate of drug release from the two main oral dosage forms, tablets and capsules. These are immersed in an aqueous solution, and the concentration of the active ingredient is monitored as a function of time [1]. Unfortunately, dissolution testing provides limited or no information on chemical processes that take place within a dissolution vessel. One of the challenges the industry is faced with is to increase an understanding of the mechanisms governing dissolution. The current approach relies heavily on a data-driven approach. The health authorities have challenged the pharmaceutical industry to understand dissolution and make the dissolution test more biologically relevant [2,3]. In order to have a better understanding of dissolution, the industry needs to explore other applications that could give insight into what is happening inside of the vessel. Attempts have been made to improve the characterization of the controlled release formulations by FT-IR imaging in ATR-IR mode [4]. Van der Weerd and Kazarian explored FT-IR imaging as a useful application for studying the distribution of different components in the tablet, e.g., drug, polymer and water as a function of time. It was shown that the release profile obtained by FT-IR imaging is comparable to that obtained by the flow-through dissolution test with UV spectroscopic detection. In addition, FT-IR imaging was used to investigate the release of a poorly soluble drug from pharmaceutical tablets [5].

The majority of the methods for monitoring dissolutions utilize UV–vis spectroscopy or HPLC with UV–vis detection based on manually withdrawn aliquots. However, the sampling process is disruptive to the dissolution profile since removal of aliquots from the vessel disturbs the solution. In addition, there are instruments that allow real-time analysis using *in situ* UV–vis probes. For example, fiber optic dissolution testing is being used in the industry to monitor pharmaceutical drug product release. Fiber optic dissolution is also being used for formulation development [6].

There is interest in the development of new methods that do not require manual sampling. Also, for multi-component formulations it is important to be able to observe the dissolution profile of each active pharmaceutical ingredient (API). Thus, there is also interest in the development of new spectroscopic methods that enable observation of multiple components. The use of attenuated total reflection FT-IR (hereafter ATR-IR) for analyzing aqueous samples is limited by the relatively high concentration of analyte required for detection [7]. Hence, this research aims to investigate the use of ATR-IR as a potential application for monitoring and understanding the dissolution.

ATR (attenuated total reflection) spectroscopy is a sampling technique that is based on molecular vibration and the curvature of light beams when passing through different media. An ATR spectrum is generated by transmitting radiation, which can be IR (from 0.1×10^{-5} cm to 7.5×10^{-5} cm), VIS (from 7.0×10^{-5} cm to 4.0×10^{-5} cm), or UV (from 4.0×10^{-5} cm to 2.2×10^{-5} cm),

* Corresponding author. Tel.: +1 973 761 9034.
E-mail address: john.sowa@shu.edu (J.R. Sowa Jr.).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.04.023

through an optical crystal in contact with a sample and then determining what portion of the incident radiation is attenuated by the sample at a particular wavelength. ATR spectrometry is used extensively in clinical assays, medical diagnostics, and laboratory testing [8]. Since the depth of penetration for the evanescent wave in ATR spectrometry is shallow, there is a low incidence of Fresnel reflection. Thus, reliable spectral analysis of murky, semisolid, turbid, and optically dense solutions is possible with ATR spectrometry. Moreover, the ATR crystal is a relatively chemically resistant Zn–Se crystal that can be coated with an additional chemically resistant material which enables IR spectroscopy to be performed in aqueous solution.

Therefore, *in situ* ATR-IR spectroscopy has the unique potential to simultaneously address problems associated with manual sampling and multi-component analysis discussed above. Thus, this is a potentially useful and novel method for dissolution testing. Modern infrared instruments can be equipped with fiber optic probes containing ATR crystals that are chemically robust, provide excellent sensitivity and, with respect to *in situ* UV–vis, are not affected by turbidity. In addition, since IR is very sensitive to specific functional groups, it has greater versatility in differentiating components in a multi-component mixture than UV–vis spectroscopy [9]. In this paper, we describe the successful development of dissolution tests using *in situ* ATR-IR spectroscopy to analyze single and multi-component mixtures.

