lyophilized, or spray dried was examined by scanning electron microscopy (Fig. 4). All samples showed a platey structure, although it was most evident in the lyophilized sample. The spray-dried sample showed spherical particles that are inherent to spray drying. However, the spheres were made up of thin plates. The air-dried samples formed scroll-like sheets due to the more rapid rate of water loss from the top surface during drying. Johansson (9), in proposing the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ complex, noted the formation of plate-like crystals during the study of a basic aluminum sulfate that was built up from the same kind of aluminum–oxygen complexes. It is suggested that the high uneven charge on the spherical units minimize contact with adjacent units so that a planar configuration provides the most favorable spatial and electrostatic arrangement.

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Automated Dissolution Testing of Combination Drug Product Using High-Pressure Liquid Chromatography

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Abstract □ An automated high-pressure liquid chromatographic (HPLC) system compatible with any standard tablet dissolution apparatus was developed. This system allowed the individual drug concentrations within a product to be determined simultaneously, even when the drugs had similar structures and UV spectra. This automated system permitted unattended sampling and concentration determination at predetermined time intervals. The dissolution medium was pumped continuously through a fixed-volume, microprocessor-controlled injector and returned to the USP rotating-basket dissolution apparatus. No corrections for the changing dissolution medium volume were necessary since each injection onto a reversed-phase HPLC column consumed just 10 μl of medium. Dissolution tests were performed on three brands of trisulfapyrimidines tablets. Sample injections were made automatically at 5.1-min intervals for ~2 hr. Dissolution profiles were determined for each drug in each product. Statistically significant differences were found in the mean concentration–time values between drugs within a drug product and between drug products.

Keyphrases □ High-pressure liquid chromatography—with automated dissolution testing of a combination drug product □ Dissolution—automated high-pressure liquid chromatography, combination drug product □ Combination drugs—dissolution testing using automated high-pressure liquid chromatography □ Trisulfapyrimidines—combination drug product, dissolution testing using automated high-pressure liquid chromatography

Automated procedures for dissolution testing of pharmaceuticals have been of interest since such procedures are labor saving and increase analytical reproducibility. These automated procedures usually involve pumping the dissolution medium directly through a flowcell mounted in a UV spectrophotometer (1–6). An inherent problem with such an arrangement is the lack of drug specificity. If two or more drugs in a drug product have similar UV spectra, this procedure is useless.

In view of the number of combination products on the market, an automated technique is needed that allows each drug in a product to be quantitated individually during a dissolution run.

BACKGROUND

High-pressure liquid chromatography (HPLC) is a versatile analytical technique that combines the specificity of chromatography with the sensitivity of refractive index, UV, fluorescence, or electrochemical detection. Optimal retention times for the separation of active ingredients and dosage form excipients may be obtained by appropriately varying the mobile phase composition, pH, and/or flow rate. Changing the chromatographic temperature and utilization of gradient elution also are viable options. Other than filtration, aqueous samples require no special preparation prior to injection onto a reversed-phase HPLC column.

While various components of an HPLC system have been used to automate drug analysis procedures (3, 7) during dissolution, the actual chromatographic process has been included only in a manual (8) or semiautomated (7) procedure.

This report describes a totally automated HPLC method that has been used successfully to characterize the dissolution profile of each drug entity in a trisulfapyrimidines USP tablet. Completely unattended dissolution analysis is possible using this technique.

EXPERIMENTAL

A USP rotating-basket dissolution apparatus, a dissolution stirrer drive[1], and a water bath[2] were used. The basket was rotated at 150 rpm, and the temperature of the dissolution medium (0.1 *N* HCl) was 37.0 ± 0.1°. A pump[3] circulated the dissolution medium through a fixed-volume (10-μl loop) microprocessor[4]-controlled injector[5] and returned the me-

[1] Model 53, Hanson Research Corp., Northridge, CA 91234.

[2] Precision Scientific, Chicago, Ill.

[3] Milton Roy Mini-Pump, Laboratory Data Control, Riviera Beach, FL 33404.

[4] Model 740 Control Module, Micromeritics, Norcross, GA 30093. (Depending on the interfacing procedure, the Micromeritics model 753 ternary solvent mixer may also be required.)

