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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 469–476

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Multiple fiber-optic dual-beam UV/Vis system with application to dissolution testing

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Received 1 January 2002; received in revised form 8 February 2002; accepted 24 February 2002

Abstract

A system for fiber-optic probing in dissolution testing of solid pharmaceutical formulations has been constructed. The system is based on an imaging spectrometer and a charged coupled device (CCD) detector and includes 12 fiber-optic probes with a novel dual-path design. UV light was produced by a small arc deuterium lamp illuminating an optical fiber bundle. Twelve fiber-optic dipping probes were constructed with a reflection geometry. A 5 mm diameter lens was used to achieve a parallel light beam. The light passed back and forth through the flow-through cuvette defined by a sapphire window and a coated aluminium mirror. The mirror was cut in half and each segment was tilted and set at different distances from the window to obtain two separate paths with different lengths. Two receiver fibers were used for each probe to collect the transmitted light. The 24 receiver fibers from the 12 probes were bunched to a linear bundle and fed to an imaging spectrometer and the corresponding spectra were detected with a 512×512 pixel cooled CCD detector. The sampling interval was typically a few seconds for all probes. A software package was developed for data recording and on-line analysis. The program includes tools for multi-component analysis. The system was tested for different tablet formulations. Prednisone 50 mg tablets, normally used for control tests of dissolution baths, were followed for 3 h. Secondly, an extended release low dosage tablet was followed for 7 h resulting in a linear dissolution profile. Finally, a combination tablet containing two active drugs was tested for 60 min profiles. In the latter case, separate dissolution curves for the two active components were obtained. Future work will mainly focus on further development of the multi-component capability of the system. © 2002 Published by Elsevier Science B.V.

Keywords: Drug dissolution; Fiber-optic probe; UV/VIS spectroscopy

1. Introduction

Dissolution testing of pharmaceutical preparations are performed for quality control purposes and to obtain information about the rate of release of active compounds into the human body [1]. This is normally done in vitro, but by choosing the experiment parameters with care, a good in vivo correlation can be reached. The standard methods for dissolution testing of pharmaceutical preparations are both time consuming and labor intensive. The typical procedure involves dissolu-

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0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(02)00091-2

tion of the tablets in glass vessels under heating and rotation of the dissolution medium. The rotation speed and amount and composition of the medium can be chosen to fit the in vivo correlation, while the geometric parameters, the shape and position of the paddle and the vessels, are strictly regulated by Pharmacopoeia guidelines. Samples are withdrawn from the vessels at selected times, filtered and analysed with HPLC or spectrophotometrically. As an alternative, automatic flow-through sampling can be used in combination with a spectrometer. However, these methods suffer from several disadvantages. As already mentioned the overall analysis time is long. Furthermore, the total number of samples are limited both by the sampling time, 0.5–1 min, and by the sampling volume that will decrease the total vessel volume by repeated sampling. Thus, the time resolution is on the order of 5 min for a typical dissolution testing involving six samples. Another drawback of the automatic flow-through spectrometers is that some substances adsorb to the tubings. The poor precision and reliability of the pumps is yet another reason for avoiding such systems.

A different approach is to perform the measurements by in situ probing rather than by extracting samples from the vessels. Fiber-optic probing has previously been reported in connection with dissolution testing of pharmaceutical preparations. Single-probe systems were developed for on-line fiber-optic spectroscopy in dissolution testing [2,3]. In one system a PLS model was utilised to correct for sample turbidity [2]. Later, multi-probe systems based on either sequential sampling [4–6] or multiple-track CCD detection [7,8], were reported. In the latter case, multivariate techniques were evaluated for parallel detection of two different constituents.

In this paper we report on a dissolution testing system with 12 parallel fiber-optic probes. The system is based on CCD detection and an imaging spectrometer with a correction unit for lowest image aberrations. Each of the probes includes two parallel optical paths differing only in the path length. Depending on analyte concentration, either the short or the long path can be utilised. Alternatively, a ratio of the signal from the two paths can be used. A description of the system with special attention to the probe design is given in this paper. A comparison of data obtained with a single path and with the ratio of the two paths will be given.

