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Automated analytical systems for drug development studies. II — A system for dissolution testing*

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Abstract: Microdialysis is a non-equilibrium dynamic sampling method in which the analytes diffuse across a semipermeable membrane due to a concentration gradient and are carried away by the constantly pumping perfusion medium for on-line analysis. A BAS, Inc. microinfusion pump/injector and an on-line LC system were interfaced with a dissolution apparatus to automate dissolution testing of tablets. A DL-5 microdialysis loop probe was suspended in the dissolution medium for sampling. The automated system was used reproducibly (RSD <2%) to measure the dissolution of acetaminophen and Sulfatrim tablets. Drug recovery from the microdialysis probe was a function of the perfusion rate at constant temperature. However, microdialysis recovery was independent of drug concentration over the linear ranges of the assays for the analytes of interest. Dissolution profiles determined by microdialysis sampling were compared with manual sampling. Identical profiles were obtained for acetaminophen tablets in water at 37°C and 50 rpm by both sampling methods. Dissolution of Sulfatrim tablets was determined in 0.1 M hydrochloride acid at 37°C and 75 rpm. Microdialysis sampling permitted the use of a specially designed perfusion medium to buffer the acidic samples before injecting onto the LC column. Dissolution profiles of sulphamethoxazole were comparable for both sampling methods; however, microdialysis sampling indicated slightly higher release of trimethoprim from the Sulfatrim tablets, which was attributed to release of adsorbed drug from the connecting tubing.

Keywords: *Automation; microdialysis; liquid chromatography; dissolution; acetaminophen; sulphamethoxazole; trimethoprim.*

Introduction

Biological activity of a drug can be related to the rate at which it becomes available to the body after administration. The determination of the release rate of drug from a dosage form has become an essential part of pharmaceutical development, research and quality control laboratories. The knowledge of dissolution behaviour is also useful in selecting an optimum formulation of drug.

Dissolution testing of pharmaceutical dosage forms is a laborious process that generates a large number of samples. Automated dissolution systems are desirable since they are labour saving and often improve analytical reproducibility. Various approaches have been proposed in the past to automate this testing procedure and there are a few automated systems available commercially [1, 2]. Most of these systems involve pumping the dissolution medium directly through a flow cell mounted in a UV spectrophotometer [3–5]. An inherent problem with such systems is the lack of specificity. LC systems have been interfaced with dissolution apparatus for automated on-line analysis of complex, multicomponent samples [6–8]. However, none of the systems provide provisions for rebuffering the samples that are at extreme pH (e.g. if dissolution medium used is 0.1 M hydrochloric acid), to avoid rapid deterioration of LC columns. Several robotic systems, such as the Zymate™ dissolution apparatus, have been developed to achieve complete automation [9–11]. However, rapid profiling of the dissolution curve limits the use of robots, which are also expensive and complicated to set up for routine analyses.

Previously, microdialysis sampling has been conveniently interfaced with a reaction vessel and an LC system to automate drug stability determinations [12]. This was the first system of its kind capable of rapid sampling (2

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samples/min or higher) and continuous re-buffering of the solutions to pH values that were compatible with silica-based LC columns. The present paper describes the development of a similar system for automated tablet dissolution testing. The system was designed to study only one tablet per run and critical factors affecting the performance of the system, such as system calibration, were identified. A more complicated system designed to sample six tablets simultaneously, using six different microdialysis probes and a multi-position switching valve, is currently under development.

This paper describes the initial development of an automated dissolution testing system comprised of a microdialysis sampling system and an on-line liquid chromatograph interfaced with a dissolution apparatus. At this point, provisions were provided for testing only one tablet at a time. Microdialysis sampling is the unique feature of the system. It involves a non-equilibrium dynamic exchange of analytes across a semipermeable membrane. The constantly pumping perfusion medium carries the analytes that enter the dialysis probe to an on-line LC system for analysis.

Microdialysis sampling technique affords several advantages over other means of sampling. It represents an inexpensive and efficient way to automate sampling and analyses. With the appropriate design of the perfusion medium, samples at extremes of pH can be buffered to a pH that is more compatible with LC column materials for on-line analysis.

