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Automated analytical systems for drug development studies 3. Multivessel dissolution testing system based on microdialysis sampling☆

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Abstract

An automated system consisting of a six-vessel dissolution apparatus, microdialysis sampling, STT E6 multiposition switching valve and a liquid chromatograph was assembled to measure dissolution profiles of immediate and sustained-release tablets. A DL-5 microdialysis loop probe (BAS, Inc.) was immersed in each dissolution vessel and perfused with a suitable medium for sampling. The dialystate from each vessel was injected sequentially onto an on-line liquid chromatography (LC) system for automated analysis. The STT E6 multiposition switching valve was used to sample up to six vessels simultaneously. After addressing issues related to sample carry-over and between-probe variability, the automated system was used in a reproducible manner (RSD < 3%) to measure the dissolution of immediate-release acetaminophen tablets and Accutrim® (containing 75 mg phenylpropanolamine HCl) 16 h Precision Release™ tablets. An uneven injection time sequence was used to monitor three acetaminophen tablets per dissolution run using the automated system and each vessel was sampled about every 6.5 min. However, with Accutrim® 16 h Precision Release™ tablets, a longer sampling interval (10 min) was used, the six tablets could be tested in each dissolution run. The dissolution profiles of acetaminophen and Accutrim® tablets measured using the automated multivessel dissolution system compared well with manual and automated single-vessel dissolution systems.

Keywords: Automation; Dissolution testing; Flow-injection analysis; Immediate release; Liquid chromatography; Microdialysis sampling; Multivessel; Sustained release

1. Introduction

Dissolution testing is used as a routine Compendial test to monitor the lot-to-lot variability of solid dosage forms. The testing procedure is laborious and generates a large number of samples for analysis. Previously, a microdialysis sampling system and an on-line liquid chromatograph were interfaced with a single-vessel dissolution apparatus to automate the dissolution testing of tablets [1]. The automated system was kept simple during the initial developmental stages by studying only one tablet per dissolution run. The effect of critical factors, such as system calibration, linearity and microdialysis drug recovery, on the performance of the automated system was studied. This paper describes the development of a more complicated system designed to sample up to six dissolution vessels simultaneously.

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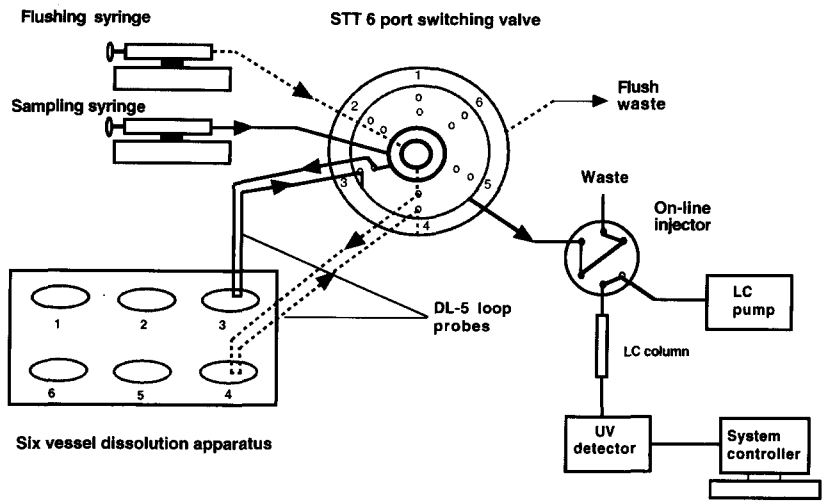

Fig. 1. Schematic diagram of the automated multivessel dissolution testing system.

The advantages of microdialysis sampling to automate sampling and analyses have been described before [1,2]. Essentially, microdialysis sampling provides an easy and inexpensive tool for automation. It is a continuous sampling process that does not involve any volume change and allows on-line buffering of the samples to make them compatible with silica-based liquid chromatography (LC) column packing materials. The microdialysis drug recovery can be modified by changing the perfusion rate or membrane surface area to avoid an additional dilution step for high-dose drug products. The main limitations associated with microdialysis sampling are the adsorption of drugs onto the dialysis membrane and/or connecting tubing and the between-probe variability that necessitates calibration of individual probes.

2. Experimental

2.1. Materials

Acetaminophen and phenylpropanolamine hydrochloride (PPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Monobasic and dibasic sodium phosphate and methanol were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Acetaminophen tablets (325 mg) and Accutrim® 16 h Precision Release™ tablets (containing 75 mg total PPA, 20 mg bolus and 55 mg controlled-release core) were obtained from a local pharmacy. Water processed through the Milli-Q water system (Waters Corp., Bedford, MA) was used in all the experiments.

