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DISSOLUTION TESTING: SEMIAUTOMATIC SAMPLING AND ANALYSIS FOR $700

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ABSTRACT

A semiautomatic sampling and analysis system (SASAS) has been developed for dissolutions of formulations containing a UV(VIS) absorbing active ingredient. The basis of the system is a peristaltic pump which transfers samples from the dissolution vessels to a flow cell in a UV(VIS) spectrophotometer. Sample selection is performed by the analyst, reducing processing time to less than 30 seconds per sample. SASAS was first applied to Theophylline tablets and sustained release capsules at a cost of $700. As many as 36 samples were tested by one analyst concurrently.

INTRODUCTION

Automation of dissolution testing is being vigorously pursued by the pharmaceutical industry and related institutions because of growing demands for dissolution testing and its labor intensive nature. Several companies have designed and marketed microprocessor controlled instrumentation to automate sample acquisition and analysis[1-3], and in some instances, miscellaneous options such as report writing. The primary drawbacks of these automated systems are the high cost and limited capacity. Many

institutions and smaller laboratories simply cannot bear large expenditures for an automated system, especially since the current technology is still in a period of development. Other laboratories, including our own, have also found that their testing requirements exceed the capacities of commercial automated systems.

Much of the effort in many automated systems[1-4] involves selecting the proper sample and making the appropriate dilutions and transfers necessary to move it into a cell where it can be analyzed. Alternatively, the continuous flow systems, which have separate flow cells for each dissolution vessel, are limited because a separate spectrophotometer is required for each six vessel unit[5]. By developing a system that can transfer and analyze a single sample and employing an analyst to do the sample selection, most of the cost associated with automated systems can be avoided. This allows for more flexibility in the system while placing only a minimal burden on the analyst. Following this concept, an economic, semiautomatic sampling and analysis system (SASAS) has been developed. The basis of SASAS is a narrow path flow cell fitted into a UV(VIS) spectrophotometer, and a peristaltic pump. Samples are pumped from the dissolution vessel to the flow cell where the UV absorbance is measured. The results are recorded graphically.

MATERIALS

Theophylline Tablets USP (100 mg and 200 mg) and Theophylline sustained release capsules (60 mg, 125 mg and 250 mg), (William H. Rorer, Inc., Fort Washington, Pennsylvania, USA).

Theophylline, USP reference standard.

USP XX[6] Apparatus #2 (paddle).

Bausch and Lomb 210UV Spectrophotometer, (Shimadzu Scientific Instruments, Columbia, Maryland).

Recordall Series 5000 Chart Recorder, (Fisher Scientific Co.).

Flow Cell with ports at the top, 0.5mm pathlength, #170-QS, (Hellma Cells, Jamaica, New York, USA).

Peristaltic Pump and Pumphead Catalog Numbers 7553-00 and 7014, (Cole-Parmer, Chicago, Illinois).

1.6mm ID and 0.8mm ID Tygon tubing, (Cole-Parmer).

20 micron polyethylene filter tip, Catalog Numbers Ft-4004 or Ft-6006, (Centaur Sciences, Inc., Stamford, Connecticut). Ft-4004 fits tubing with an OD of 2.5mm and Ft-6006 fits tubing with an OD of 5.0mm.

SYSTEM DESIGN

The spectrophotometer, with flow cell, and the dissolution units were positioned as close to one another as practically possible (See Figure 1).