2. Material and methods

2.1. Fiber-optic dissolution system

The layout of the dissolution testing system is shown in Fig. 1. It consists of a Deuterium (D_2) lamp, 12 fiber-optic dipping probes, an imaging spectrometer and a CCD detector. The D_2 lamp (Oriel Instruments, Stratford, CT) was a 30 W small arc (0.5 mm) lamp mounted in a lamp house with optics for coupling into optical fibers. An electronically driven shutter controlled by the CCD detector controller was mounted on the lamp house to minimise the illumination onto the optical fibers. Two different fiber-optic probe configurations were used. In one arrangement the probes were placed next to the shaft above the paddles (Fig. 2a). In the other arrangement (Fig. 2b), the probes were inserted through the hollow paddle shaft for each vessel. A flow-through channel was drilled through the paddles with the inlet

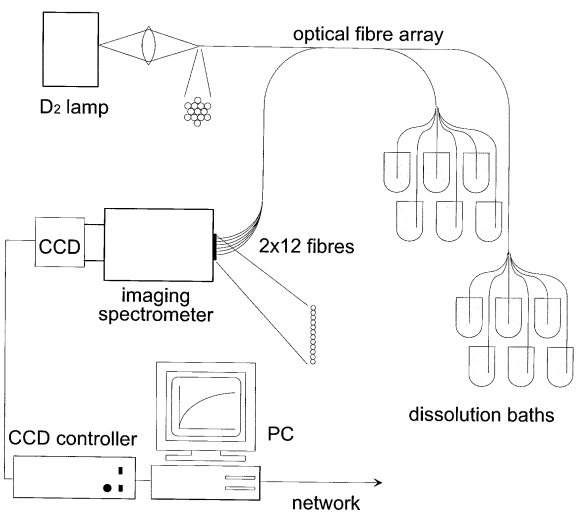

Fig. 1. System set-up for fiber-optic dissolution testing system.