Microdialysis is a continuous sampling process that does not involve volume change during sampling and the semipermeable membrane acts as a filter to separate the particulates from sample solution. Drug recovery from microdialysis probes can be adjusted according to perfusion rates or membrane surface area, to avoid an additional dilution step for analysis of high-dose drugs. The maximum sampling interval with this system was limited only by the chromatographic run time. Fast LC columns with 3-μm packing materials were used to reduce the analysis time while maintaining adequate separations of the active ingredients from the tablet excipients.

Experimental

Materials

Acetaminophen, sulphamethoxazole and trimethoprim were purchased from the Sigma Chemical Co. (St Louis, MO, USA). Mono- and dibasic sodium phosphate, sodium citrate, methanol, acetonitrile and hydrochloric acid were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). Triethylamine was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Citric acid was purchased from MCB Manufacturing Chemists, Inc. (Cincinnati, OH, USA). Acetaminophen tablets (325 mg), and Sulfatrim tablets (400 mg sulphamethoxazole, 80 mg trimethoprim) were

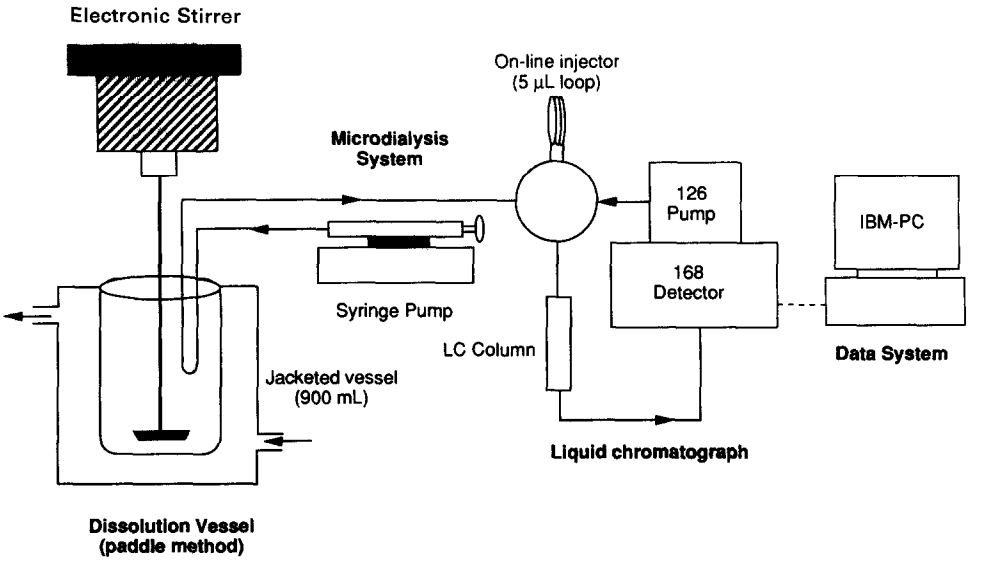

Figure 1
Diagram of the automated system for dissolution testing.

obtained from a local pharmacy. Water processed through the Milli-Q water system (Waters Corp., Bedford, MA, USA) was used in all the experiments.

Instrumentation

The automated system (Fig. 1) consisted of a dissolution apparatus, a microdialysis sampling system and a liquid chromatograph. A Hanson Research Corporation (Chatsworth, CA, USA) model SR2 dissolution apparatus was used for the acetaminophen tablets; whereas, a single vessel apparatus, built in-house by Interx (Lawrence, KS, USA), with a Cole–Parmer Instrument Co. (Chicago, IL, USA) motor and a Van-Kel Industries Inc. (Iselin, NJ, USA) model 2500 circulator was used for the Sulfatrim tablets. The microdialysis system consisted of a BAS, Inc. (West Lafayette, IN, USA) model CMA/100 syringe pump and a model CMA/160 on-line injector. Sampling was achieved with DL-5 microdialysis loop probes, with 5-cm long dialysis membranes, purchased from BAS, Inc. The inlet end of the probe was connected to the syringe pump for continuous perfusion and the outlet was connected to the on-line injector. The LC system consisted of a Beckman (Fullerton, CA, USA) model 110A pump, a Kratos Analytical (Ramsey, NJ, USA) model 757 ultraviolet detector, and a Shimadzu (Kyoto, Japan) model C-R3A integrator. An ODS Hypersil column (3 μm, 30 mm × 4.6 mm i.d.) was purchased from Keystone (Bellefonte, PA, USA).

