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AUTOMATED DISSOLUTION TESTING WITH FLOW-INJECTION ANALYSIS. DISSOLUTION PROFILES FOR THE ANTIVIRAL DRUGS, DHPG AND ACYCLOVIR, IN CAPSULE FORMULATIONS.

Richard A. Kenley*, Stuart E. Jackson, Gary C. Visor, and John S. Winterle

Syntex Research
3401 Hillview
Palo Alto, Ca
94304

ABSTRACT

We have designed, assembled, and tested an automatic dissolution apparatus using flow-injection analysis (FIA) techniques with spectrophotometric detection. The components (pumps, switching valves, detector, and so forth) that comprise the system are all readily-available items used primarily for high-performance liquid chromatography. The system performs well as evidenced by the usual tests for precision, response linearity, and dissolution behavior of standard U.S.P. calibrator (salicylic acid and prednisone) tablets, and offers significant advantages over conventional continuous-flow dissolution testing methods with respect to simplicity, cost, and versatility. Dissolution tests on capsules of the

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antiviral drugs, DHPG and acyclovir, showed very similar drug release profiles for both formulations.

INTRODUCTION

Pharmaceutical compendia[1-3] and accepted formulation development practices require dissolution testing of oral dosage forms to ensure uniform and reproducible drug release rates. As the popularity of dissolution testing has increased over the last two decades, so has the number of reported[4-12] methods for automating the test procedures. Although various commercial and literature designs for automated dissolution testing differ in detail, most current procedures share a common analyte detection method, namely spectrophotometric detection of dissolved drug in a continuous dissolution medium stream.

The continuous-flow technique is reliable and easily adapted to modern grating and diode-array spectrophotometers, but nevertheless suffers three important disadvantages. First, some continuous-flow methods consume relatively large sample volumes for each analysis and require complicated sample-return mechanisms to prevent analyte concentration changes via dissolution medium depletion. Secondly, the continuous-flow method does not easily adapt to alternate detection schemes such as electrochemical, spectrofluorimetric, and analyte derivatization techniques. Finally, conventional continuous-flow systems are relatively costly.

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As an alternative to continuous-flow spectrophotometric detection for automated dissolution testing, we have considered flow-injection analysis (FIA). FIA is a technique wherein precise sample volumes are sequentially injected into a liquid carrier stream and transported to a detector with or without the introduction of additional processes (extraction, derivatization, and so forth) required for analyte quantitation. By carefully controlling the FIA transport parameters (e.g. carrier flow rate and volume), analyte diffusional dispersion remains constant between injections, and a transient signal proportional to analyte concentration obtains as the sample passes through the detector. Since its introduction[13,14] in 1975, FIA has enjoyed considerable success for organic and inorganic analysis in general[15–18] and for pharmaceutical analysis in particular[19].

Koupparis et al.[20] previously described an automated dissolution apparatus based on FIA, but the reported system used only a single dissolution vessel. Because compendial, statistical and sample throughput considerations recommend multiple vessels for dissolution testing, we have extended the application of FIA to include serial sampling from six dissolution vessels. The system reported below combines "off-the-shelf" hplc components with a standard dissolution vessel-stirrer-bath assembly to provide a simple, relatively

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inexpensive, and useful alternative to continuous-flow dissolution test equipment.

We find that the FIA-based dissolution system performs well as indicated by the usual tests for precision, response linearity, and drug release profile using U.S.P. calibrator (salicylic acid and prednisone) tablets. Additionally, we report dissolution profile data for the antiviral drugs, DHPG[21] and acyclovir[22], in capsule formulations.

EXPERIMENTAL DETAILS

Materials. Salicylic acid (300 mg, Lot H) and prednisone (50 mg, Lot G) tablets were used as supplied by the U.S.P. Acyclovir (trademark Zovirax), 200 mg, capsules were from Burroughs-Wellcome. DHPG prototype 200 mg hard gelatin capsules were prepared by the Syntex Institute of Pharmaceutical Sciences.