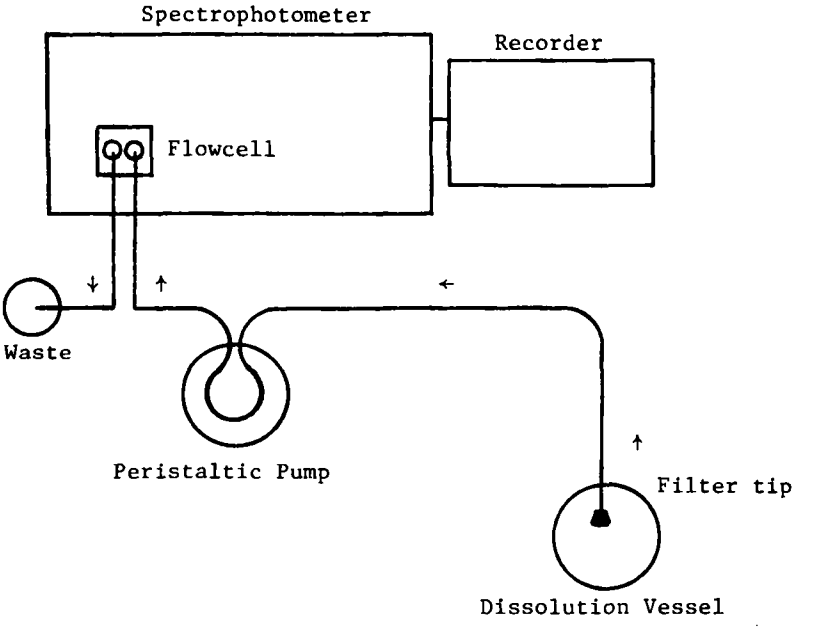

FIGURE 1

System Layout

Tubing (1.6mm ID by 0.5m) was attached to the inlet port of the flow cell and fed through the peristaltic pump. A second piece of tubing (0.8mm ID by 3.0m) was connected to pump inlet and the free end was fitted with a filter tip. Three meters of tubing was long enough to reach all the dissolution vessels in the laboratory. A third piece of tubing was attached to the outlet port of the cell and extended to waste. With this system a solution was rapidly sampled and analyzed by placing the probe (filter tip at end of tubing) into the solution for about 25 seconds. As the solution was drawn through the cell the absorbance was recorded continuously at 271nm, the absorbance maximum. A flat topped peak was drawn as the sample passed through the cell and a steady-state was established. The absorbance value was then determined from the horizontal line at the top of the peak (Figure 2). The flow cell pathlength of 0.5mm was chosen because it scaled the absorbances into a reasonable range (0.017-0.850).

Tubing diameters were minimized in order to keep sample carryover to a minimum. High carryover associated with large tubing diameters necessitated undesirably large volumes of sample to flush the sample path (filter tip, tubing, pump, and flow cell). Since the volume of sample required to flush and equilibrate the sample path was equal to the volume of the system, the following equation relating system volume (V), flow rate (R), and minimum sampling time (T) was derived:

$$T = V/R. \qquad \text{(Eq. 1)}$$

The flow rate was optimized at about 10 mls/min and the volume of the system was 2.8mls, thus the minimum sampling time was 17 seconds (0.28 minutes). A sampling time of 25 seconds was adopted for the procedure allowing for a margin of safety and resulting in about 4mls of solution taken for each sample. Compensation for sample volumes removed was made in the calculations.