Tablet dissolution

Dissolution testing of the acetaminophen and Sulfatrim tablets was performed according to USP XXII specifications, using the paddle method. Dissolution profiles of six tablets were determined by manual and microdialysis sampling each, but only one tablet was studied at a time.

Acetaminophen tablets. The dissolution medium used for the acetaminophen tablets was 900 ml water at 37°C and 50 rpm. A DL-5 microdialysis loop probe was suspended in the dissolution medium for sampling and the probe was continuously perfused with water at a rate of 10.5 μl min^{-1}. The dialysate was collected in a 5-μl injection loop and injections were made every minute. Thus the injection loop was flushed with at least twice its own volume of perfusion solution between each injection. The samples were analysed on-line using a short LC column and UV detection at 280 nm. The mobile phase used to elute acetaminophen consisted of methanol–phosphate buffer (pH 6.2, 50 mM) (15:85, v/v) at a flow rate of 2 ml min^{-1}. In a separate study, the dissolution of tablets was followed by sampling manually the medium every 3 min and analysing the samples off-line.

Sulfatrim tablets. The dissolution medium used for the Sulfatrim tablets was 900 ml of 0.1 M HCl at 37°C and 75 rpm. A DL-5 probe was immersed in the dissolution medium and perfused with citrate buffer (1 M, pH 4.5) to rebuffer the acid before injection onto the LC column. A perfusion rate of 11.5 μl min^{-1} was used and injections were made every 1.4 min. Under these conditions the injection loop was flushed with approximately twice its own volume of perfusion solution between each injection. The samples were analysed on-line using a short LC column and UV detection at 230 nm. A mobile phase containing acetonitrile–citrate buffer (0.1 M, adjusted to pH 5 with 25 mM triethyalmine) (20:80, v/v) was used at a flow rate of 2 ml min^{-1} to elute sulphamethoxazole and trimethoprim. Tablet dissolution was also followed by manually sampling the dissolution medium every 5 min and diluting the samples with equal amount of mobile phase before analysis.

Independent determinations of the drug content of the tablets

Twenty tablets were weighed to determine the average tablet weight and then crushed with a mortar and pestle. An amount equivalent to the average tablet weight was accurately weighed and suspended in 1 l of the dissolution medium. The mixture was sonicated for 10–15 min to ensure complete dissolution of the drug(s) from tablets. The undissolved materials were separated by filtration and the filtrate was analysed by LC with UV detection. Each analysis was conducted in triplicate.

Results and Discussion

Acetaminophen tablets

Single component acetaminophen tablets were used to check the feasibility of microdialysis sampling to automate dissolution

testing. Although the dissolution samples of acetaminophen did not require LC analysis (flow injection or continuous UV absorbance measurement would have adequate), separation capabilities were incorporated to demonstrate the feasibility of this approach for the analysis of more complex samples (e.g. Sulfatrim tablets).

Analytical recovery and system reproducibility. The analytical recovery and system reproducibility were studied as a function of perfusion rate and volume of the perfusate introduced into the injection loop. In these experiments the acetaminophen was first added to microdialysis perfusion solution and pumped through the injection loop at a constant flow rates ranging between 3 and 10 μl min^{-1}. Injections were made at predetermined intervals so that the loop was flushed with either 150 or 200% of its own volume between each injection (i.e. 7.5 or 10 μl). The analytical recovery (R_A) was then determined from the equation:

$$R_A = \frac{P_P}{P_D} \times 100 \quad (1)$$

where P_P is the peak height of acetaminophen following injection of the drug when added to the perfusion medium and pumped through the loop and P_D is the peak area of acetaminophen following direct introduction of the drug through the injection port.