[5] Model 735 with model 725 automatic injector valve, Micromeritics, Norcross, GA 30093.

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Table I—Parameter Estimates for Individual Dissolution Runs

Manufacturer[a]	Drug	Rate of Appearance of Drug in Solution (K), min^{-1}								Asymptote of Drug Concentration (C_{max}), mg/900 ml							
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Mean	SD	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Mean	SD
A	I	0.061	0.070	0.063	0.057	0.064	0.062	0.063	0.004	141.6	151.7	151.2	146.9	152.3	158.4	150.3	5.6
	II	0.109	0.108	0.111	0.091	0.100	0.112	0.105	0.008	161.9	160.9	161.9	156.4	163.0	165.9	161.7	3.1
	III	0.181	0.143	0.164	0.130	0.147	0.150	0.152	0.018	164.0	164.6	163.4	156.2	162.9	167.3	163.1	3.7
B	I	0.038	0.042	0.042	0.045	0.056	0.052	0.046	0.007	158.3	158.1	152.3	147.6	150.6	161.9	154.8	5.4
	II	0.067	0.062	0.065	0.067	0.080	0.076	0.070	0.007	171.2	165.0	168.0	165.7	166.3	177.0	168.9	4.5
	III	0.083	0.079	0.081	0.081	0.099	0.090	0.085	0.008	173.3	158.1	165.7	166.3	167.3	175.6	167.8	6.2
C	I	0.036	0.031	0.033	0.042	0.046	0.052	0.040	0.008	169.5	138.2	137.6	135.0	135.1	152.0	144.6	13.8
	II	0.067	0.044	0.048	0.062	0.064	0.075	0.060	0.012	163.5	164.7	166.8	164.5	159.6	167.8	164.5	2.9
	III	0.095	0.061	0.064	0.090	0.091	0.090	0.082	0.015	161.1	162.1	166.1	163.1	156.7	167.7	162.8	3.9

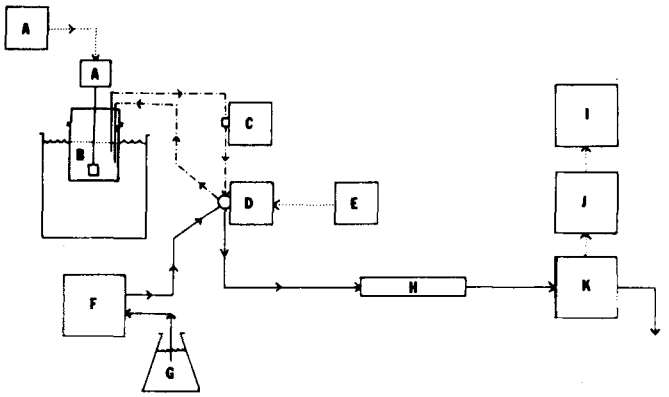
[a] Manufacturers, trade names, lot numbers, and expiration dates are as follows: A, Eli Lilly & Co., Neotrizine, 3FV36A, September 1, 1974; B, E. R. Squibb & Sons, Terfonyl, 8L238, July 1, 1983; and C, Wyeth Laboratories, Sulfose, 1790577, January 1984.

dium to the dissolution flask. All dissolution medium was filtered through a 2-μm low-pressure solvent filter as it entered the external circulation system. Only 1.05 ml of dissolution medium was external to the bulk (900 ml) at any given moment, and the circulation rate was 2.25 ml/min. The time required for a sample of bulk dissolution medium to reach the injector loop was determined experimentally to be 0.3 min.

Another pump[6] delivered the mobile phase (180 ml of acetonitrile[7]–820 ml of 0.05 *M* sodium acetate[8] buffer, pH adjusted to 5.7 with acetic acid) through the injector and onto a 25-cm long × 4-mm i.d. reversed-phase[9] column at a flow rate of 1.50 ml/min. The chromatographic analysis was conducted at room temperature. The absorbance of the column effluent was measured[10] at 254 nm. Peak areas were integrated[11] automatically, and a recorder[12] was used to display chromatographic peaks (Scheme I).