Moreover, dissolution testing has evolved into a highly regulated activity in the pharmaceutical industry. Health agencies have placed a greater emphasis on regulating dissolution methods. The dissolution test is the only one that can give an *in vitro* snapshot of how the drug product may behave *in vivo*. Because of this function, the number of dissolution methods in the United States Pharmacopeia (USP) has grown substantially. In fact, in 1970 there were 12 dissolution methods in the USP while in 2008 there were 686 [10]. In addition, the US Food and Drug Administration (FDA) has placed greater importance on the dissolution test. The FDA official website is full of material related to dissolution testing from guidance documents to warning letters [11,12]. Thus, the potential impact of a new analytical technique that permits *in situ* analysis of multiple active ingredients is large.

2. Experimental

2.1. Chemical and materials

Acetaminophen reference material (batch no. 104K0154) was purchased from Sigma–Aldrich. Salicylic acid reference material (batch no. 04708HE) was purchased from Sigma–Aldrich. Acetaminophen tablets (Tylenol batch no. SLA175) were purchased from a local pharmacy. Excedrin caplets (Back and Body Brand, Lot #10067371: acetaminophen 250 mg, aspirin 250 mg) were purchased from a local pharmacy. Salicylic acid calibrator tablets (USP batch no. Q0D200) were purchased from the USP. Methanol, acetone and acetonitrile (HPLC grades) were purchased from Pharmaco-Aaper. Sodium hydroxide (batch no. 064214BH), used to prepare the pH buffered solutions, was purchased from Sigma–Aldrich. Potassium phosphate monobasic (batch no. 103K0060), used to prepare the pH buffered solutions, was purchased from Sigma–Aldrich. Glacial acetic acid was purchased from Sigma–Aldrich. All solutions were prepared using water treated by a Milli-Q Millipore purification system. All purified water aliquots have resistivity of not less than 18 MOhm cm^{-1}.

2.2. Instrumentation

Samples were tested using Mettler Toledo's iC10 FT-IR system using a fiber optic probe equipped with a 1 mm diamond coated ATR probe. The IR system was operated by Mettler Toledo's iC IR version 3.0 and 4.0 software. pH determinations were carried out using a pH meter from VWR (model no. Symphony SB70P). HPLC analysis was carried out using Hewlett Packard 1050 (operated by ChemStation) and Waters 2695 (operated by Empower). All UV measurements were carried out using a Hewlett Packard UV instrument (model no. 8452A diode array). The UV instrument was operated using HP's Softer-Olis Spectralworks. All manual dissolutions were tested using Vankel's Dissolution Batch (model no. 700). All analytical weight measurements were carried out using Mettler Toledo's DeltaRange and AG204 DeltaRange.

2.3. Buffered solutions

The pH 5.8 (200 mM) and 7.4 (50 mM) phosphate buffered solutions were prepared with monobasic potassium phosphate in accordance with the USP [13,14], respectively.

2.4. Dissolution experiments

Single-component analysis: 300 mg salicylic acid tablets were tested in pH 7.4 phosphate buffered solutions. Dissolution was conducted using a vessel volume of 500 ml and at ambient temperature. All dissolutions were conducted using USP Apparatus Type II (paddles) with an agitation speed of 100 rpm. The dissolution run time was 7 h and 30 min.

Multi-tablet analysis: 300 mg salicylic acid and 500 mg acetaminophen tablets were tested in pH 5.8 phosphate buffered solutions. Dissolution was conducted using a vessel volume of 500 ml and at ambient temperature. All dissolutions were conducted using USP Apparatus Type II (paddles) with an agitation speed of 50 rpm. The dissolution run time was 6 h.

Multi-component analysis: three Excedrin tablets, each composed of 250 mg aspirin (acetylsalicylic acid) and 250 mg acetaminophen were tested in pH 7.4 phosphate buffered solutions. Dissolution was conducted using a vessel volume of 500 ml and at ambient temperature. All dissolutions were conducted using USP Apparatus Type II (paddles) with an agitation speed of 100 rpm. The dissolution run time was 1 h and 30 min.

2.5. HPLC analysis

The single-component analysis experiment was performed using the following HPLC parameters; HP 1050 HPLC, Symmetry 300 C18 5 μm column (4.6 mm × 50 mm), mobile phase was 60/40/1 (water/methanol/glacial acetic acid), flow rate was 2.0 ml/min, isocratic mode, injection volume was 5 μl and UV detector was set at 296 nm.