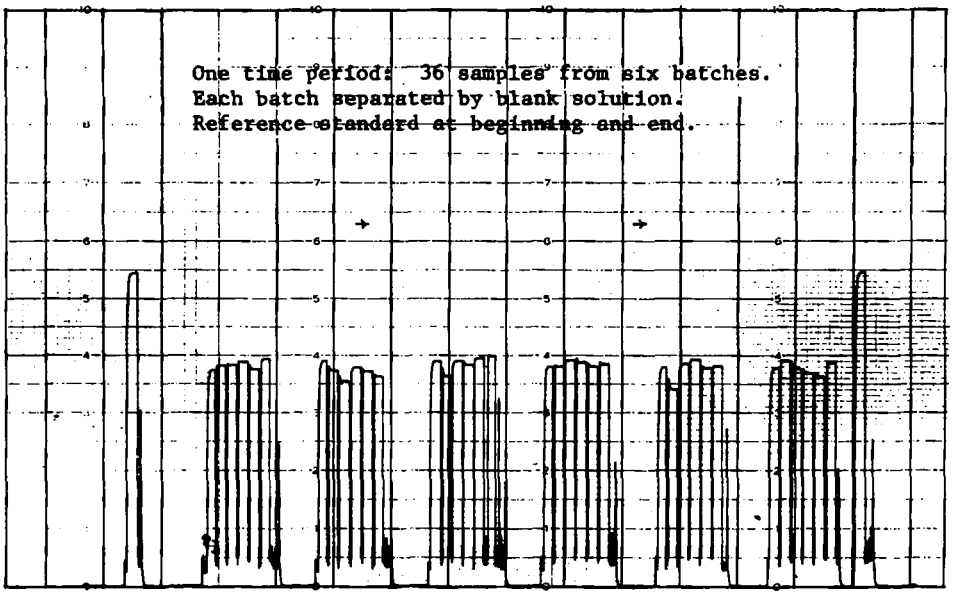

Figure 2

Typical recording: 36 samples from six batches.

Flow rates were limited by the filter tip, and depended upon the types of excipient material present in the formulation being tested. High flow rates and/or clogged filter tips caused excess bubbles to appear in the lines, resulting in unstable, erratic absorbance readings. At the optimum flow rate of 10mls/min, few bubbles could be seen in the tubing except between samples when air was aspirated. Although the filter tips could normally be used for 24 samples, occassionally clogging would occur after three or four samples and was indicated by the presence of air bubbles in the tubing. Since the filter tips were very inexpensive (about 4¢ each), a policy of changing them after six samples were aspirated was adopted.

METHODOLOGY

The USP XX[6] procedure, adapted to these particular products, was followed. The tablets were sampled at two time periods and the capsules at four. Six tablets (capsules) were analyzed per test. Individual samples were introduced into each dissolution vessel at intervals of 30 seconds with slightly longer intervals between test units (six vessels/unit). Prior to sampling times the pump and recorder were started and the instruments were calibrated with blank and reference solutions. At the appropriate times, samples were taken by placing the probe into the prescribed region of the vessel for a period of 25 seconds. This left five seconds to move to the next vessel. Blank and reference solutions were redetermined after the sampling for a time period was completed. Concurrently, sampling and analysis were performed by manually withdrawing samples by pipet, diluting them and determining the absorbance in a 10mm cell for comparison.

RESULTS AND DISCUSSION

Linearity of the system was determined by regression analysis[7] on a 15 point calibration curve bracketing the expected concentration range (0.01mg/ml - 0.36mg/ml). See Figure 3. The y-intercept was 0.001 and the correlation coefficient was 0.999993. The coefficient of variation of the actual data from the predicted was 0.2%. Comparison of the SASAS analysis method with the manual method revealed no differences of any practical significance (Tables 1 and 2). The comparison data was analyzed by applying paired student's t-test to the variables of time, dosage type and dosage strength. Of the 30 t values calculated only the value for the first period of the 250 mg capsules was significant at the 95% level of confidence. However, since the level of difference was only 0.2% no practical significance was attributed to this point.

An interlaboratory reproducibility study was performed to determine system ruggedness. Six standard solutions of varying con-