Dissolution testing of three brands (six runs each) of trisulfapyrimidines tablets USP (500 mg) was performed according to USP XX guidelines to demonstrate the utility of the system. Each tablet consisted of 167 mg each of sulfadiazine (I), sulfamerazine (II), and sulfamethazine (III). Sample injections were made automatically at 5.1-min intervals for ~2 hr. Dissolution profiles were determined for each drug in each drug product. Six dissolution runs at 100 rpm also were performed on one formulation to assess the impact of the basket rotation rate on the dissolution profile. The dissolution medium and mobile phase (one batch each) were prepared in sufficient quantity for all runs.

A standard curve for each drug was constructed by making four solutions ranging from 0.045 to 0.185 mg/ml. Each standard solution was placed in the resin flask of the dissolution apparatus, and the apparatus was assembled in the same manner as if a dissolution test were to be conducted. When the standard solution reached 37°, solution circulation through the injector loop and basket rotation were initiated. Injections were controlled by the microprocessor, and the same injector loop was used as in the actual dissolution runs.



Scheme I—Schematic diagram of the analytical system. Key: A, motor and control unit for basket rotation; B, USP dissolution flask and basket; C, dissolution medium recirculation pump; D, sample injector; E, microprocessor for injector control; F, mobile phase delivery pump; G, mobile phase container; H, reversed-phase HPLC column; I, strip-chart recorder; J, peak integrator; K, UV detector; —, mobile phase; - · - ·, dissolution medium; and · · ·, electrical signal.

[6] Model 750 solvent delivery system, Micromeritics, Norcross, GA 30093.
[7] HPLC grade, Fisher Scientific Co., Fair Lawn, NJ 07410.
[8] Certified ACS, Fisher Scientific Co., Fair Lawn, NJ 07410.
[9] RP-18, 10-μm particle size, E. Merck, Darmstadt, West Germany.
[10] Model 790 UV detector, Micromeritics, Norcross, GA 30093.
[11] CDS-111, Varian Instruments, Sunnyvale, CA 94086.
[12] Fisher Recordall, Houston Instruments, Austin, Tex.

The standard curves were linear and exhibited coefficients of determination (r^2) of 0.998 for I and II and 0.997 for III. This excellent linearity allows single-point calibrations before each dissolution run to guard against changing detector response and column behavior. The coefficients of variation at each concentration were <2.0% for I and II and <2.7% for III.

RESULTS AND DISCUSSION

Content uniformity tests were performed on each formulation according to USP XX guidelines (9). All products passed the specifications of 95.0–105.0% of the labeled amount of total sulfapyrimidines, with each drug providing not less than 31.5% nor greater than 35.0% of the total.

Figure 1 illustrates the typical chromatographic output of one dissolution run. The peaks (in the order shown) represent the amounts of I, II, and III present at a specific time. Machine integration (*Experimental*) yielded the area of each peak. Drug concentrations were determined from these integrated peak areas.

The dissolution data for each drug present in each run were fitted to:

$$C = C_{max}(1 - e^{-Kt}) \qquad \text{(Eq. 1)}$$

where C is the experimentally determined sulfa drug concentration at time t, C_{max} is the asymptote of the dissolution profile, and K is the rate constant for the appearance of drug in solution, as opposed to a rate constant for dissolution, since it includes the process of disintegration.

A nonlinear least-squares regression analysis program[13] was used for fits. All coefficients of determination (r^2) fell in the 0.972–0.998 range, with most ≥0.990. Parameter estimates (K and C_{max}) appear in Table I. The rate constant K should be characteristic for a given drug in a particular formulation when evaluated by a specific dissolution testing procedure. The estimated rate constants presented in Table I indicate that this is the case.

Concentration values at 30.0 and 60.0 min were generated from the parameter estimates for each drug within each run (Table II). These times were chosen based on guidelines proposed by the Food and Drug Administration (10), which suggested that 50% (83.5 mg/900 ml) and 80%

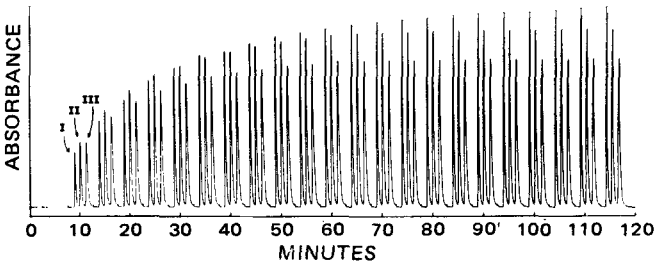


Figure 1—*Typical chromatographic output of one dissolution run. The time axis represents the summation of the dissolution time and the chromatographic time. The molar absorptivities of the three compounds are not equal in the present experimental conditions.*

[13] PROC NLIN, Statistical Analysis System, Raleigh, NC 27605.