2.2. Equipment

The automated multivessel dissolution testing system (Fig. 1) consisted of a dissolution apparatus, a microdialysis sampling system, a multiposition switching valve, and a liquid chromatograph. A Hanson Research Corporation (Chatsworth, CA) Model SR2 six-vessel dissolution apparatus with temperature-controlled water bath was used in the dissolution experiments. The sampling system consisted of DL-5 microdialysis loop probes (5 cm membrane length) obtained from BAS, Inc. (West Lafayette, IN), and a Harvard Apparatus (South Natick, MA) Model 55-4140 dual syringe pump for perfusion of the probes. The dialysate was passed through a 2 μl injection loop mounted on a Valco Instruments Co. (Houston, TX) Model EF 60 on-line injector. A multiposition switching valve Model STT E6 from Valco Instruments Co. was interfaced between the dissolution apparatus and the on-line injector for the simultaneous sampling of six vessels. The LC system consisted of a Beckman Instruments Inc. (Fullerton, CA) Model 110A pump, System Gold® programmable UV detector Module 166, and an IBM PS/2 Model 56SX system controller installed with System Gold® software. An ODS Hypersil column (3 μm, 30 mm × 4.6 mm i.d.) was purchased from Keystone (Bellefonte, PA).

2.3. Dissolution of tablets

Dissolution testing of immediate release acetaminophen tablets and Accutrim® 16 h Preci-

sion Release™ tablets was performed according to USP XXII specifications, using the paddle method. In an attempt to demonstrate two different analytical techniques, the acetaminophen was determined by on-line LC and the phenylpropanolamine was determined by flow-injection analysis (FIA).

Acetaminophen tablets

The dissolution of acetaminophen tablets was studied in 900 ml of water at 37 °C and 50 rpm. A DL-5 microdialysis loop probe was suspended in each dissolution vessel and perfused continuously with water at a rate of 50 μl min^{-1}. The dialysate was collected in a 2 μl on-line injection loop and injections were made every 96 s, followed by a blank injection for 36 s. The latter injection was necessary to flush the system and eliminate carryover. Only three tablets could be studied per dissolution run using the STT E6 valve owing to the rapid sampling intervals necessary for the adequate description of the dissolution profile of acetaminophen which was released rapidly from the tablets. Each vessel was sampled once every 6.5 min. The samples were analyzed on-line using a fast LC column with UV detection at 280 nm. The mobile phase used to elute acetaminophen consisted of methanol–phosphate buffer (50 mM, pH 6.2) (15:85, v/v) at a flow rate of 2 ml min^{-1}. The dissolution profiles measured using the automated multivessel dissolution testing system were compared to manual and automated single-vessel dissolution testing systems.

Accutrim® 16 h Precision Release™ tablets

The dissolution of Accutrim® 16 h Precision Release™ tablets was studied in 1 l water at 37 °C and 100 rpm. Dissolution profiles were measured by the automated multivessel dissolution system described here, by the single-vessel dissolution system described in a previous paper [1], and by manual sampling. Six tablets could be monitored per dissolution run using the automated multivessel dissolution system. A DL-5 loop probe was suspended in each dissolution vessel and perfused with water at 10 μl min^{-1}. The perfusate was collected in a 2 μl injection loop and analyzed on-line using FIA with water at 1 ml min^{-1} as a carrier and UV detection at 215 nm. The six dissolution vessels were sampled sequentially every 10 min. Therefore, each dissolution vessel was sampled every hour using the automated multivessel dissolution system. Similar perfusion rate and injection intervals were used with the automated single-vessel dissolution system, but only one tablet per dissolution run was studied and the dissolution vessel was sampled every 10 min. Dissolution profiles of Accutrim® tablets measured using various systems were compared to the previously reported dissolution profile [3].