Table 1 shows that the precision was acceptable (RSD <2%) if a volume of perfusion solution equal to more than 150% the volume of the loop was passed through the injector between each injection. However, Table 1 also shows that it was necessary to flush the loop with at least 200% of its own volume of perfusion for complete recovery of the analyte from the microdialysis perfusate.

Recovery of the microdialysis probe. The recovery of the microdialysis probe was determined in a separate set of experiments in which a 100 μg ml^{-1} solution of acetaminophen was sampled by microdialysis at a several flow rate (1–13 μl min^{-1}). The relative recovery of the microdialysis probe (R_m) was defined by:

$$R_m = \frac{P_i}{P_e} \times 100 \quad (2)$$

where P_i and P_e are the peak heights of acetaminophen in the microdialysis medium and in the external solution, respectively. The absolute recovery (A_m, in μg ml^{-1} min^{-1}) was defined by equation 3

$$A_m = C_e \cdot F_v \cdot R_m \quad (3)$$

where C_e and F_v are the concentration of analyte in the external solution and the flow rate through the microdialysis probe, respectively. Figure 2 shows the effect of perfusion rate on relative and absolute recovery of acetaminophen at 37°C. The relative recovery decreased with increasing perfusion rates, consistent with previous results [12]. On the other hand, the absolute recovery, defined as the concentration of analyte recovered per unit time, increased within increasing perfusion rate up to 12 μl min^{-1} and then reached a plateau

Table 1
Effect of perfusion rates and loop fill volumes on analytical recovery and precision of system

Perfusion rate (μl min^{-1})	Fill volume* (μl)	Peak height† (μV × 10^3)	Analytical recovery‡ (%)	RSD§ (%)
Manual	100	77.4	100.0‖	0.15
3	7.5	76.9	99.4	1.40
3	10	77.4	100.0	0.26
6	7.5	74.3	96.0	0.20
6	10	77.8	100.5	0.98
10	7.5	73.0	94.3	0.65
10	10	76.3	98.5	1.50

* Volume of solution passed through loop between each injection.
† Concentration of acetaminophen = 100 μg ml^{-1}.
‡ $R_A = \frac{P_P}{P_D} \times 100$ (equation 1).
§ $n = 5$.
‖ 100% by definition.

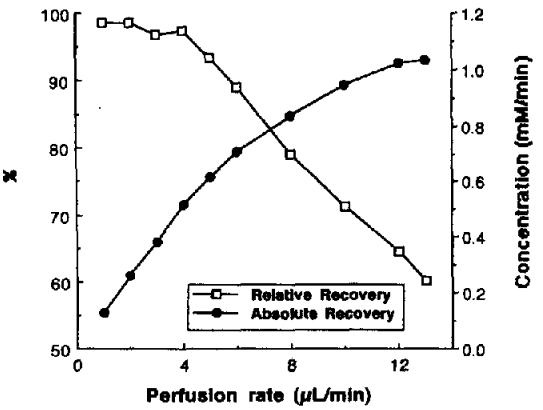

Figure 2
Effect of microdialysis perfusion rate on relative and absolute recoveries of acetaminophen at 37°C.

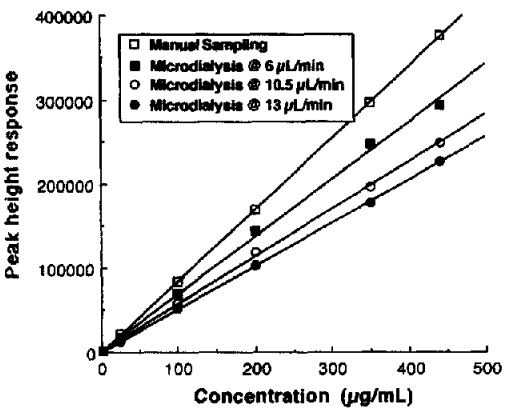

Figure 3
Calibration curves of acetaminophen determined by manual sampling and microdialysis sampling at 37°C and various perfusion rates.

due to ultrafiltration of the analyte through microdialysis probe at higher perfusion rates.