Table II—Drug Concentrations at 30 and 60 min Obtained with Parameters in Table I

Manufacturer	Drug	Concentration at 30 min, mg/900 ml								Concentration at 60 min, mg/900 ml							
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Mean	*SD*	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Mean	*SD*
A	I	118.8	132.8	128.3	120.2	129.8	133.6	127.2	6.3	137.9	149.4	147.8	142.0	149.0	154.5	146.8	5.9
	II	155.7	154.7	156.2	146.2	154.9	160.1	154.6	5.1	161.6	160.7	161.7	155.7	162.6	165.7	161.3	3.2
	III	163.2	162.3	162.2	153.1	160.9	165.5	161.2	4.2	164.0	164.6	163.4	156.2	162.8	167.3	163.0	3.7
B	I	106.9	112.8	109.6	109.7	122.5	127.9	114.9	9.3	141.6	145.1	140.3	137.9	145.4	154.7	144.2	5.9
	II	148.1	139.6	144.1	143.2	151.4	159.0	147.6	6.9	168.1	161.1	164.6	162.6	165.0	175.1	166.1	5.0
	III	159.0	143.1	151.0	151.9	158.7	163.6	154.5	7.4	172.1	156.7	164.4	165.1	166.8	174.8	166.6	6.4
C	I	111.1	84.0	86.3	96.9	101.1	119.8	99.9	13.9	149.4	117.0	118.4	124.2	126.6	145.2	130.2	13.8
	II	141.8	121.2	127.2	138.6	136.4	149.9	135.8	10.3	160.6	153.2	157.4	160.4	156.3	165.9	159.0	4.4
	III	151.9	135.8	141.7	152.2	146.5	156.5	147.4	7.6	160.6	157.8	162.5	162.3	156.0	167.0	161.0	3.9

Table III—Results of Multiple Comparison Test for 30- and 60-min Mean Drug Concentrations [a]

Drug	Product Ranking, Highest to Lowest					
	30-min Concentrations			60-min Concentrations		
I	A	B	C	A	B	C
II	A	B	C	B	A	C
III	A	B	C	B	A	C

[a] Products underlined by a common line did not differ significantly ($p > 0.05$).

Table IV—Dissolution Test Results of Formulation A at 100 and 150 rpm [a]

Parameter	Drug	100 rpm		150 rpm	
		Mean	*SD*	Mean	*SD*
K, min^{-1}	I*	0.045	0.004	0.063	0.004
	II*	0.072	0.004	0.105	0.008
	III*	0.105	0.004	0.152	0.018
C_{max}, mg/900 ml	I	146.9	5.0	150.3	5.6
	II	162.5	2.3	161.7	3.1
	III	163.1	2.4	163.1	3.7
30-min concentration, mg/900 ml	I*	108.2	8.0	127.2	6.3
	II*	143.7	3.0	154.6	4.6
	III**	156.1	2.7	161.2	4.2
60-min concentration, mg/900 ml	I**	136.6	6.9	146.8	5.9
	II	160.3	2.4	161.3	3.2
	III	162.8	2.4	163.0	3.7

[a] Results were analyzed for significance using an unpaired t test ($\alpha = 0.05$); * = significant difference ($p < 0.001$), and ** = significant difference ($p < 0.05$).

(133.6 mg/900 ml) of the drug should be in solution after 30 and 60 min, respectively.

An analysis of variance[14] was performed on both the 30- and 60-min data for each drug entity to detect significant differences between formulations. When a significant difference was found, Tukey's critical difference ($\alpha = 0.05$) was used to identify the mean values responsible (Table III).

Ranking the formulations with respect to the amount of drug in solution at 30 and 60 min showed that Formulation C consistently exhibited lower levels. In all but one case, these levels were significantly lower than the formulation with the number one ranking and often were significantly lower than the second-ranked formulation. The predicted values of C_{max} for Formulation C were not significantly different from those of Formulations A and B.