The multi-tablet analysis experiment was analyzed using the following HPLC parameters; Waters 2695, Symmetry Shied RP C18 3 μm column (3.5 mm × 50 mm), mobile phase was 70/30/1 (water/acetonitrile/trifluoroacetic acid (TFA)), flow rate was 1.5 ml/min, isocratic mode, injection volume was 5 μl and UV detector was set at 296 nm.

The multi-component analysis experiment was performed using the following HPLC parameters; HP 1050 HPLC, Phenomenex Intersil ODS C18 5 μm column (4.6 mm × 150 mm), mobile phase was 60/40/1 (methanol/water/trifluoroacetic acid (TFA)), flow rate was 1.0 ml/min, isocratic mode, injection volume was 25 μl and UV detector was set at 280 nm.

2.6. ATR-IR analysis

The ReactIR™ iC10 FT-IR instrument is composed of an MCT detector (liquid nitrogen cooled) and the FiberConduit™. The

FiberConduit™ is comprised of flexible IR transparent silver chloride/silver bromide optical fibers. The fiber optic probe interface (AgX 9.5 mm × 1.5 m fiber (Silver Halide)) contains a diamond tip-DiComp ATR crystal. The resolution was set to 8 wavenumbers. The optical range used by the system is: 1900–650 cm^{-1}. The gain adjustment was set to normal (1×) and the apodization method was set to Happ-Genzel. The system uses compressed air (house air, filtered and de-humified) to purge the optics.

For the single-component testing, data treatment was carried out using the following methodology. The data were first subjected to baseline correction. An absorption band at 1388 cm^{-1} was selected for salicylic acid. The height was calculated using a baseline band correction set at 1370 cm^{-1}. The ATR-IR system was configured to collect spectra every 5 min.

For *multi-tablet testing*, the data were first subjected to baseline correction. An absorption band at 1388 cm^{-1} and baseline band at 1370 cm^{-1} were selected to calculate the peak height for salicylic acid. An absorption band at 1246 cm^{-1} and a baseline band of 1276 cm^{-1} were selected to calculate the peak height for acetaminophen. The ATR-IR system was configured to collect spectra every 5 min.

For *multi-component testing*, the data were first subjected to baseline correction. An absorption band at 1388 cm^{-1} and a two-point baseline set at 1370 cm^{-1} and 1414 cm^{-1} were selected to calculate the peak area for aspirin (acetylsalicylic acid). An absorption band at 1246 cm^{-1} and a two-point baseline set at 1217 cm^{-1} and 1265 cm^{-1} were selected to calculate the peak area for acetaminophen. The ATR-IR system was configured to collect spectra every 3 min.

For all ATR-IR experiments, 256 scans were collected and co-added for each spectral point. On average, every spectral point, took about 2 min to complete. For all testing, the calculated peak response was subjected to mathematical smoothing using the iC_{10} software. The data were compared to reference standards measurements collected prior to start of the linearity and dissolution experiments. Dissolution data were plotted vs. time and all dissolution experiments were allowed to equilibrate in the buffer for at least half an hour prior to the start of a dissolution experiment.

3. Results and discussion

3.1. Single-component analysis

3.1.1. Linearity results for salicylic acid using pH 7.4 phosphate buffer

Salicylic acid was thoroughly studied in pH 7.4 phosphate buffered solution. During the method development phase, linear dilutions of salicylic acid reference standards were prepared and analyzed using ATR-IR spectroscopy. The linearity experiments served two purposes; (1) they determined whether IR spectroscopy provided a linear response to the different concentrations of salicylic acid and (2) whether IR spectroscopy can be used for very low concentration levels of salicylic acid. Initially, eight levels of salicylic acid standards were prepared. Based on the IR spectra obtained, salicylic acid has an IR frequency of interest at 1388 cm^{-1} (Fig. 1). The linearity experiments were further analyzed using linear regression. Based on the analysis, it was determined that salicylic acid had excellent linear correlation using IR spectroscopy with a 0.998 correlation coefficient (r^2). Linear regression for salicylic acid was calculated using the IR absorption band at 1388 cm^{-1} and subtracting the baseline absorption band at 1370 cm^{-1}.