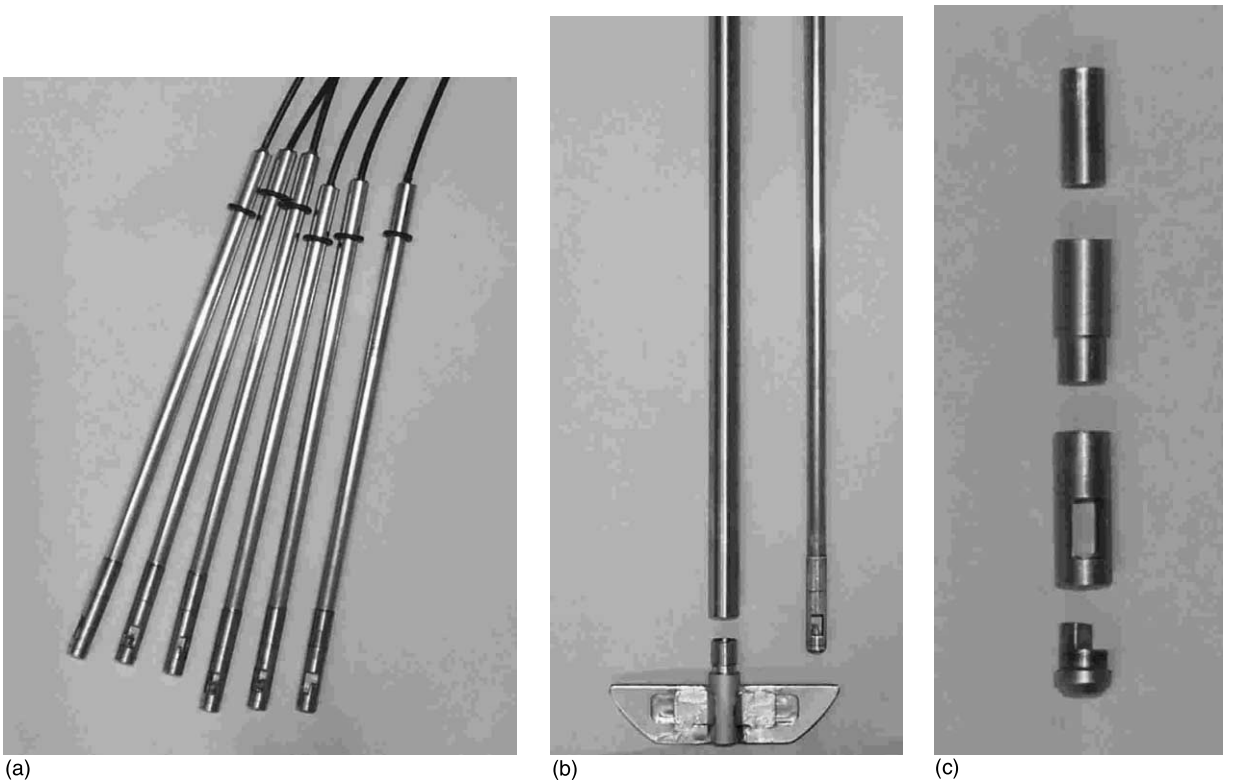

Fig. 2. Photograph showing details of the dissolution testing system. Dipping probe version, six probes (a), axial probe version with shaft and paddle (b) and disassembled probe head (c). The diameter of the probes is 6.5 mm.

at the high-pressure side and the outlet at the low-pressure side. The probe head was placed down through the hollow paddle perpendicular to the flow-through channel. The probe head, shown in Fig. 2c and Fig. 3, includes an illumination fiber, a UV lens (Melles Griot, Irvine, CA), a sapphire window, a Al-coated mirror divided into two half-circular parts, and two detection fibers all mounted in stainless steel cylinders. The flow-through probe cuvette was defined by the window and the Al mirrors. The two mirrors were fixed at 3 and 6 mm from the window, respectively in order to obtain two separate optical path ways. Each mirror was tilted 2° so that the light transmitted through the detection cuvette was focused onto two focal points corresponding to the respective detection fiber. The mirrors were made from a UV quartz substrate and mounted with the Al layer against the brass support to protect the Al layer from acidic solutions. The detection fibers were deliberately withdrawn about 1 mm from the focal plane in order to have a less critical optical alignment. The light from the D_2 lamp was directed through an optical fiber bundle (Fiber-Guide Ind., Stirling, NJ) consisting of 12 200 μm diameter fibers, that was split into 12 separate fibers and connected to the probes. The detection light was collected with 24 fibers of the same type, two from each probe, and re-arranged to a linear array that was connected to the spectrometer (Acton Research, Acton, MA). The spectrometer set-up consists of a 275 mm spectrometer (model spectraPro-275) and an imaging assembly (model FC-459) that corrects for imag-

ing aberrations. A 512 × 512 pixel, back-illuminated CCD detector (model TEA/CCD 512, Princeton Instruments, Trenton, NJ) cooled to −40 °C was connected to the spectrometer.

2.2. Software

A software package was developed in LabVIEW 5.0 (National Instruments, Austin, TX) and used for data transfer and communication with the CCD, for data calculation and presentation. Instrumental settings such as analytical wavelength, sampling time and sampling interval are set and dissolution curves are displayed live on the screen for the selected probes. The software includes graphical routines for calibration against blank and reference solutions. The dissolution test can be executed using one or more analytical wavelengths and a background wavelength. Using a post-processing routine, the wavelengths or the pixel averaging may be changed and switching between long path, short path or ratio data is allowed. A report may be printed after dissolution with the dissolution data including statistics as well as the instrumental settings.

2.3. Reference methods

Reference measurements were normally performed using a spectrophotometer (HP 8453, Hewlet Packard, Palo Alto, CA). Samples were extracted from the dissolution vessels and filtered through 0.80 μm filters (MFS-25 CA0.80, Micro Filtration Systems, Dublin, CA) prior to measurements in a 1 cm quartz cuvette. The absorbance at a reference wavelength was measured and subtracted from the absorbance at the UV absorbance maximum. For the dissolution testing of the combination product, no sample aliquots were collected during dissolution testing. Instead, mean values ($n = 6$) of content from previous HPLC separations were used.