Dissolution System Components. The six-vessel stirrer and bath assembly was a Distek Model 2000 system (U.S.P. rotating-paddle Apparatus 2). Teflon tubing, 0.1-mm i.d., was used throughout for sample transfers, and Gelman Acrodisc CR 1 micrometer filters were used in-line between the dissolution vessels and

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sampling valves. The sample selection valve (Valve 2, see below) was a Rheodyne Model 7066 1-into-6 rotary valve with Model 5704 pneumatic actuator. The sample injection valve (Valve 1, see below) was a Rheodyne Model 7010 six-port valve with Model 7001 pneumatic actuator. Rheodyne Model 7163 solenoid air valves operated the pneumatic actuators, and an Autochrome Model 201 Solenoid Interface connected the air valves to the system timer-controller (Minarik Micromaster microprocessor-controller). An Eldex Model 1001/E low-pressure pump (Pump 2, see below) delivered samples from the dissolution vessels to the sample injection valve, and a Waters Model P6000 pump transported sample from the injection loop to the detector (Spectra-Physics Model 8200 variable-wavelength spectrophotometric detector). A Spectra-Physics Model 4290 integrator acquired and recorded the detector signals.

Dissolution System Configuration. Figure 1 is a schematic representation of the overall system configuration. Figure 1 shows both the sample delivery pump (Pump 2) and the sample injection pump (Pump 1) connected to the sample injection valve (Valve 1). Pump 2 draws sample from the dissolution vessels and through the 10-uL sample loop on Valve 1. Under system timer control, Valve 2 sequentially samples from each of the six dissolution vessels that are immersed in the thermostatted sample bath. Switching Valve 1 from the "Load" to the "Inject"

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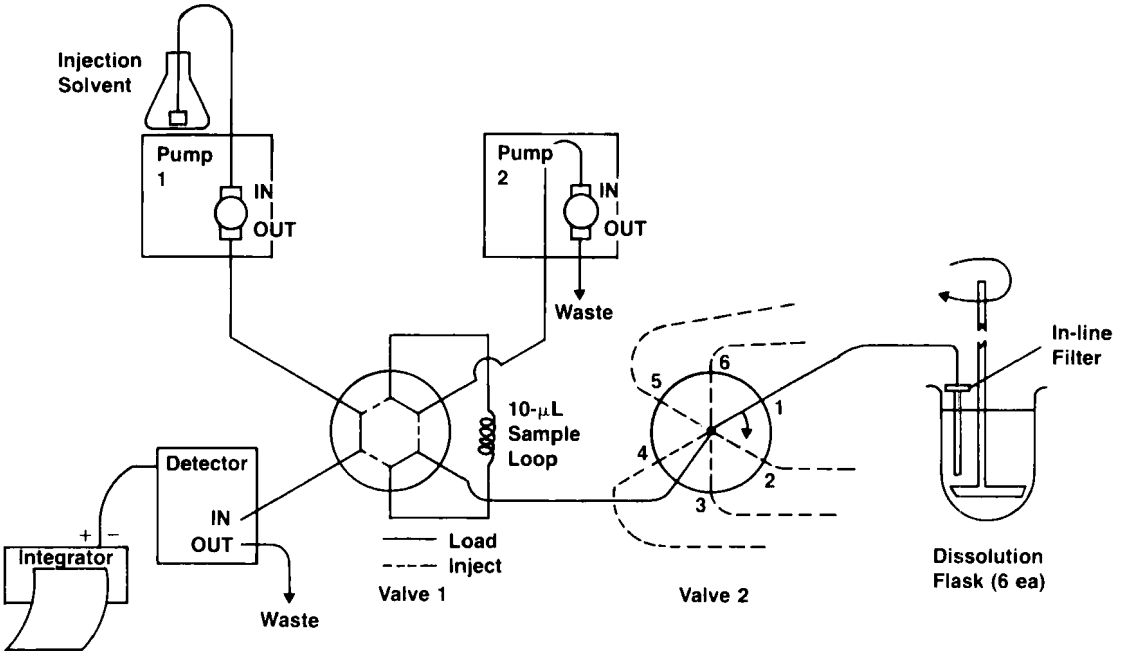

FIGURE 1.
Schematic Representation of the FIA Dissolution Apparatus. Only one of six dissolution vessels is shown for clarity. Pump 1 is the sample injection pump, and Pump 2 is the sample delivery pump. Valve 1 diverts sample from the sample loop into the Pump 1 carrier stream and, thence, to the detector. Valve 2 sequentially selects the dissolution vessel for sampling.

position (again under system timer control) diverts the sample loop contents to the Pump 1 carrier stream and, thence, to the detector. Figure 2 details the Valve 1 configurations in the "Load" and "Inject" positions. Figure 3 is a schematic of the electrical and pneumatic connections in the system timer, controller, and valve assemblies.