3.1.2. Dissolution results for salicylic acid using pH 7.4 phosphate buffer

The next phase of method development was to determine the accuracy of ATR-IR spectroscopy as a technique for measuring dissolution rates of a drug. This was determined by comparing the IR spectroscopy results with HPLC results. Although USP methods recommend a volume of 900 ml, our dissolution vessel was filled with 500 ml of pH 7.4 phosphate buffer in order to increase the signal-to-noise ratio. The paddle was lowered to the USP recommended position (25 ± 2 mm from the bottom of the vessel) and rotated at 100 rpm at ambient temperature. To minimize hydrodynamic effects, the ATR probe was inserted approximately 5 mm below the surface of the medium [15]. The first 30 min was used to establish the spectral baseline, the shaft was momentarily stopped and one salicylic acid (300 mg) tablet was dropped into the vessel. Rotation was resumed and IR data were acquired every 5 min for a period of 7 h. During this time, aliquot samples were removed and stored in HPLC vials for subsequent analysis. As shown in Fig. 2, the results from the IR and HPLC systems match extremely well. In fact, the relative accuracy is within ±3%.

3.2. Multi-tablet analysis

3.2.1. Linearity results for salicylic acid and acetaminophen in pH 5.8 phosphate buffer

The USP monograph specifies pH 5.8 phosphate buffer as the medium of choice for acetaminophen. Thus, acetaminophen and salicylic acid were studied in pH 5.8 phosphate buffer. As expected, regression analysis for salicylic acid indicated an excellent linear correlation (r^2 = 0.994) of IR peak intensity vs. concentration. For acetaminophen, at low concentrations (≤0.11 mg/ml, 0.7 mM), the relationship between absorbance and concentration is effectively

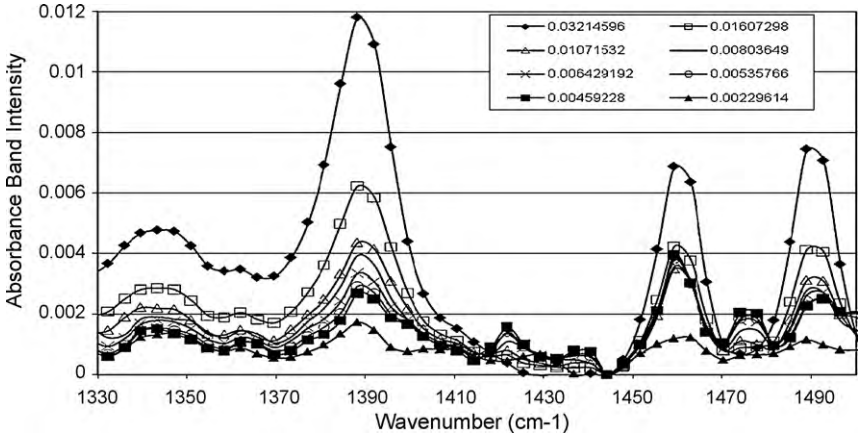

Fig. 1. Salicylic acid spectra at different concentrations in pH 7.4 phosphate buffered solution.

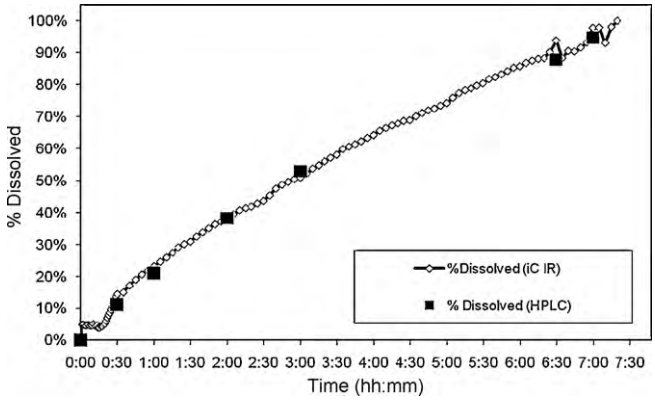

Fig. 2. Comparison of *in situ* ATR-IR and HPLC methods for dissolution of a salicylic acid tablet.