2.4. Chemicals

The water was prepared using an ELGA system. Phosphate buffer solution was prepared from sodium dihydrogen phosphate (601 ml, 1 M) and disodium hydrogen phosphate (433 ml, 0.5 M) both obtained from Merck (Darmstadt, Germany). Ethanol 95% was obtained from Kemtyl (Stockholm, Sweden). Prednisone (1-dehydrocortisone) substance was obtained from Sigma-Aldrich (Dorset, UK).

2.5. Tablets

Prednisone tablets 50 mg Lot L were obtained from the United States Pharmacopeial Convention (Rockville, MD).

Extended release tablets 2.5 mg and the combination tablet were manufactured at AstraZeneca R&D Mölndal (Mölndal, Sweden). The tablets were stored at room temperature prior to use.

3. Results and discussion

A system for dissolution testing of pharmaceutical solids based on multiple fiber-optic probes and CCD detection has been constructed. The system incorporates 12 dipping probes with a design with two separate optical paths with different path lengths. A fiber-optic dissolution testing starts by acquiring a background image with the lamp off, which corrects for the dark current and for potential ambient light interferences. After that, the probes are dipped in a blank solution and a standard solution corresponding to 100% released and the corresponding recordings are averaged over typically five recorded CCD readouts. The dissolution test is then initiated and the dissolution curves from up to 12 vessels are followed in 'live' mode on the computer screen. The dissolution data are calculated according to the following:

$$\%\ \text{released} = (A_{\text{dis}} - A_{\text{bl}}) \cdot \frac{100}{A_{\text{std}} - A_{\text{bl}}} \quad (1)$$

where A_{dis}, A_{std} and A_{bl} correspond to the measured absorbance for the dissolution experiment, the standard solution and the blank solution, respectively. These are calculated according to:

$$A = \log(I_{\text{an}}) - \log(I_{\text{ref}}) - \log(I_{\text{offset}}) \quad (2)$$

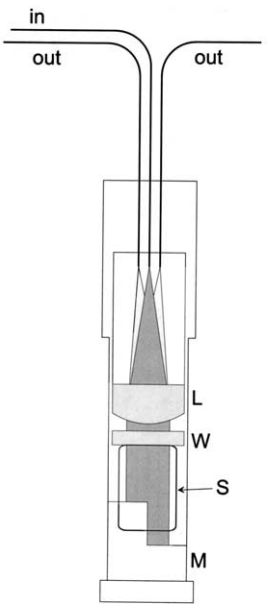

Fig. 3. Set-up of the 2-path dipping probe. M, mirror; S, sampling flow-through cell; W, sapphire window; L, lens. The light is emitted from the middle fiber and reflected off the slightly tilted mirrors (M) into the receiving fibers.

where I_{an}, I_{ref} and I_{offset} correspond to measured intensities at the analytical and reference wavelengths, respectively, as well as the remaining offset measured at a wavelength between 150 and 200 nm. I_{an} and I_{ref} are background subtracted values. The aspect of the offset will be discussed below. Since all probes are individual they yield different calibration data. Thus, all active probes are used for both blank, standard solutions and dissolution testing. When the dissolution test is completed the curves and the underlying data is printed. Since the full spectra are stored for the 12 probes, post-processing such as changing the analyte wavelength, is possible.

The system was used for dissolution testing of various tablets including USP calibrator prednisone tablets. In Fig. 4 raw data spectra are shown for dissolution testing of prednisone according to dissolution apparatus USP II using a single probe. The CCD exposure time here was 1 s and the sampling interval was 15 s. The original transmission spectrum (thin line) through the probe dipped in the water-filled vessel as well as

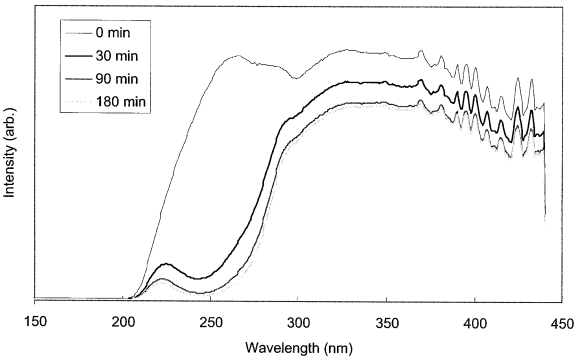

Fig. 4. Spectral raw data from dissolution testing of 50 mg prednisone tablets in H_2O. The temperature was 37 °C and the stirring speed was 50 rpm.