Dissolution System Specifications. Table I summarizes the dissolution media, stir rates, and detector wavelengths used for

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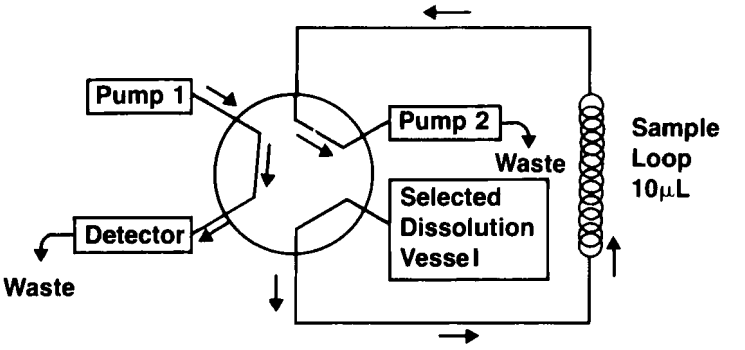

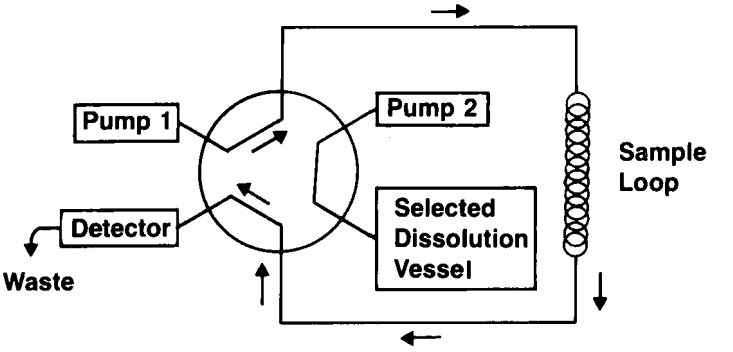

FIGURE 2.
Schematic Representation of Valve 1 in the "Load" and "Inject" positions. In the "Load" position, Pump 2 fills the sample loop. In the "Inject" position, Pump 1 flushes the sample loop contents to the detector. Pump 2 remains off while Valve 1 is in the "Inject" position.

dissolution testing of DHPG, acyclovir, salicylic acid, and prednisone solid dosage forms. For all determinations, the samples were thermostatted at 37 ± 0.5 °C, and samples were withdrawn via tubing positioned 5 cm above the vessel bottom.

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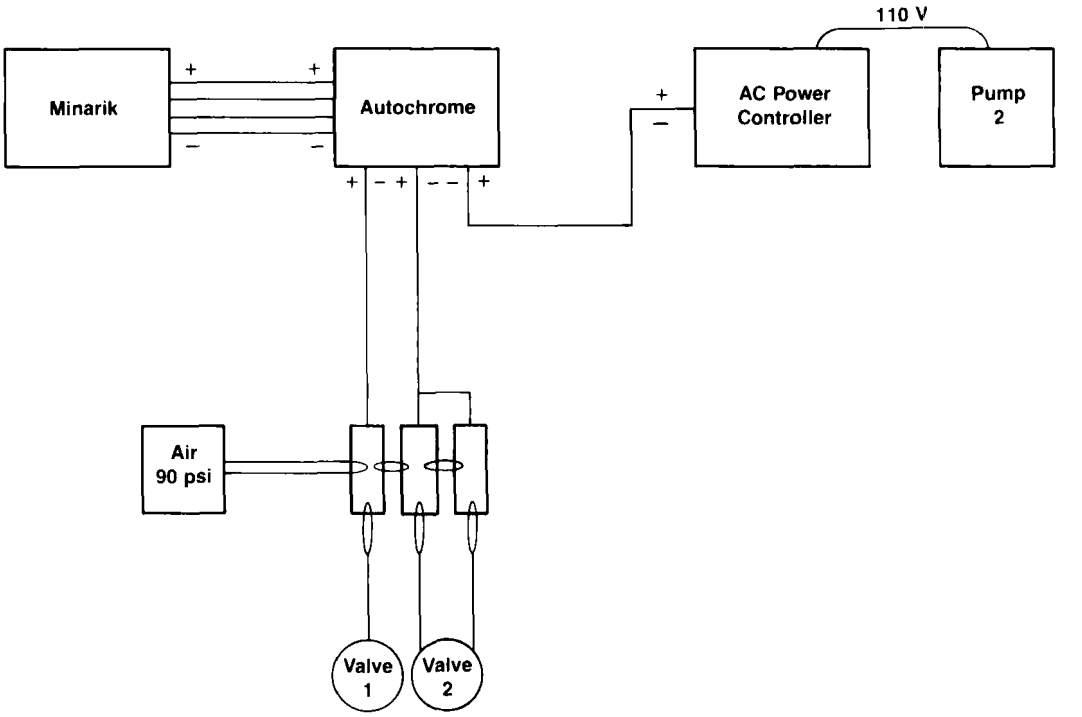

FIGURE 3.
Schematic Representation of the Electrical and Pneumatic Connections in the System Controller, Pump, and Valve Assemblies.