3. Results and discussion

3.1. Principle of operation

The STT E6 switching valve has two sets of inlets and outlets and six pairs of ports for sampling up to six dissolution vessels simultaneously (see Fig. 1). The syringes mounted on the dual syringe pump were filled with the perfusion medium and connected to the two inlets of the STT E6 valve. A DL-5 microdialysis probe was suspended in each dissolution vessel and both ends of the probe were connected to a pair of ports on the STT E6 valve. At any instance, two probes were perfused — one probe was flushed to waste and the other connected to the injector. An uneven injection time sequence was built into the detector method to sample and analyze the dissolution medium in an automated fashion. At the end of the injection interval, the STT E6 valve, rotated counter-clockwise, and an on-line injector, which was an integral part of the LC system, were switched simultaneously through a relay on the system controller. The on-line injector made an injection into the LC column, whereas the STT E6 valve moved to the next position. Fig. 1 shows port #4 in the flush mode (dashed line) and port #3 in the sample mode (solid line). Once an injection had been made and the STT E6 valve moved counter-clockwise, port #5 would be in the flush mode and port #4 in the sample mode. The sampling sequence was designed so that the stagnant probe was flushed prior to the actual sampling from that probe. The flush interval was designed to allow the previously stagnant probe to reach a steady-state condition necessary for reproducible sampling. Once the injection sequence was initiated, the data and chromatograms were recorded and stored automatically in the system controller.

3.2. System characterization

Analyte carry-over studies

Carry-over experiments were conducted by placing an acetaminophen solution (350 μg ml^{-1}) in dissolution vessel #1. A DL-5 loop probe was suspended in the vessel for microdialysis sampling and both ends of the probe were connected to port #1 on the STT E6 valve. The remaining five ports were linked with connecting tubing to pump the perfusion medium directly through the valve. Analyte carry-over from the previous injection through the STT E6 valve was studied as a function of perfusion rate and injection interval. Table 1 summarizes the results of the carry-over study. The analyte carry-over was significant at lower perfusion rates and reduced at higher perfusion rates. Less than 1% carry-over was observed at a perfusion rate of 100 μl min^{-1} and a 1 min injection interval. The sample carry-over also decreased with increase in injection interval. At 10 min injection interval and 10 μl min^{-1} perfusion rate, the sample carry-over was below 1%.

Carry-over of sample may have occurred as a result of the common passages of dissolution samples through the STT E6 valve, connecting tubing and/or the on-line injector. Technical information on an STT E6 multiposition switching valve provided by the manufacturer confirmed that the two sets of inlet/outlet lines are separated in such a way that no sample mixing could have occurred between the ports. However, each inlet/outlet line had a dead volume of up to 30 μl. Port #1 used in the carry-over study had the largest dead volume. Accounting for the extra-valve dead volume arising from the connecting tubing and the on-line injector, the total dead volume of the system was calculated to be about 45 μl or less. A volume of perfusate that is twice the dead volume must be passed through the system to ensure acceptable precision in the measurements [1,2]. If the volume of perfusate pumped through the system is lower than the system dead volume, mixing of the samples could occur. As seen from Table 1, doubling the perfusion rate from 30 μl min^{-1} did not reduce the sample carry-over by half. At higher perfusion rates, the dialysis membrane of the DL-5 loop probe showed significant ultrafiltration, resulting in leakage of the dialysate to the dissolution vessel. At a perfusion rate of 60 μl min^{-1} through the probe, only 45 μl of the dialysate was collected per minute at the other end. This reflected in a reduced absolute recovery of the drug at higher perfusion rates, as shown in Fig. 2. Higher perfusion rates were also detrimental to the physical integrity of the dialysis membrane. By choosing a larger sampling interval, lower perfusion rates can be used with negligible carry-over effect. Larger sampling intervals are ideal for dissolution testing of sustained-release formulations, but may not be adequate for immediate release dosage forms, if a dissolution profile is desired.

Table 1
Effect of perfusion rate and injection interval on the sample carry-over from the STT E6 multiposition switching valve

Perfusion rate (μl min^{-1})	Injection interval (min)	Sample carry-over (%)
20	1	30
30	1	21
30	2	10
30	3	5.7
60	1	14
75	1	9.0
100	1	<1
10	10	<1

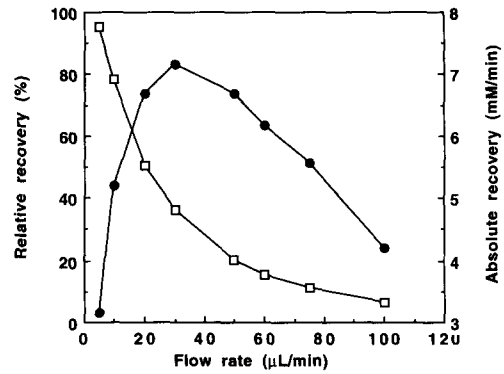

Fig. 2. Effect of perfusion medium flow rate on the relative (□) and absolute (●) recoveries of acetaminophen through a DL-5 loop probe. The concentration of acetaminophen was approximately 350 μg ml^{-1} in water at 37 °C. The perfusion medium was water.