Figures 2–4 illustrate the product performances. In these figures, all of the concentration–time data for each drug entity within each drug product were fitted simultaneously to Eq. 1. The mean concentration points and standard deviations are superimposed on the fitted curves.

A comparison of the dissolution performance of Formulation A at basket rotation rates of 100 and 150 rpm is presented in Table IV. It has long been recognized that the dissolution rate of a drug may be greatly influenced by the degree of agitation present in the dissolution flask. Carstensen *et al.* (11) stated that rotation speeds of <150 rpm in USP Apparatus I may result in solution nonhomogeneity. For this reason, a basket rotation rate of 150 rpm was chosen for most determinations in this study.

[14] PROC ANOVA, Statistical Analysis System, Raleigh, NC 27605.

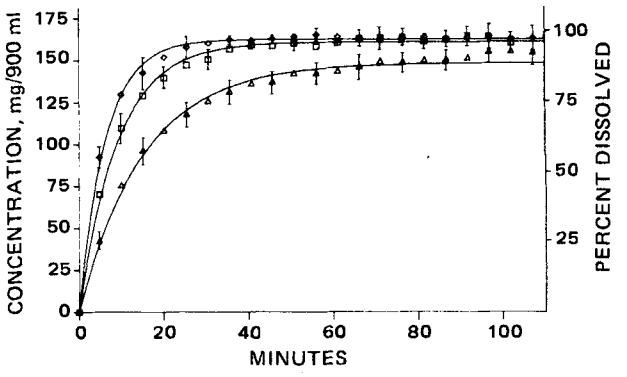


Figure 2—*Concentrations of drugs in solution* versus *time for Formulation A (average of six runs, basket rotation rate of 150 rpm). Standard deviations are superimposed on the plots. Key:* △, *I;* □, *II; and* ◇, *III.*

Figures 2 and 5 provide a visual comparison of the performance of Formulation A with basket rotation rates of 150 and 100 rpm, respectively. Although the rate constant for drug appearance in solution significantly increased with an increase in the basket rotation rate (Table IV), the predicted maximum concentration of drug in solution (C_{max}) was independent of the rotation rate.

As expected, it is possible for each active ingredient to have a unique dissolution rate (Tables I–III). Significant differences in the dissolved amounts were found between active drug entities and between manufacturers' formulations at both 30 and 60 min. Such differences necessitate the simultaneous determination of the dissolution profiles of each active component in a drug product. The typical automated dissolution apparatus, lacking the chromatographic procedure, can seldom accomplish such an analysis.

Although the system described was used with USP Apparatus I, USP Apparatus II or III can be employed equally well as long as the intake and outlet for the circulated dissolution medium are in the positions recommended by the USP. Since each injection consumes only 10 μl of dissolution medium, there is no need for volume replacement nor calculational compensation for decreasing dissolution medium volume. The 0.3 min required for a sample of bulk dissolution medium to reach the injector

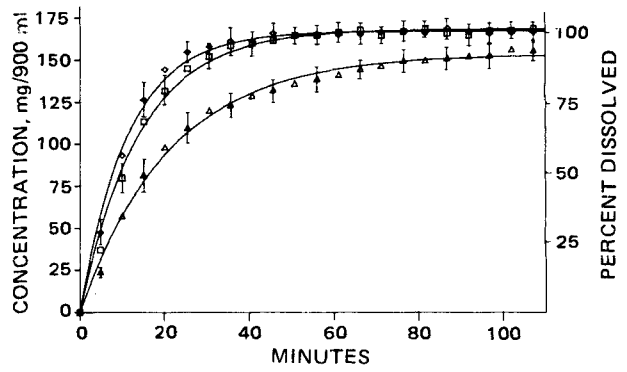


Figure 3—*Concentrations of drugs in solution* versus *time for Formulation B (average of six runs, basket rotation rate of 150 rpm). Standard deviations are superimposed on the plots. Key:* △, *I;* □, *II; and* ◇, *III.*