Table I. Experimental Conditions for Dissolution Testing.[a]

DRUG	DOSE mg	DISSOLUTION MEDIUM	VOLUME mL	STIR RATE r.p.m.	WAVELENGTH nm
Prednisone	50	water	900	50	242
Salicylic Acid	300	50 mM, pH=7 Phosphate	900	50	296
DHPG	200	1 N HCl	900	50	254
Acyclovir	200	1 N HCl	900	50	254

a. All at 37.0 ± 5 °C. See Experimental Details section.

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The injection solvent (carrier stream from Pump 1) was 0.1% aqueous phosphoric acid. Pump 1 operated at 1.5 mL/min and Pump 2 at 3.0 mL/min. The system dead volume was 0.80 mL and 1.5-mL sample aliquots were drawn through the 10-uL sample loop prior to each injection.

System Operation. Table II lists the timed events used for automated dissolution testing of acyclovir and DHPG capsules. The entire sequence (Steps 2 through 5) takes 0.83 min, and with the delay step (Step 1),allows sampling the six dissolution vessels at 5-min intervals. For salicylic acid and prednisone tablets (which were sampled at a single timepoint only), the delay step was set to 30 min, and the sampling sequence terminated after withdrawing single samples from each dissolution vessel. The following paragraphs detail general aspects of the system operation and a specific procedure for experimental determinations.

Procedures. Prior to operation, prime all sampling lines and Pump 2 with degassed dissolution medium to ensure consistent sampling. Wet the in-line filters to prevent air lock. Also prior to each run, advance Valve 2 to vessel #6. Initiating the timing sequence advances Valve 2 to vessel #1 before withdrawing the first sample. After each run, flush the tubing and pump parts completely with water to remove acid and salts contained in the dissolution media. Flush with methanol or

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Table II. Timed Event Sequence For Automated Dissolution Testing.

STEP #	TIME min	EVENT(s)
1	0 to 5.00	Delay before first sample
2	5.00 to 5.02	Advance Valve 2, Valve 1 to "Load"
3	5.02 to 5.52	Pump 2 on, sample loop fills
4	5.52 to 5.83	Pump 2 off, Valve 1 to "Inject" Pump 1 pumps sample to detector
5	5.83	Return to Step # 2

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acetonitrile between runs. The flushing procedure prevents corrosion in the stainless steel pump and valve components.

To begin a determination, fill each dissolution vessel to volume with medium, bring the system to temperature, set Valve 1 to the "Inject" position, and introduce a capsule into vessel #1. Upon introducing the first capsule, initiate the timing sequence (Table II) in the system timer-controller and then sequentially add individual capsules to vessels 2 through 6 at 0.83-min intervals. After the delay interval (Step 1) has elapsed, the system controller: advances Valve 2 to the next dissolution vessel, and sets Valve 1 to the "Load" position (Step 2), and then turns on Pump 2 to fill the sample loop (Step 3). The 0.5-min duration of Step 3 allows Pump 2 to displace two dead volumes with sample and fill the injector loop with

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fresh sample. Step 4 stops Pump 2, and sets Valve 1 to the "Inject" position whereby Pump 1 forces the sample aliquot downstream to the detector cell. At 5.83 min into the sequence, Step 5 returns the system to Step 2 and the sampling procedure begins again. The operating sequence is designed to sample each of six dissolution vessels at 5-min intervals. A "HALT" key on the system timer allows manually restarting the sequence at any interval.

RESULTS AND DISCUSSION

System Validation. Injecting standard drug solutions at known concentrations provided checks on system linearity and precision. For DHPG, six standards (in duplicate) at 70 to 120 percentage of labeled strength (% LS) gave the linearity plot shown in Figure 4. The data in Figure 4 adhere to equation (1):

$$\text{Peak Height} = (21600 \pm 13000) + (4870 \pm 140)*(\%LS) \qquad (1)$$

where the error limits are 95% confidence intervals and the least-squares correlation coefficient = 0.9992.