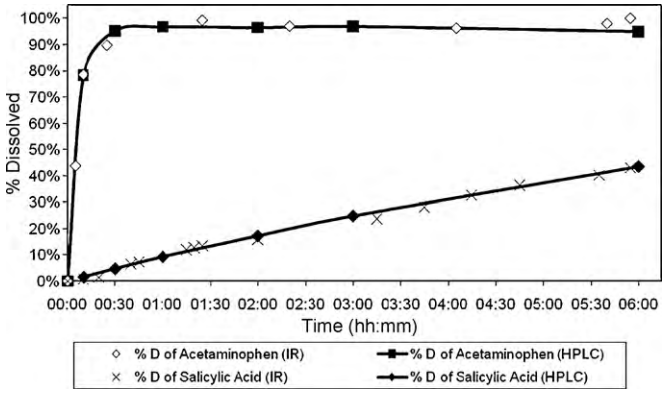

Fig. 4. Simultaneous dissolution of acetaminophen and salicylic acid tablets: *in situ* ATR-IR vs. HPLC.

linear ($r^2 = 0.998$). However, to our surprise, non-linear behavior for both IR and UV–vis methods was observed at higher concentrations. As shown in Fig. 3, both methods can be modeled using second-order polynomial equations [16].

3.2.2. Dissolution results for salicylic acid and acetaminophen in pH 5.8 phosphate buffer

To demonstrate the ability of *in situ* ATR-IR to successfully monitor dissolution of two different tablets, the dissolution profile of salicylic acid and acetaminophen tablets was determined by simultaneously adding one tablet of each and monitoring the dissolution rate of both components over 7 h. Although salicylic acid is not used clinically, it serves as an USP recommended standard [13] for a slowly eroding tablet and acetaminophen serves as an example of a tablet that disintegrates. As shown in Fig. 4, the *in situ* ATR-IR method is clearly able to distinguish the dissolution of each component. For example, the acetaminophen tablet disintegrates and releases acetaminophen more rapidly than the salicylic acid tablet which slowly erodes over time. Thus, *in situ* ATR-IR method is successful in determining the dissolution profile of a two tablet system.

To compare the *in situ* ATR-IR method with HPLC and UV–vis methods, aliquots were taken throughout the experiment and analyzed. The results from the *in situ* ATR-IR and HPLC systems are overlaid in Fig. 4 and an excellent correlation is observed between the two methods. Since both salicylic acid and acetaminophen absorb at approximately 296 nm (pH 5.8), it was not feasible to monitor the dissolution of these two components using UV/vis alone.

3.3. Multi-component analysis

3.3.1. Dissolution results for Excedrin caplets (aspirin and acetaminophen) in pH 7.4 phosphate buffer

To demonstrate the ability of *in situ* ATR-IR to successfully monitor multiple components within the same tablet, dissolution testing was carried out on Excedrin caplets containing acetylsalicylic acid (250 mg) and acetaminophen (250 mg) actives (note: caffeine is not present is this formulation). The release profile was determined by simultaneously adding three Excedrin caplets to a 500 ml vessel volume containing pH 7.4 phosphate buffer. Three caplets were necessary to ensure satisfactory signal-to-noise ratio. Refer to Fig. 5 for ATR-IR spectra for Excedrin caplets. The dissolution rates of both components were monitored over 1.5 h. Based on the data, it was determined that acetylsalicylic acid and acetaminophen were fully released within 1.5 h as shown in Fig. 6. *In situ* ATR-IR method is clearly able to distinguish the dissolution of the acetylsalicylic acid and acetaminophen components.

To compare the *in situ* ATR-IR method with HPLC, aliquots were taken throughout the experiment and analyzed. The results from the *in situ* ATR-IR and HPLC systems are overlaid in Fig. 6 and an excellent correlation is observed between the two methods. Overall, this set of experiments indicates the versatility of *in situ* ATR-IR for dissolution testing as it allows automated observation of a multi-component system without manual sampling and by successfully tracking the dissolution behavior of two of the major components.