the corresponding spectra after 30, 90 and 180 min of dissolution, are shown. Fig. 4 clearly shows the increasing light absorption at 242 nm caused by the dissolved prednisone substance. It also shows a substantial increase in absorbance (decreased intensity) at longer wavelength that is attributed to larger particles in the solution. These particles from the disintegrated tablet matrix will not primarily cause a wavelength dependent light scattering but rather a wavelength independent decrease in collected light intensity caused by the shadowing effect of these particles. More difficult to see, is a small remaining off-set after background subtraction. We believe that this off-set originates from a faint reflection from the CCD window and back onto the CCD chip. This reflected imaged will be out of focus and will appear almost as an off-set. Thus, this can to a first degree be corrected for simply by off-set subtraction.

A set of six simultaneously recorded dissolution curves are shown in Fig. 5. The tablets are again prednisone and the dissolution conditions were the same as in Fig. 4. Sample aliquots were collected after 30, 90 and 180 min as indicated by the different markers for the individual dissolution tests. The samples were filtered and the absorbance was measured at 242 nm after the dissolution experiment. As can be seen, there is a good agreement between fiber-optic data and the reference values with slightly higher deviations at 30 min. The accuracy was calculated for the amount

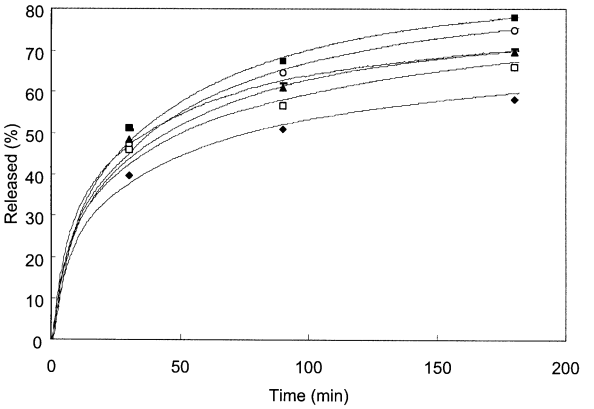

Fig. 5. Dissolution curves from testing of 50 mg prednisone tablets in H_2O. The temperature was 37 °C and the stirring speed was 50 rpm. The markers correspond to reference values from sample extraction and measured on a standard spectrophotometer.

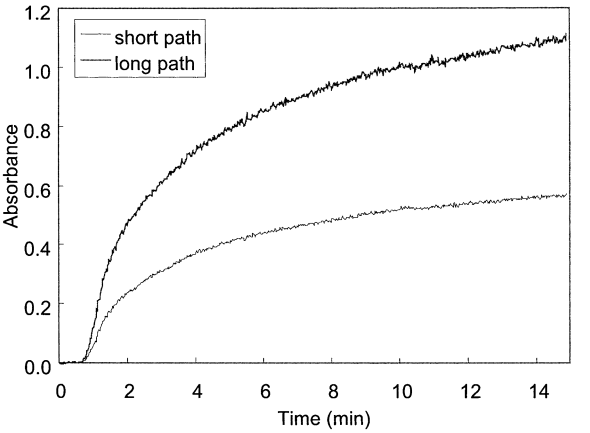

Fig. 6. Two simultaneously measured dissolution curves corresponding to the short path and long path of a single probe.

released at 180 min and found to be 99.6% ($n = 6$). The corresponding value at 30 min was 103.8%.

The linearity of the system was investigated by recording dissolution spectra for six probes of solutions of prednisone at five different dilutions and a blank. The linearity data are shown in Table 1. A correlation coefficient of $R = 0.9997$ was found for an absorbance interval of two absorbance units. However, if multiple CCD read-outs were added to get a higher signal, the useful linear range was found to be extended. The reproducibility of the system was determined by calculating the RSD of absorbances using the data at 0.030 mg/ml. The reproducibility was found to be 1.1% ($n = 6$).