Probe-to-probe variations

Multivessel sampling using the STT E6 valve required use of one microdialysis probe per vessel. Inter-probe variations were determined using three DL-5 loop probes. The probes were immer-sed in an acetaminophen solution (350 μg ml^{-1}) at 37 °C and stirred at 50 rpm to simulate the conditions of the dissolution ex-

periments. Each probe was perfused with water at 50 μl min^{-1} and the dialysate was analyzed to determine the drug recovery through the probe. The range for the recoveries of the three probes was approximately 15%. Therefore, it was necessary to calibrate each probe individually.

The automated multivessel dissolution system was calibrated as follows. Before the dissolution study, the drug recovery from each microdialysis probe at the condition of the experiment was determined using a standard solution. Next, dissolution studies were conducted on a required number of tablets. The experiment was concluded by repeating the same drug recovery experiment. Average peak heights of the standard solution before and after the dissolution experiments were used to quantify the results of the dissolution study using a single-point calibration method.

Linearity and repeatability

The linearity of the integrated sampling and chromatographic system for acetaminophen was determined using three DL-5 microdialysis loop probes and a STT E6 multiposition valve. Standard solutions of acetaminophen in the concentration range 25–500 μg ml^{-1} were placed in the dissolution vessel at 37 °C and stirred at 50 rpm (i.e. the conditions of the dissolution study). A probe was immersed in each dissolution vessel and perfused with water at 50 μl min^{-1} for on-line sampling. The calibration curves of acetaminophen were linear in the concentration range studied, as shown in Fig. 3. Since microdialysis recovery varied from probe to probe, a slight different curve was obtained with each probe. The range of the slopes was approximately 15%, consistent with the inter-probe variability described in the previous section. The linear calibration curves suggested that the microdialysis recovery did not change with drug concentration in the range studied.

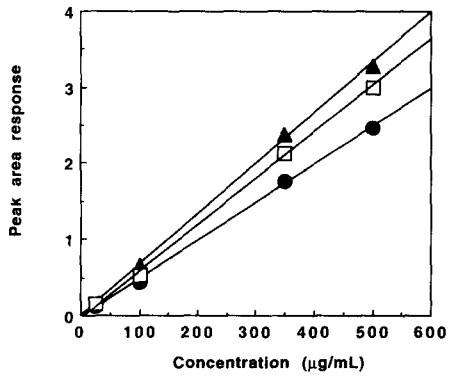

Fig. 3. Calibration curves of acetaminophen determined by three different DL-5 loop probes simultaneously using the automated multivessel dissolution system. Study conditions: 37 °C and 50 rpm; perfusion rate, 50 μl min^{-1}; perfusion medium, water.

Similarly, the calibration curves of phenylpropanolamine were linear in the concentration range of 10–100 μg ml^{-1}. However, below 10 μg ml^{-1} the response was not linear with concentration, presumably owing to adsorption of the drug onto the connecting tubing and/or the dialysis membrane. The average microdialysis recovery of drug from aqueous solutions at 37 °C and 10 μl min^{-1} perfusion rate was about 45% over the linear range of the assay (10–100 μg ml^{-1}).

The repeatability of the multivessel automated system was assessed by injecting each standard solution in triplicate during the calibration study. The RSD was less than 3% for all the standards measured by manual and microdialysis sampling, except for the 100 μl ml^{-1} standard measured by probe 2 where the RSD was 4.3%. In general, the RSD value measured at each concentration by microdialysis sampling was lower than that by manual sampling.

3.3. Dissolution of acetaminophen tablets

The dissolution profiles of acetaminophen tablets obtained with an automated multivessel dissolution testing system compared well with profiles measured by manual and automated single-vessel dissolution systems [1], as shown in Fig. 4. The sampling interval with the automated multivessel dissolution system was limited by the dead volume of the STT E6 switching valve. An uneven injection sequence consisting of a 96 s sampling mode alternated by a 36 s flush mode was used to avoid carry-over of the sample from the previous injection. The flush time of 36 s was determined by the retention time of acetaminophen on the LC column. With the present configuration, only three tablets per dissolution run could be studied and each vessel was sampled about every 6.5 min. Faster sampling rates could be achieved if a switching valve with a lower dead volume were available.