Linearity

The linearity of the chromatographic system was established over a concentration range of 2–440 μg ml^{-1} by manual and by microdialysis sampling. Three different perfusion rates were used for the microdialysis sampling. Linear calibration curves were obtained with both sampling techniques (Fig. 3 and Table 2). The *y*-intercept for each calibration curve was less than 2% of the peak height response at the highest concentration (440 μg ml^{-1}), suggested a negligible (zero) intercept on the *y*-intercept. The ratio of the slopes of microdialysis sampling at each perfusion rate to the slope obtained by manual sampling gave an additional measurement of the acetaminophen recovery at that perfusion rate. The average recovery values calculated from the slopes compared well with values obtained from the flow rate study (Fig. 2). Linearity of the calibration curves confirmed that the microdialysis drug recovery did not change with concentration in the range studied.

Dissolution study. The dissolution of acetaminophen tablets in 900 ml water at 37°C and 50 rpm was studied using the automated system developed in this study. Six tablets were analysed by each method and the dissolution profiles are reported as the mean, with the associated standard errors, at the mid-point of each sampling interval.

The automated system was calibrated as follows: at the beginning of the experiment, the drug recovery from microdialysis probe at the conditions of the dissolution study was determined at the lowest and the highest concentrations of the calibration curve. Then, the dissolution profiles of six tablets were determined using the same probe in an automated fashion. The experiment was concluded

Table 2
Parameters for calibration curves of drugs measured by manual and microdialysis sampling

Drug	Sampling method	Perfusion rate (μl min^{-1})	Calibration parameters		Peak height* (μV × 10^{-4})
			Slope	*y*-intercept	
Acetaminophen	Manual	—	852	−318	37.6
	Microdialysis	6.0	681	2052	29.4
	Microdialysis	10.5	564	1596	24.9
	Microdialysis	13.0	512	450	22.7
Sulphamethoxazole	Manual	—	1178	3737	89.5
	Microdialysis	11.5	552	785	27.9
Trimethoprim	Manual	—	1482	1933	15.0
	Microdialysis	11.5	372	207	3.8

*Peak height of the highest standard used in the calibration curve. The highest standards used for acetaminophen, sulphamethoxazole and trimethoprim were 440, 500 and 100 μg ml^{-1}, respectively.

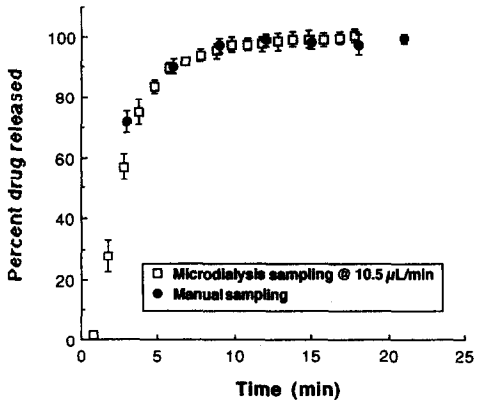

Figure 4
Dissolution profiles of acetaminophen tablets in water determined by manual and microdialysis sampling. Study conditions: 37°C and 50 rpm; perfusion rate — 10.5 µl min^{-1}; perfusion medium — water.

by repeating the drug recovery experiment with the same standard solutions. The average peak heights of the standards were used to define the dissolution profiles.

Figure 4 shows the dissolution profiles of acetaminophen tablets obtained with microdialysis and manual sampling. The sampling interval with microdialysis system was limited by the chromatographic run time. With a mobile phase of methanol–phosphate buffer (50 mM, pH 6.2) (15:85, v/v) and a flow rate of 2 ml min^{-1} the retention time for acetaminophen was 0.6 min, which allowed a run time of about 1 min before the next sample could be injected into the chromatograph. The microdialysis probe was perfused with water during sampling. The analytical recovery experiments described previously in this report indicated that a perfusion rate of 10.5 µl min^{-1} was necessary if the samples were to be sampled every 60 s and still permit an analytical recovery (R_A, equation 1) of 100%. With manual sampling, the dissolution medium was sampled every 3 min and the samples were analysed by direct injection. Dissolution profiles obtained with both sampling techniques were similar (Fig. 4). All the tablets met the USP specification of not less than 80% drug released in 30 min.