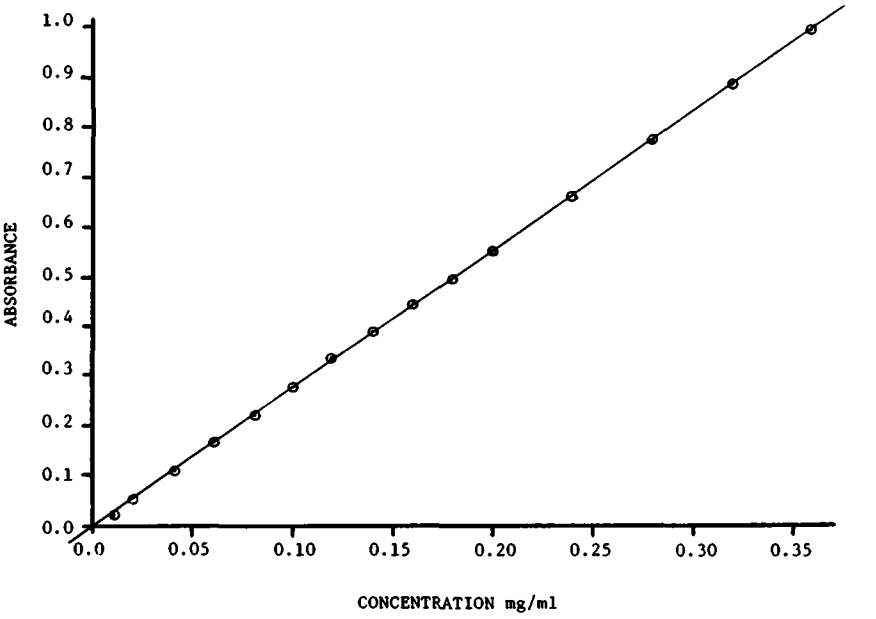

FIGURE 3

Plot of Absorbance versus Concentration of Anhydrous Theophylline

centration were analyzed by eight analysts from three laboratories. All but one analyst made duplicate determinations (Table 3). The greatest standard deviation was .0012 (0.4 mg). The greatest standard deviation for any dosage strength at applicable concentration levels was less than 0.5 percent of label claim.

The cost of implementing SASAS was under $700 (last four items under Materials). The UV(VIS) spectrophotometer and recorder were not considered in the cost since they were already present in the laboratory and were used with the manual system. The efficiency of SASAS enabled analysts to double their productivity, and test from 1 to 36 samples concurrently. Analysts were limited to a maximum of 36 samples because only six six-vessel dissolution apparatuses were available during the study.

TABLE 1

Method Comparison - Capsules

Δ = Manual result - SASAS result for the average of six capsules in percent label claim

t = Paired student's t

$\bar{\Delta}$ = Average differences

60 mg

Period	Lot 1 Δ	Lot 2 Δ	Lot 3 Δ	$\bar{\Delta}$	t
1	0.79	-0.16	-0.04	0.2	0.7
2	0.86	-0.68	-0.22	-0.0	-0.0
3	0.18	-1.17	-0.60	-0.5	-1.4
4	0.70	-0.81	-0.14	-0.2	-0.2

125 mg

Period	Lot 4 Δ	Lot 5 Δ	Lot 6 Δ	$\bar{\Delta}$	t
1	0.41	-0.09	-0.11	0.1	0.4
2	0.45	0.01	-0.17	0.1	0.5
3	0.70	-0.01	-0.05	0.2	0.9
4	-0.08	-0.03	-0.14	-0.1	-2.6

250 mg

Period	Lot 7 Δ	Lot 8 Δ	Lot 9 Δ	Lot 10 Δ	$\bar{\Delta}$	t
1	-0.25	-0.12	-0.33	-0.20	-0.2	-5.1
2	0.13	-0.98	-0.49	-0.29	-0.4	-1.8
3	0.03	-0.19	-0.39	-0.15	-0.2	-2.0
4	0.27	0.63	0.01	0.06	0.2	1.7

Capsules

Period	$\bar{\Delta}$	t		$\bar{\Delta}$	t
1	-0.01	-0.1	60 mg	-0.1	-0.6
2	-0.1	-0.8	125 mg	0.1	0.9
3	-0.2	-1.1	250 mg	-0.1	-1.6
4	0.04	0.3	All	-0.07	-0.9

TABLE 2

Method Comparison - Tablets

Δ = Manual result - SASAS result for the average of six capsules in percent label claim

t = Paired student's t

$\bar{\Delta}$ = Average differences

100 mg

Period	Lot 11 Δ	Lot 12 Δ	Lot 13 Δ	$\bar{\Delta}$	t
1	-0.71	-0.20	0.13	-0.3	-1.1
2	-0.32	-0.15	0.49	-0.01	-0.03