Table III summarizes precision statistics for replicate injections of drug standard solutions made to known

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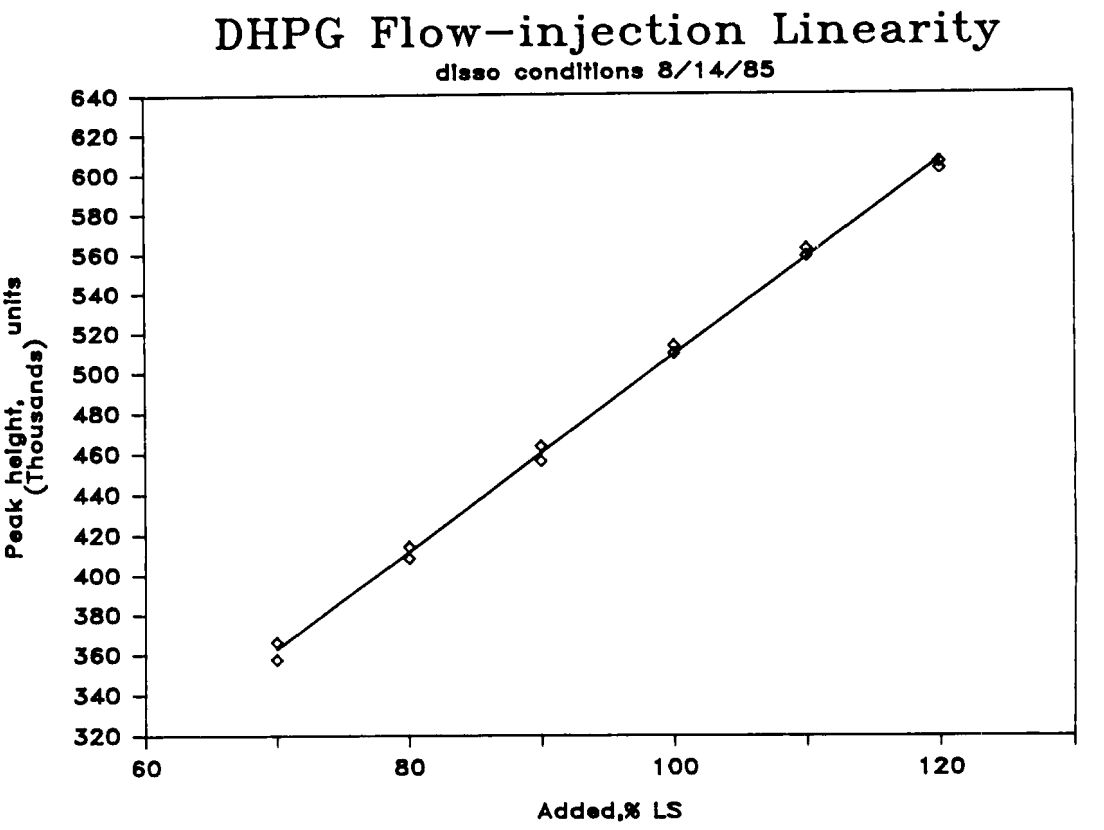

FIGURE 4.
Response Linearity Plot of Peak Height versus % of Labeled Strength (% LS) of DHPG Standard Solutions Made to Known Concentrations.

Table III. Precision Statistics For Replicate Injections of Drug Standard Solutions With FIA Dissolution Apparatus

DRUG	# OF REPLICATES	[STANDARD] % LS [a]	RELATIVE STANDARD DEVIATION
Prednisone	5	100.7	1.17
Salicylic Acid	5	93.6	0.380
DHPG	10	108	0.29
Acyclovir	5	118	0.18

a. % LS = % of dosage form labeled strength, see Table I.

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concentrations. For all drugs tested, the relative standard deviations were less than 1.5%.

As a final check on the dissolution system validity, we determined the percentage of drug dissolved at 30 min for U.S.P. salicylic acid and prednisone calibrator tablets. For prednisone the fraction dissolved was 40.44 ± 0.01 % of labeled strength (error limits expressed as the standard deviation about the mean). For salicylic acid the same test gave 14.4 ± 0.81% of labeled strength dissolved after 30 min. Both prednisone and salicylic acid tablets passed the dissolution specifications provided by the U.S.P.