Table 1
Test of linearity for solutions of prednisone in H_2O/EtOH (100:1)

Concentration (mg/ml)	Absorbance
0.000	0.002
0.010	0.406
0.020	0.805
0.030	1.206
0.040	1.587
0.050	1.953
n	6
R^2	0.9997
Intercept	0.0149
95% CI of intercept	−0.0149–0.0447
Slope	0.0391
95% CI of slope	0.0381–0.0401

Another possibility to extend the useful absorbance sample concentration range is to take advantage of the dual path design of the fiber probes. As was shown in Fig. 3, the probe head is constructed with two parallel mirrors that make up two different optical paths. These paths are in flow contact with each other but they have different path lengths. In this case the path lengths were chosen to be 6 and 12 mm (3 and 6 mm back and forth). In Fig. 6, two dissolution curves of a fast disintegrating model tablet, are shown. These curves originate from the long and short path from the same probe, recorded simultaneously. As can be observed, the two curves follow basically the same curve shape. Hence, we have the possibility to choose the suitable probe path length after the experiment when the exact absorbance levels are known. Furthermore, if the short path is optimised for the region of 20–100% released, the long path can be used to enhance the precision of the low-concentration region.

One purpose with the two optical paths was to utilise the signals to form a ratio of the two absorbance values. By forming a ratio, the dissolution curves would be less sensitive to lamp fluctuations or intensity variation due to deposi-

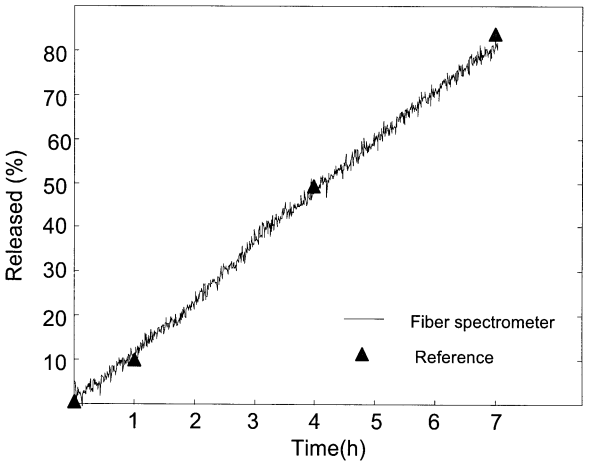

Fig. 7. Dissolution curve for a 2.5 mg extended release tablet. The temperature was 37 °C and the stirring rate was 100 rpm. The dissolution medium was 500 ml phosphate buffer pH 6.5 with 0.4% CTAB. The curve for the fiber-optic probe is calculated using the long path dissolution data. The reference samples (triangles) were collected during the dissolution testing and analysed with the standard spectrometric reference method.

tions of excipients on the mirrors. However, what is gained in less noise due to dissolution perturbations is lost by an increased noise from forming a ratio. Hence, when this was done the overall signal-to-noise was the same or lower for the ratio than for any of the single paths. Our interpretation of this is that the quality of the dissolution curves were surprisingly high (e.g. very low level of perturbations) for this system and thus the ratio became less attractive.

The dissolution testing system was utilised for an extended release tablet, 2.5 mg, with a dissolution profile of 7 h. An example of a dissolution curve is shown in Fig. 7. Reference samples were collected and analysed using a spectrophotometer at 362 nm. The agreement between the fiber-optic recording and the reference values seem to be very good. The dissolution curve is in this case somewhat noisy. This is not due to noisy spectra but rather to a small difference in spectral shape of the blank and standard solutions; the maximum absorbance was about 0.1 AU. The sampling interval was, however, in this case 30 s and can easily be lowered to 5 s. This would allow a

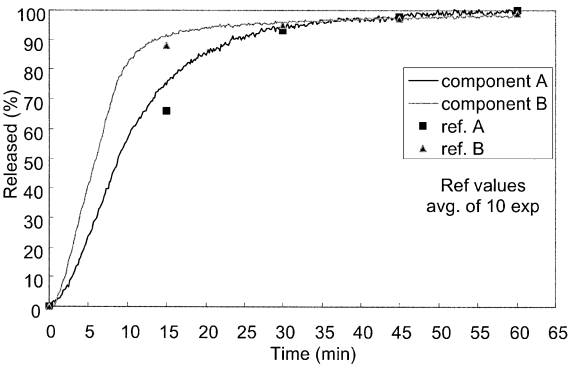

Fig. 8. Dissolution curves of the two active components of a combination tablet. The temperature was 37 °C and the stirring rate was 100 rpm. The dissolution medium was 900 ml phosphate buffer with 0.5% of a surfactant. The reference values are average values from six prior HPLC analyses.

substantial curve smoothing still keeping the time resolution of about 1 sample/min.