200 mg

Period	Lot 14 Δ	Lot 15 Δ	Lot 16 Δ	Lot 17 Δ	$\bar{\Delta}$	t
1	-0.66	0.48	0.55	0.03	0.1	0.4
2	-0.19	-0.15	-0.66	0.11	-0.2	-1.4

All Tablets

	$\bar{\Delta}$	t
Period 1	-0.05	-0.3
Period 2	-0.1	-0.9
100 mg	-0.1	-0.8
200 mg	-0.1	-0.4
All	-0.1	-0.8

All Tablets and Capsules

$\bar{\Delta}$	t
-0.07	-1.2

More apparatuses would have increased testing capacity until it would have been limited by the time between test intervals. The tablets, for example, were sampled at 30 minutes and 60 minutes. Consequently, all sampling for a time period had to

TABLE 3

Interlaboratory Reproducibility

Vessel #	1	2	3	4	5	6
Anhydrous Theophylline mg/ml	0.0500	0.00600	0.1000	0.2000	0.0200	0.3000
			Reported Absorbance Values			
Test #						
1	.139	.017	.277	.552	.056	.824
2	.139	.017	.277	.552	.056	.824
3	.139	.017	.277	.551	.057	.823
4	.139	.017	.277	.553	.056	.824
5	.139	.018	.277	.551	.056	.823
6	.139	.017	.275	.551	.055	.823
7	.139	.017	.277	.552	.056	.825
8	.139	.018	.278	.552	.056	.825
9	.140	.018	.278	.553	.056	.827
10	.140	.018	.278	.553	.056	.824
11	.140	.017	.277	.552	.056	.826
12	.139	.017	.277	.552	.056	.824
13	.139	.018	.277	.552	.055	.824
14	.140	.017	.278	.553	.056	.826
15	.139	.017	.277	.553	.056	.826
Average	.1393	.0173	.2771	.5521	.0559	.8245
ASD	.0004	.0005	.0007	.0007	.0004	.0012
ASD in mg	0.14	0.16	0.24	0.24	0.14	0.41

be accomplished within 30 minutes. As many as 60 vessels could be sampled by sampling at 30 second intervals. Inserting additional time between units of six would decrease that number to about 40. The number of sustained release samples tested would be virtually unlimited because of the longer intervals between samplings. Using SASAS six lots of tablets (36 tablets) were easily tested in less than three hours, including preparation and clean up. For sustained release products less than half an hour per time period was required for processing 36 samples, freeing the analyst for other duties such as media

preparation or report writing. Another benefit of SASAS was increased accuracy. Experimental error was reduced by eliminating most of the sample manipulation (eg. pipeting, diluting, filling the cell, etc.) of the manual method.

OTHER APPLICATIONS

The SASAS concept could easily be adapted to other paddle or basket dissolution systems. Rapid or sustained release formulations could be analyzed provided that the other components in the formulation do not have UV(VIS) absorbances at the same wavelength as the ingredient of interest. Flow cells with pathlengths of 0.01mm to 10mm are readily available[8] so that most components can be analyzed directly with no need for dilution or concentration of samples. If a moderate to long pathlength is necessary a micro flow cell is advisable. The volume of the flow cell should not exceed 0.5ml. Tubing material and filter porosity can be selected according to the specific needs of the application. Filter tip porosities range from 0.5 to 90 microns, though at porosities below 20 microns, vacuum problems escalate with aqueous media. Variable speed peristaltic pumps with small diameter tubing are recommended unless the pump is placed on the outlet side of the flow cell. Minimum sampling times can be determined from equation 1 and are largely a function of the distance from the spectrophotometer to the dissolution units.

CONCLUSION

SASAS is an economic way to increase an analyst's productivity two fold. It yields high testing capacities, is essentially maintenance free, simple to operate, and may be applied to other compounds besides theophylline.

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