In summary, dissolution testing system with FIA performs very well as measured by the usual criteria for linearity, precision, and standard tablet dissolution profiles. The following section describes the dissolution behavior of two new oral dosage forms for the antiviral drugs, DHPG and acyclovir.

DHPG and Acyclovir Dissolution Profiles. Figure 5 is a FIA trace for acyclovir capsules. The figure shows detector response plotted on the vertical axis versus sampling interval on the horizontal axis. Each group of six peaks corresponds to responses from the six dissolution vessels sampled at the indicated time intervals.

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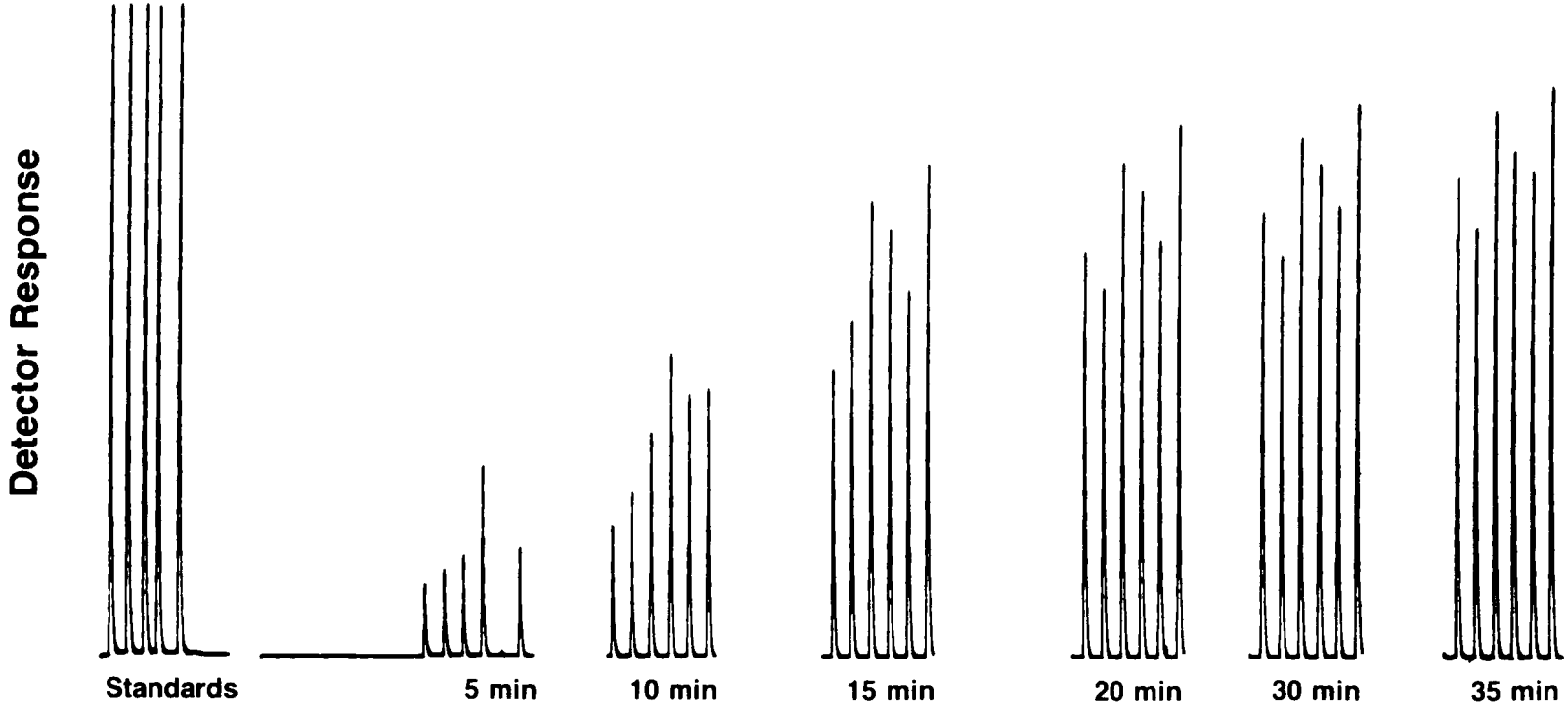

FIGURE 5.
Flow-Injection Trace for Acyclovir Capsule Dissolution. The Detector Response is Plotted on the Vertical Axis versus Sample Time on the Horizontal Axis. Each Group of Six Peaks Represents the Contents of Individual Dissolution Vessels at the Indicated Timepoints.