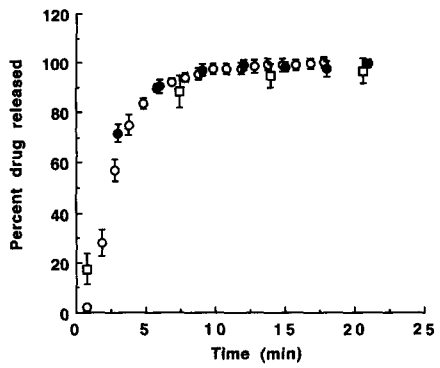

Fig. 4. Dissolution profiles of acetaminophen tablets in water determined by an automated multivessel dissolution system and compared to automated single-vessel and manual dissolution systems. Study conditions: same as in Fig. 3. ○, Single vessel microdialysis sampling; □, multivessel microdialysis sampling; ●, manual sampling. Data from Ref. [1].

3.4. Dissolution of Accutrim® 16 h Precision Release™ tablets

Dissolution profiles of phenylpropanolamine from Accutrim® tablets were measured using the automated multivessel and single-vessel dissolution systems based on microdialysis sampling, and a manual sampling system. The automated systems were calibrated by a single-point calibration method using a 75 μg ml^{-1} standard solution of phenylpropanolamine. The percentage of drug released was calculated based on the labeled amount of 75 mg PPA per tablet. Because of the non-linearity of the assay at less than 10 μg ml^{-1}, results obtained below that value were not reported. Fig. 5 shows the dissolution profiles of Accutrim® 16 h Precision Release™ tablets measured by different systems. Similar drug release profiles were obtained by all the systems and the results compared well with the previously reported release profiles [3]. The percentage of drug released was within the allowable Compendial limits for once-a-day tablets [4], i.e. between 15 and 45% released in 3 h, between 40 and 70% released in 6 h, and not less than 70% in 12 h. Larger sampling intervals were possible for the measurement of the dissolution profile of a slow-releasing tablet. Therefore, dissolution of six tablets could be studied simultaneously using the multivessel dissolution system. In this case, the dissolution vessels were sampled sequentially and the same dissolution vessel was sampled every hour.

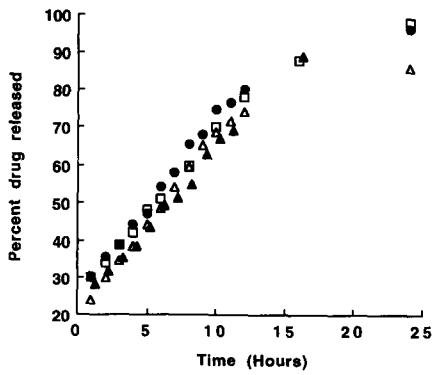

Fig. 5. Dissolution profiles of phenylpropanolamine from Accutrim™ tablets determined by automated multivessel and single-vessel dissolution testing systems and a manual sampling system. Study conditions: 37 °C and 100 rpm; perfusion rate, 10 μl min^{-1}; perfusion medium, water. ●, Manual sampling; △, single-vessel microdialysis sampling; ▲, multivessel microdialysis sampling; □, reference data from Ref. [3].

4. Conclusions

After resolution of the carry-over problem, the six-port STT E6 switching valve was used in a reproducible manner to follow the dissolution of up to six tablets simultaneously. The microdialysis probes were permanently mounted on the switching valve and the probes were suspended in the dissolution vessel at the time of the study. The automated multivessel dissolution system could be switched to a single-vessel or manual system by simply removing the multiposition valve mounted with probes. Except for cleaning of the dissolution vessels and filling the dissolution medium, the system was completely automated. The multiposition switching valve, on-line injection valve and detector were controlled through a computer such that once the testing was initiated, samples were removed and analyzed automatically and the data was stored in the computer. The multiposition switching valve used in this study could be expanded to 16 ports to study the dissolution of up to 16 tablets simultaneously. However, a valve with a lower dead volume must be designed to overcome the carry-over problems observed in this study.

References

[1] K.P. Shah, M. Chang and C.M. Riley, J. Pharm. Biomed. Anal., 12 (1994) 1519–1527.
[2] K.P. Shah, J. Zhou, R. Lee, R.L. Schowen, R. Elsbernd, J.M. Ault, J.F. Stobaugh, M. Slavik and C.M. Riley, J. Pharm. Biomed. Anal., 12(8) (1994) 993–1001.

[3] H.M. Abdou, in A. Gennaro, B. Migdalof, G.L. Hassert and T. Medwick (Eds.), Dissolution, Bioavailability and Bioequivalence, Mack Publishing Co., Easton, 1989, p. 232.
[4] The United States Pharmacopeia, XXII revision, Mack Publishing Co., Easton, 1990.