In another case, a combination tablet consisting of a homogenous mixture of two active compounds within a single tablet matrix, was tested. The resulting dissolution curves are shown in Fig. 8 for a single probe. Reference measurements were not performed in this case, however, reference values are shown for an average of six dissolution experiments from another occasion using the same conditions and the standard analysis method. As can be observed, the dissolution rates for the two components differ, which is clearly displayed by the high time-resolution provided by the fiber-optic measurement. The two separate dissolution curves were calculated using multiple analytical wavelengths as is allowed in the post-processing feature of the LabVIEW program. The two components exhibit partially overlapping spectra. At one wavelength the absorbance of component B was almost zero this allowed the calculation of the dissolution curve of component A at this wavelength. Once that was done, the dissolution curve of component B could be calculated by subtracting the contribution from component A. This was calculated at a second wavelength corresponding to maximum absorbance of component B. The background and remaining off-set was subtracted prior to the calculation in the same fashion as for previous cases.

The use of imaging spectrometers in combination with CCD detection enables parallel fiber-optic probing at a high time resolution, in the order of a sample/second. An alternative to parallel detection is to use stepper motor switching between fiber probes and a single spectrophotometer for serial detection. The advantage of parallel detection and a high time resolution is rather obvious although we report on a problem related to straylight in the CCD detector. A possible explanation to this is a back-reflection in the protective window yielding an out-of-focus image of the fiber pattern. This would appear almost as a constant off-set with slightly lower intensity closer to the edges. We were able to partially correct for this pattern by subtracting an off-set as measured at around 200 nm where no light could have been transmitted through the optical fiber. However, it would surely be possible to optimise the spectrometer/CCD coupling to lower the straylight.

The key to robust fiber-optic dissolution testing is the design and placement of the probes. In this paper a 2-path design was tested for two different probe locations. A USP standard paddle was modified with a flow-through channel from the high-pressure side of the paddle to the low-pressure side. The probes were inserted through the hollow shaft all the way down to a hole drilled vertically into the centre of the paddle, perpendicular to the flow channel. When the paddle was rotating a flow could be visually confirmed using small particles in the dissolution medium. For non-disintegrating tablets a sufficient flow was established and a time lag of 10–15 s of the dissolution curves was found between probing inside the paddle and probing next to the axis. For disintegrating tablets, on the other hand, problems often occurred with tablet matrix debris building up on the probe mirrors. Obviously, the flow was not always sufficient to remove the debris from the mirrors and this would affect the dissolution curves. Hence, we believe that fixing the probe next to the axis is a better and more robust alternative.

4. Conclusions

Fiber-optic dissolution testing was proven useful for pharmaceutical solid formulations. A system was constructed in-house and used to study both instrumental parameters as well as for tablet dissolution tests. By using parallel detection and a high time-resolution (2–3 s), simultaneous data registration was obtained. The high time-resolution can become particularly useful for early development of complex formulations, but also in routine analysis as it yields more information than with conventional methods. The use of multivariate analysis may increase the robustness of the method. Hence, an on-line multivariate module is now being tested for more complex formulations. The linearity was investigated and found sufficient for the requested concentration interval. Furthermore, the novel design with a 2-path flow-through cell extends the useful concentration range further. The use of a dimensionless ratio of the two paths did, however, not significantly improve the quality of the dissolution profiles. We also investigated the placement of the fiber probes and found that placing the probes some distance from the axis is required for a reliable operation. The potential influence of the probe on the flow profile of the dissolution media and thus on the dissolution rate has still to be investigated.

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