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Table IV. Dissolution Profiles for Acyclovir and DHPG [a,b].

Drug	Vessel #	% Labeled Strength Dissolved at Time (min) = 5	10	20	25	30	60	120	135
Acyclovir	1	13.1	23.3	74.4	81.6	87.5	96.2	102.1	106.4
	2	16.0	30.6	67.1	72.9	78.7	88.9	94.8	105.0
	3	19.0	40.8	90.4	94.8	100.6	105.0	107.9	112.3
	4	35.0	55.4	84.6	90.4	93.3	97.7	99.1	107.9
	5	—	48.1	75.8	83.1	88.9	99.1	105.0	110.8
	6	20.4	49.6	97.7	100.6	105.0	107.9	109.4	110.8
	Mean =	20.7	41.3	81.6	87.2	92.3	99.1	103.0	108.9
	SD =	8.5	12.3	11.3	10.0	9.5	6.7	5.5	2.9
	% RSD =	40.9%	29.7%	13.9%	11.5%	10.3%	6.8%	5.3%	2.6%
DHPG	1	29.6	62.6	87.2	91.8	95	101.2	98.1	98.1
	2	32.7	60.7	85.6	91.8	96.5	105.8	99.6	98.1
	3	32.7	68.5	93.4	98.1	99.6	113.3	99.6	101.2
	4	48.3	71.6	96.5	96.5	109.1	102.7	104.3	102.7
	5	43.6	66.9	93.4	99.6	101.2	104.3	105.9	105.9
	6	33.5	62.3	93.4	98.1	102.7	105.9	105.9	98.1
	Mean =	36.7	65.4	91.6	96	100.7	105.6	102.2	100.7
	SD =	7.4	4.6	4.2	3.4	5	4.3	3.5	3.2
	% RSD =	20.2	6.6	4.6	3.5	5	4.1	3.4	3.2

a. Capsules at 37.0 °C in 900 mL 1 N HCl with 100 r.p.m. agitation rate.
b. Labeled strength = 200 mg per capsule.

Table IV summarizes the dissolution data for both DHPG and acyclovir capsules, and Figure 6 portrays the dissolution (time versus % LS dissolved) profiles for both drugs. Both capsule types behaved similarly, although the DHPG capsules gave slightly faster release: >90% LS released at t = 20 min for DHPG versus t = 30 min for acyclovir. The DHPG capsules also gave slightly more reproducible results than the acyclovir

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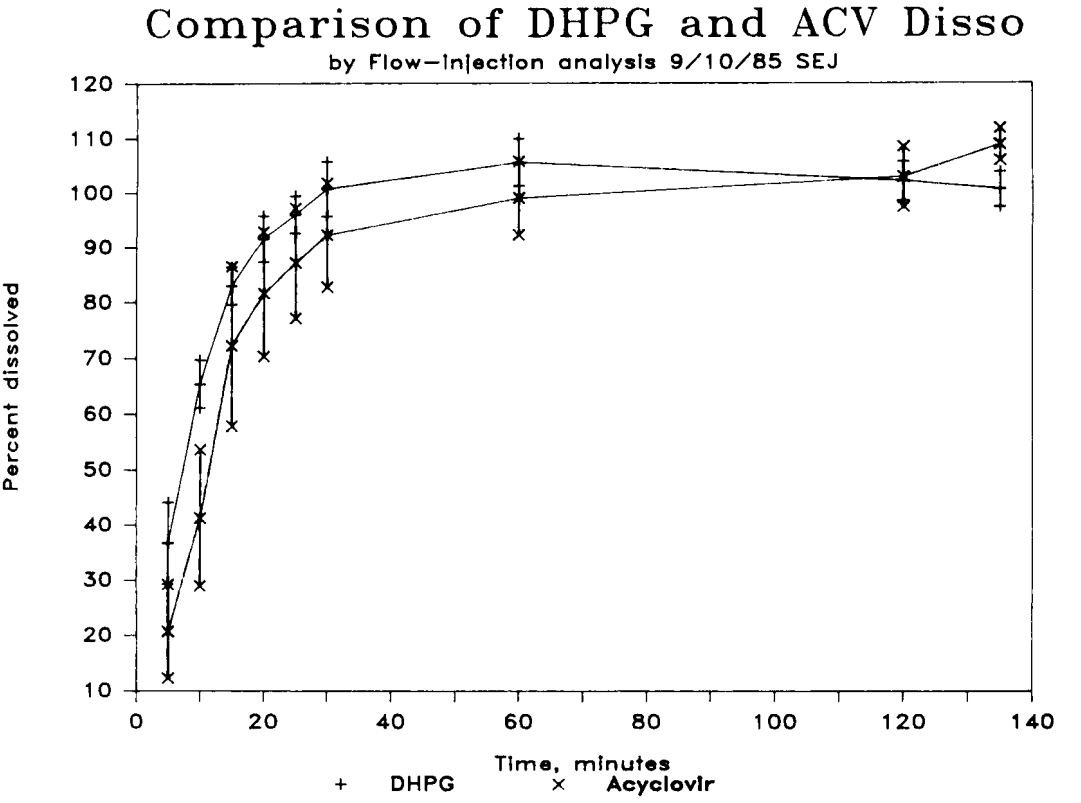