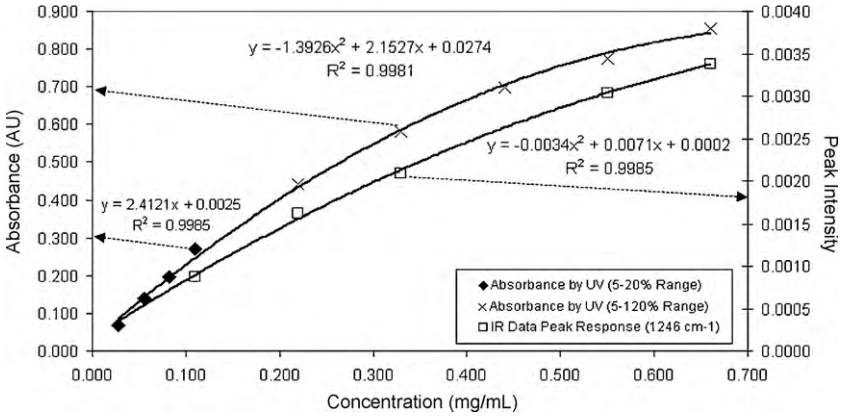

Fig. 3. Calibration curves of acetaminophen standards at pH 5.8: UV/vis absorbance at 296 nm and IR peak intensity at 1246 cm^{-1}.

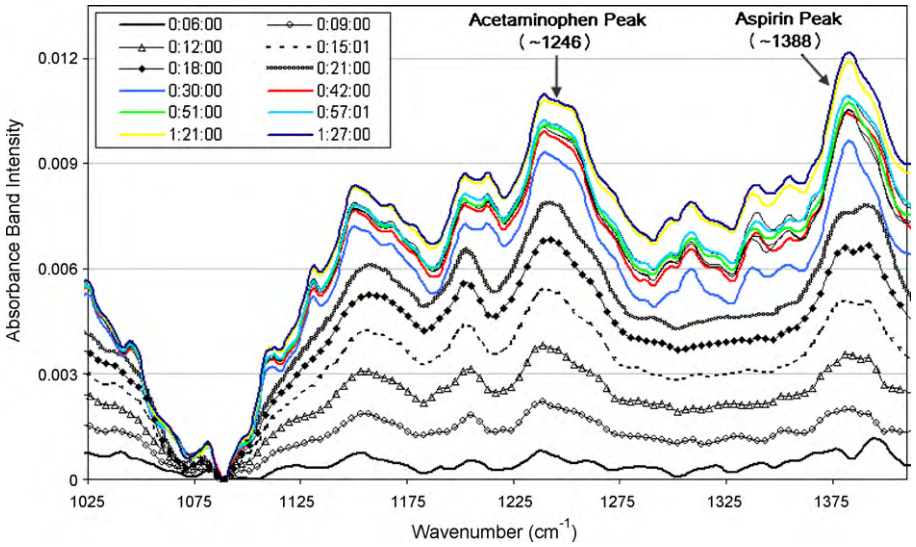

Fig. 5. ATR-IR spectra of Excedrin caplets at different time-points during dissolution in pH 7.4 phosphate buffered solution.

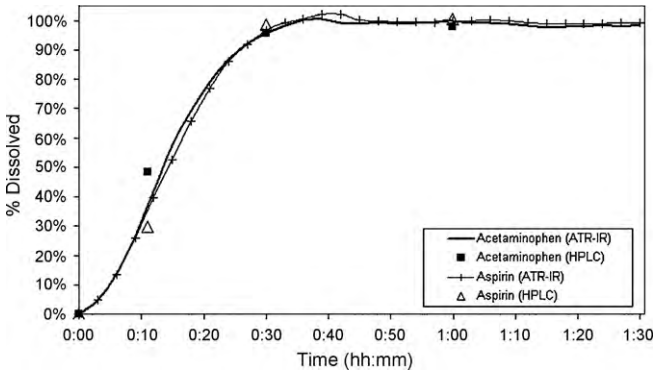

Fig. 6. Dissolution of acetylsalicylic acid and acetaminophen in Excedrin caplets: *in situ* ATR-IR vs. HPLC.