Sulfatrim tablets

A multicomponent tablet, containing sulphamethoxazole and trimethoprim (Sulfatrim tablets), was used as a model for analysing complex samples using the automated system. Certain challenges had to be overcome before the system could be used effectively for dissolution testing of these tablets. Firstly, a suitable LC assay was needed so that both drugs could be eluted and analysed within 1.5 min, before the next sample was injected. Secondly, the USP XXII specifies the use of 0.1 M HCl as a dissolution medium for Sulfatrim tablets, which is not compatible with bonded phase LC columns when used for on-line analysis [12]. A specially designed perfusion medium was, therefore, developed to adjust the pH for on-line analysis.

Method development. The LC conditions described by Mathieu *et al.* [6] were adapted to achieve rapid resolution of sulphamethoxazole and trimethoprim on a short ODS Hypersil column (3 µm, 30 × 4.6 mm, i.d.). The separation was optimized by varying the pH of the aqueous component of the mobile phase, which contained acetonitrile–citrate buffer (0.1 M, pH 4.6–6.6) (Fig. 5). The retention times of both drugs decreased with increasing pH (Fig. 5). Sulfamethoxazole is a weak acid (pK_a = 5.6) and the decrease in its retention was explained in terms of its increasing degree of ionization with increasing pH. The decrease in retention of trimethoprim, which is a weak base (pK_a = 6.6), was explained in terms of its decreasing interaction with residual silanol groups at higher pH values [13]. Whereas adequate resolution of sulphamethoxazole and trimethoprim was achieved at pH 5.0, the peak of trimethoprim was poor, supporting the hypothesis that trimethoprim was partially retained on the residual silanol groups. Figure 6 show that the peak shape of trimethoprim was dramatically improved by the addition of

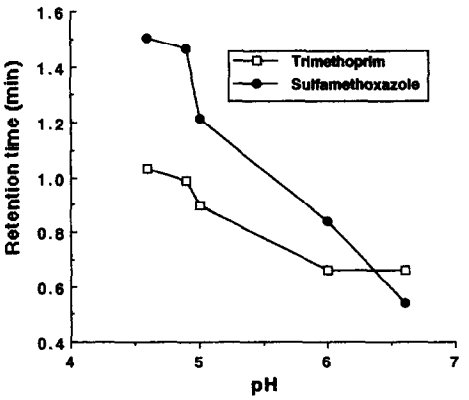

Figure 5
Effect of mobile phase pH on retention times of sulphamethoxazole and trimethoprim.