FIGURE 6.
Dissolution Profile of Percentage of Labeled Strength (% LS) of Dissolved Drug versus time for DHPG (+ ---- +) and Acyclovir (X ----- X) Capsules.

capsules: throughout the release profiles, standard deviations averaged 4.3 % LS for DHPG versus 9.0 % LS for acyclovir. In either case, it is evident from the data shown in Table IV and Figure 6 that drug dissolution is rapid and that inter-capsule variability is moderate to low.

CONCLUSIONS

We have assembled an automated dissolution apparatus that uses flow injection analysis (FIA) with spectrophotometric

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detection for analyte quantitation. The apparatus gives linear and precise results and we have used the system to demonstrate the dissolution profiles for DHPG and acyclovir capsules. Unlike the previous[20] example of a dissolution apparatus with FIA, the system described herein fully automates the serial sampling of not one, but six dissolution vessels.

We find that the FIA technique and continuous-flow techniques for dissolution testing share the common advantages of good linearity and precision with significant time savings achieved through automation. We consider, however, that the FIA technique provides these benefits at lower cost, and with greater simplicity and versatility than the continuous-flow methods.

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REFERENCES

1. The United States Pharmacopeia, 21st rev., The United States Parmacopeial Convention, Rockville, MD., 1985.

2. British Pharmacopoeia, British Pharmacopoeia Commission, London, U.K., 1980.

3. Deutscher Arzneimittel Codex, Arbeitsgemeinschaft der Berufsvertretungen Deutscher Apotheker, Frankfurt-Stuttgart, W. Germany, 1983.

4. Cakiryildiz, C.; Mehta, P.J.; Rahmen, W.; Schoenleber, D.; J.Pharm.Sci., **1975**, 64, 1692.

5. Castello, R.W.; Jellinek,G.; Konieczny, J.J.; Kwan, K.C.; Toberman, R.O.; J.Pharm.Sci., **1968**,57, 485.

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6. Shah, A.C.; Peot, C.B.; Ochs, J.F.; J.Pharm.Sci., **1973**, 62, 671.

7. Shah, A.C.; Ochs, J.F.; J.Pharm.Sci., **1974**, 63, 110.

8. Tingstad, J.; Riegelman, S.; J.Pharm.Sci., **1970**, 59, 692.

9. Schroeter, L.C.; Wagner, J.C.; J.Pharm.Sci., **1962**, 51, 957.

10. Kent, J.S.; Wong, P.P.; Hegde, G.P.; J.Pharm.Sci., **1977**, 66, 1665.

11. Walker, D.; Anal. Proc., **1983**, 20, 429.

12. Hertzfeld, C.D.; Pharm. Technol., **1984**, 72.

13. Ruzicka, J.; Hansen, E.H.; Anal. Chim. Acta, **1975**, 78, 145.

14. Stewart, K.K.; Beecher, G.R.; Hale, P.E.; Anal. Biochem., **1976**, 70, 167.

15. Betteridge, D.; Chem. Brit., **1980**, 16, 646.

16. Ranger, C.B.; Anal. Chem., **1981**,53, 20A-26A.

17. Stewart, K.K.; Anal. Chem.,**1983**, 55, 931A-940A.

18. Ruzicka, J.; Hansen, E.H.; Flow Injection Analysis, Wiley-Interscience, New York, 1981.

19. Rios, A.; De Castro, M.D.L; Valcarcel, M; J. Pharmaceut. Biomed. Anal., **1985**, 3, 105.

20. Koupparis, M.; Macheras, P.; Reppas,C.; Int. J. Pharmaceut., **1984**,20, 325.

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