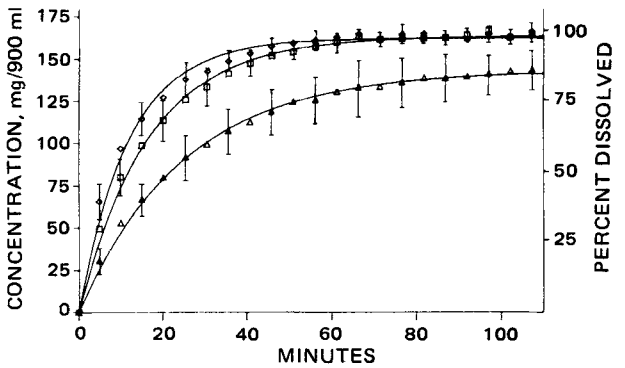


Figure 4—*Concentrations of drugs in solution* versus *time for Formulation C (average of six runs, basket rotation rate of 150 rpm). Standard deviations are superimposed on the plots. Key:* △, *I;* □, *II; and* ◇, *III.*

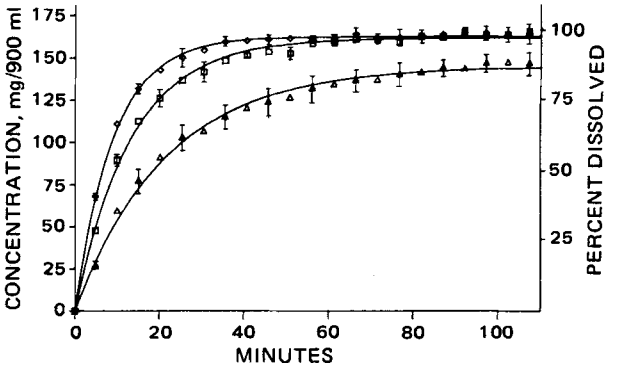


Figure 5—*Concentrations of drugs in solution* versus *time for Formulation A (average of six runs, basket rotation rate of 100 rpm). Standard deviations are superimposed on the plots. Key:* △, *I;* □, *II; and* ◇, *III.*

loop, while accounted for in the calculations, is actually insignificant if the dissolution time is in excess of 10 min. This rapid exchange of dissolution medium is accomplished with a relatively slow flow rate, and this slow flow rate is advantageous since it does not provide dissolution medium agitation in excess of that provided by the rotating basket. The 2-μm low-pressure solvent filter at the inlet port prevented particulate matter from scoring the injection valve and also prevented solid particulate buildup at the head of the column.

This system exhibits great flexibility for adaptation to the problems encountered with a particular product. Various drug and excipient entities can be accommodated by variations in the injection programming, the mobile phase composition, and pH and by gradient elution. Gradient elution is especially feasible in this system because it can be controlled by the same microprocessor that controls sample injections. No system is completely universal, however, and there are undoubtedly compound combinations for which adequate separations cannot be made with retention times short enough to allow injections at the intervals required by compendial specifications. The authors believe this latter case is relatively rare.

The HPLC system used in the present study is of the component or modular type. Substitution of a fluorescence, electrochemical, or refractive index detector in place of the UV detector would be a simple matter. The fluorescence detector could be especially useful for compounds present in very low concentrations. Additionally, the microprocessor can be programmed to change detector sensitivity during the chromatographic procedure, thereby furthering the ability to accommodate a drug product with widely varying concentrations of active ingredients.

After initial debugging, no reliability problems were encountered, and completely unattended dissolution testing was accomplished. Unattended testing is the ultimate aim of automation, and no method can be considered to be truly automated without this capability.

If the amount of drug dissolved from a dosage form need only be measured at widely spaced time intervals or at the end of the dissolution test, this system would be adaptable to monitoring six dissolution flasks. Such monitoring could be accomplished by the use of a multichannel pump and a switching valve to direct the flow from the appropriate dissolution flask to the injector loop. This switching valve would also have to permit return flow from the injector loop to the flask being sampled. If measurements were to be made on the contents of each dissolution flask at specific times (such as 30 and 60 min), initiation of the dissolution processes in the six flasks would need to be staggered.

The types of systems described readily lend themselves to computer interfacing. Such interfacing would mean that the operator's only contact with the dissolution test would be lowering the basket into the dissolution medium and collecting the completed dissolution profiles.

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