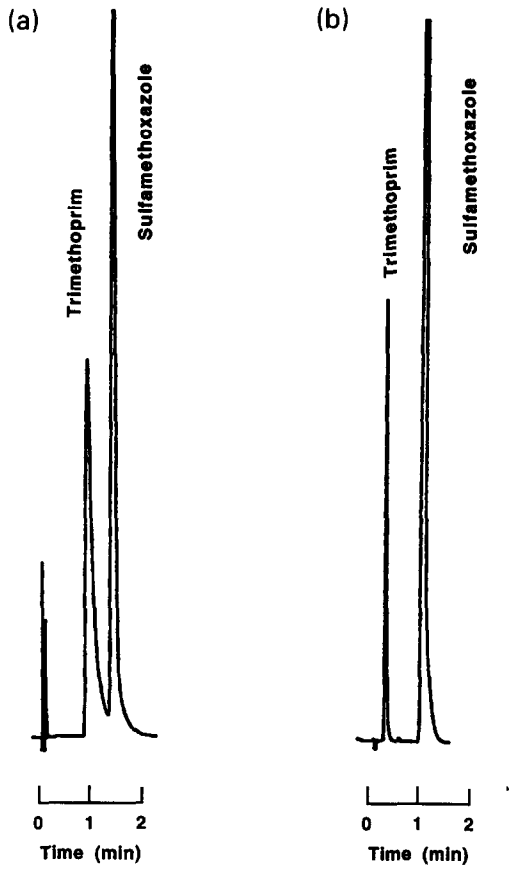

Figure 6
Typical chromatograms of the separation of sulphamethoxazole and trimethoprim: (a) in absence of TEA, (b) 25 mM TEA present in the mobile phase. Stationary phase: ODS Hypersil column (3 μm, 30 mm × 4.6 mm i.d.); mobile phase: acetonitrile–citrate buffer (pH 5.0, 0.1 M) at flow rate of 2 ml min^{-1}.

triethylamine (TEA) to the mobile phase. With a flow rate of 2.0 ml min^{-1} and a mobile phase containing acetonitrile–citrate buffer (0.1 M, pH 5) (20:80) and 25 mM TEA, the retention times for trimethoprim and sulphamethoxazole were 0.5 and 1.1 min, respectively. These conditions allowed a run time of about 1.4 min.

Design the perfusion medium. Since 0.1 M HCl was used as the dissolution medium for Sulfatrim tablets, H^+ ions diffused through the dialysis membrane and made the samples too acidic for on-line analysis using bonded phase LC columns. The main advantage of microdialysis sampling, in the present system, arose from the rebuffering of dissolution samples, which were acidic (pH 1.0), to a pH compatible for on-line LC analysis. Thus, a separate dilution step that would be required otherwise, was eliminated with this sampling technique.

Several buffers were tried, based on the pK_a of buffer species, to neutralize the samples to a suitable pH for automated LC analysis. The 0.1 M HCl by itself has a strong buffer capacity; and, therefore, a buffer at a much higher concentration (1 M) was required to adjust the pH of the perfusate to a value that was suitable for on-line LC analysis (i.e. pH > 2.0). Some of the buffers investigated at a concentration of 1 M were acetate (pH 4.75), phosphate (pH 7.0) and citrate (pH 4.5). The citrate buffer was most successful in rebuffering 0.1 M HCl at 37°C; however, it had to be used at a minimum perfusion rate of greater than 10 μl min^{-1} to achieve adequate rebuffering of the perfusate. The pH of the perfusate exiting the probe was estimated using litmus paper.

Calibration and linearity. As in the acetaminophen studies, the linear ranges for the microdialysis sampling of sulphamethoxazole and trimethoprim were identified. Calibration standards were prepared by appropriate dilution of a stock solution containing both sulphamethoxazole and trimethoprim in a 5:1 (w/w) ratio, which is the same ratio as the

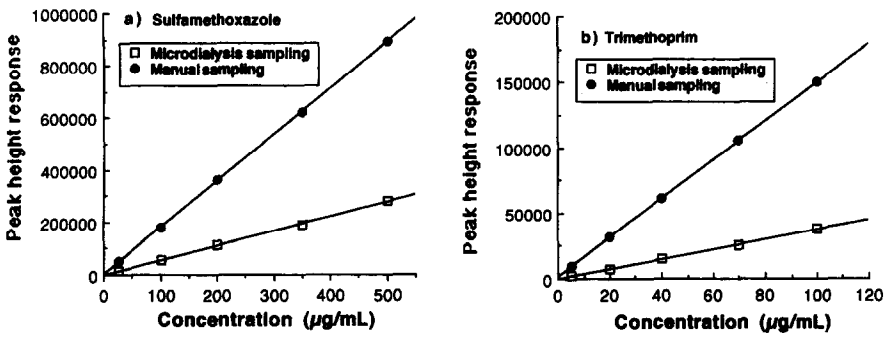

Figure 7
Calibration curves of sulphamethoxazole and trimethoprim determined by manual sampling and microdialysis sampling at 37°C and 11.5 μl min^{-1} perfusion rate.

drugs in the tablets. Figure 7 shows the calibration curves of the two drugs obtained with direct manual injection and with microdialysis sampling at a perfusion rate of 11.5 μl min^{-1}. The calibration curves were linear in the range of 25–500 μg ml^{-1} for sulphamethoxazole and 5–100 μg ml^{-1} for trimethoprim. The linear response was lost at concentrations less than these values. Both sulphamethoxazole and trimethoprim adsorbed on to the connecting Teflon tubing and/or the dialysis membrane and low concentrations. Subsequent release of adsorbed drug appeared to give rise to some carry-over and non-linear responses at low concentrations.