4. Conclusions

We demonstrate that *in situ* infrared spectroscopy is a viable alternative for measuring dissolution profiles of pharmaceutical tablets. The IR system was found to be impressive in its capability of measuring very low concentrations (0.03 mg/ml) and to distinguish separate components of a multiple component system without requiring manual sampling. This versatility is demonstrated by observing the simultaneous dissolution of acetaminophen and salicylic acid tablets. In contrast, UV–vis spectroscopy alone is unable to distinguish between these two components and HPLC must be used which requires laborious manual sampling. The *in situ* ATR-IR method has been validated by comparing dissolution profiles using UV–vis and HPLC methods. In addition, we have established an accuracy of ±3% between IR and HPLC. With the current configuration of our IR instrument, this analysis is limited by the sensitivity and wavelength range of the *in situ* fiber optic probe. However, since this paper successfully demonstrates the versatility of this novel application of ATR-IR spectroscopy, we suggest that the method has excellent potential to be improved by modification of the IR instrument and selection of a more sensitive probe. To our knowledge, *in situ* monitoring of tablet dissolution by IR spectroscopy is a novel method and this study indicates that it has excellent potential for further application in dissolution testing.

Acknowledgments

The authors thank Seton Hall University and Novartis Pharmaceuticals for financial support and Dr. Jian Wang and Mettler Toledo for technical support for the iC_{10} IR Instrument.

References

[1] (a) J.J. Dressman, J.K. Kramer (Eds.), Pharmaceutical Dissolution Testing, Taylor and Francis, Boca Raton, FL, 2005;
(b) R. Hanson, V. Gray, Handbook of Dissolution Testing, 3rd ed., Dissolution Technologies, Hockessin, DE, 2004.
[2] C. Tong, S.S. D'Souza, J.E. Parker, T. Mirza, Commentary on AAPS workshop—dissolution testing for the twenty-first century: linking critical quality attributes and critical process parameters to clinically relevant dissolution, Pharm. Res. 24 (2007) 1603–1607.
[3] C. Tong, R. Lozano, Y. Mao, T. Mirza, R. Ldbenberg, B. Nickerson, V. Gray, Q. Wang, The value of in vitro dissolution in drug development: a position paper from the AAPS in vitro release and dissolution focus group, Pharm. Technol. 33 (2009) 52–64.
[4] J. Van der Weerd, S.G. Kazarian, Combined approach of FTIR imaging and conventional dissolution tests applied to drug release, J. Controlled Release 98 (2004) 295–305.
[5] J. Van der Weerd, S.G. Kazarian, Release of poorly soluble drugs from HPMC tablets studied by FTIR imaging and flow-through dissolution tests, J. Pharm. Sci. 94 (2005) 2096–2109.
[6] T. Mirza, Q. Liu, R. Vivilecchia, Y. Joshi, Comprehensive validation scheme for in situ fiber optics dissolution method for pharmaceutical drug product testing, J. Pharm. Sci. 98 (2009) 1086–1094.
[7] F. Regan, M. Meaney, J.G. Vos, B.D. Maccraith, J.E. Walsh, Determination of pesticides in water using ATR-FTIR spectroscopy on PVC/chloroparaffin coatings, Anal. Chim. Acta 334 (1996) 85–92.
[8] Bynum, K., Kassis, A. ATR Crystal Device, Patent WO/2002/077616, March 10, 2002.
[9] D. Skoog, J. Holler, S. Crouch, Principles of Instrumental Analysis, 6th ed., Thomson Books, 2007, 455–480.
[10] USP, US Pharmacopoeia & National Formulary, United States Pharmacopeial Convention Inc., Rockville, 2009.
[11] United States Food and Drug Administration. Available from: <http://www.fda.gov/>.
[12] FDA, Guidance for Industry, Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Center for Drug Evaluation and Research, Rockville, 1997.
[13] US Pharmacopoeia & National Formulary, Monograph: USP Monograph: Salicylic Acid Tablets, USP32-NF27, United States Pharmacopeial Convention Inc., Rockville, 2009, p. 3531.
[14] USP, US Pharmacopoeia & National Formulary, Monograph: Acetaminophen Tablets, USP32-NF27, United States Pharmacopeial Convention Inc., Rockville, 2009, p. 1391.
[15] J.W. Mauger, Physicochemical and fluid mechanical principles applied to dissolution testing, Dissolution Technol. 3 (1996) 7–11.
[16] B. Dejaegher, M.S. Bloomfield, J. Smeyers-Verbeke, Y. Heyden, Validation of a fluorimetric assay for 4-aminophenol in paracetamol formulations, Talanta 75 (2008) 258–265.