Table 2 summarizes the calibration parameters for sulphamethoxazole and trimethoprim. The *y*-intercept of all the curves were below 2% of peak height of the highest standard, suggesting a zero intercept. The ratios of the slopes of microdialysis sampling to manual sampling gave 31 and 25% average recoveries (R_m) for sulphamethoxazole and trimethoprim, respectively.

Dissolution studies. Dissolution profiles of Sulfatrim tablets were determined in 900 ml of 0.1 M HCl at 37°C and 75 rpm by on-line microdialysis and by direct manual sampling. Six tablets were analysed by each method and the system was calibrated as described in the acetaminophen studies. The sampling interval for the microdialysis system was 1.4 min compared with 5 min for manual sampling. Figure 8 shows the dissolution profiles for Sulfatrim tablets obtained using both sampling methods. Complete dissolution of trimethoprim in 0.1 M HCl was obtained within 15 min; whereas sulphamethoxazole dissolved slowly over 60 min. All the tablets met dissolution specifications described in USP XXII (70% drug release in 60 min). Dissolution profiles of sulphamethoxazole obtained with microdialysis sampling compared well with manual sampling as shown in Fig. 8. However, the total amount of trimethoprim released from the tablets when measured by microdialysis sampling was slightly more than 100%. On the other hand manual sampling of the Sulfatrim tablets indicated that the amount of trimethoprim released from the tablets was 100%. The apparently higher release of trimethoprim was attributed to carry-over effect due to absorption of trimethoprim to the dialysis membrane and/or connecting tubing. Apparently the adsorption of sulphamethoxazole onto the plastic tubing did not influence its dissolution profile measured by microdialysis. Efforts are being made to search for non-reactive tubing and membrane materials to avoid the problems of adsorption.

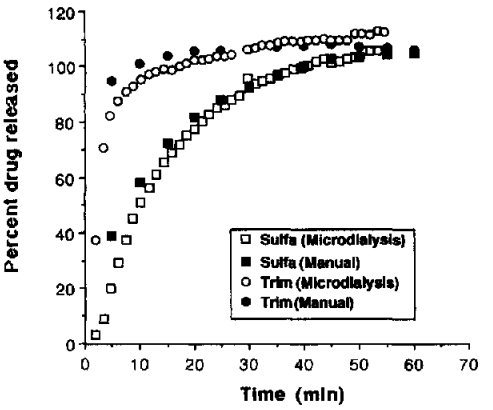

Figure 8
Dissolution profiles of Sulfatrim tablets in 0.1 M HCl determined by manual and microdialysis sampling. Study conditions: 37°C and 75 rpm; perfusion rate — 11.5 μl min^{-1}; perfusion medium — citrate buffer (1 M, pH 4.5).

Conclusion

The automated system described here can be compartmentalized to allow the selection of various sampling modes and analytical methods. For example, the microdialysis system can be directly connected to a UV detector for flow injection analysis, if separation capabilities are not required. The system is versatile and can be used with various dissolution media, regardless of pH, and over a wide range of analyte concentration. If the samples need to be removed at widely spaced time intervals or at the end of the dissolution test, this system could be adapted to monitoring six dissolution vessels simultaneously. Studies are in progress to interface a multiposition switching valve to a six station dissolution apparatus for testing controlled release tablets. Unattended testing is the ultimate aim of automation. Since the microdialysis recovery is a function of flow rate, the samples can be automatically diluted into the linear range of the measuring system by controlling the flow rate of the perfusate. The automated system described here also lends itself to computer control, such that the operator only has to add the tablets and collect the results at the end of the experiment.

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