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A widely applicable automated sampling apparatus for dissolution testing

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Summary

An automated sampling apparatus for drug release testing is described that is chemically inert and can filter particulates larger than 0.2 μm. Samples are pushed through filters with inert piston pumps capable of delivering up to 100 psi, so that small-pore membrane filters can be reliably used. These filters are now available in a variety of materials and pore sizes so that in every case a filter was identified that could remove undissolved drug and excipients while avoiding excessive adsorption. A PC-based controller provides flexibility, ease of use, and operation of multiple units. The system proved applicable with heavy particulate loading and for several hydrophobic drugs. Important considerations in designing an automated system are discussed.

Introduction

The kinetics with which therapeutic agents are released from pharmaceutical dosage forms is one factor determining in vivo performance. Several official compendia (USP, 1984; BP, 1980; DAC, 1983) specify preferred apparatuses for in vitro tests. Many test conditions are investigated during development, and numerous analytical determinations are needed to characterize release kinetics for each set of conditions. On-line analyses provide results concurrent with the test, so that decisions can be made (such as when to stop), but these methods tend to be the most costly and least flexible since specific analytical instruments are devoted to the analyses. Off-line methods use virtually any analytical method without special adaptations, although samples must be moved (manually or robotically) to the analytical instrument. In development work a variety of drugs and dosage forms is encountered. This led us to develop a versatile off-line sampler that would minimize some of the problems inherent in many commercial designs.

An off-line automated apparatus should take representative samples and deliver them to holding vessels. The system should be sufficiently inert that analytes are not adsorbed or contaminants extracted. Particulates (drug or excipients in the formulation) should be completely removed to prevent further dissolution of the drug or assay interferences (e.g. UV light scattering or HPLC column plugging). The sampling system should be easy to use and easy to adapt to different types of

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samples. We describe here a system designed to meet these objectives.

Materials and Methods

All dosages tested were experimental formulations manufactured by the Upjohn Company. Buffers were prepared from analytical or reagent grade salts. Filters were validated by comparing assays of filtered vs. "unfiltered" samples, the latter being initially freed from particulates by filtering or centrifugation. Filtrates were carefully examined for cloudiness indicative of undissolved drug or excipients. A general description of the type of testing primarily addressed may be found in the USP (1984). Several dissolution apparatuses were used (Hanson Research, Northridge, CA; Van-Kel Industries, Edison, NJ; Applied Analytical Industries, Wilmington, NC; or combinations of components).

The automated sampling system is diagrammed in Fig. 1. The sample probes were automatically withdrawn from the flasks when not sampling using pneumatic devices as described by Carrie and Sanders (1983) or as commercially available (Hanson Research). All fluid lines were 0.8 mm i.d. PTFE [1] tubing except where smaller diameters are annotated (Fig. 1). A personal computer (IBM Corp., Armonk, NY) with a control and data acquisition system (ISAAC 91-I, Cyborg Corp., Newton, MA) controlled the system. A sampler (DS-500, Medix Technologies, Inc., Jericho, NY) containing 6 inert piston pumps (FMI, Inc., Oyster Bay, NY) transported samples from the dissolution flasks to sample tubes in the fraction collector. Sampling from two 6-place test apparatuses was accomplished using double 3-way valves (V1, Fig. 1, Neptune Research, Inc., Maplewood, NJ or Rheodyne, Inc., Cotati, CA). The pumps

[1] PTFE (polytetrafluoroethylene) is used throughout this paper to denote fluoropolymers commonly referred to by the DuPont trade name "Teflon".

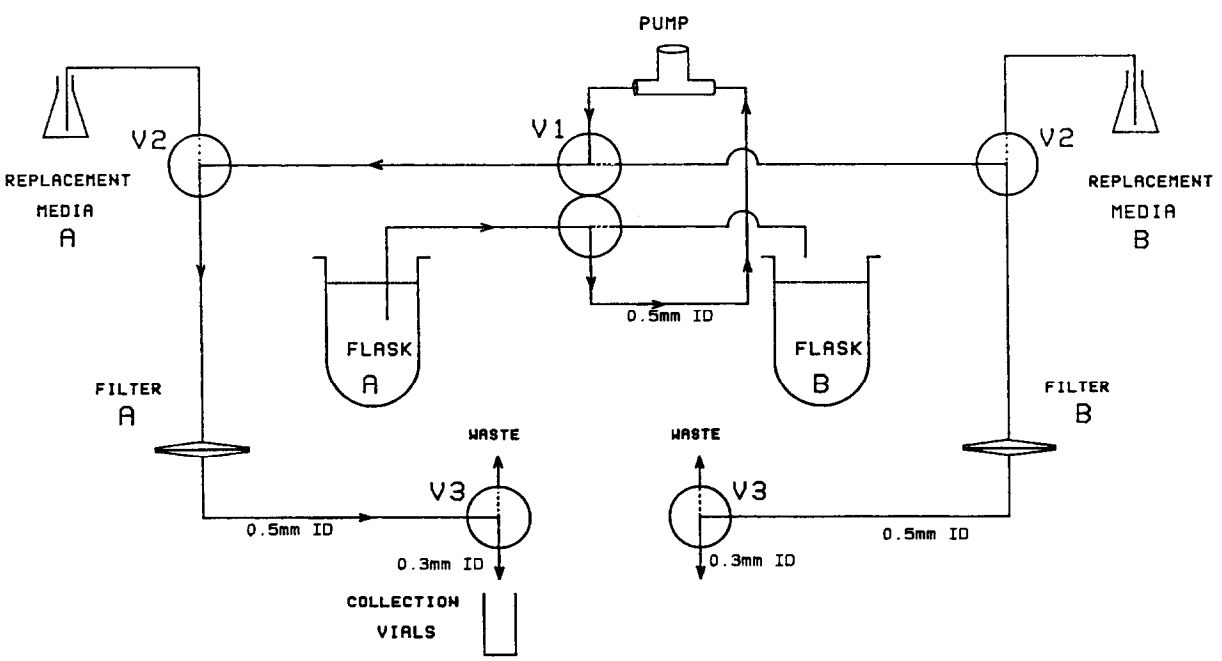


Fig. 1. Schematic for automated sampling apparatus (one of 6 such pairs): V1, double 3-way valve for switching between flasks A and B using a common pump; V2, optional 3-way valve for media replacement; V3, optional 3-way valve for preflush, allowing doubled capacity for sample collection vials; pump, valveless ceramic piston/fluorocarbon housing, bidirectional.

TABLE 1

Description of filters

Filter designation	Filter material	Pore size (μm)	Holder material
A	Cellulose acetate/cellulose nitrate	0.45	polypropylene
B	Same as A	0.45	ethylene-PVC
C	Polysulfone	0.45	acrylic
D	Acrylic copolymer cast on nylon	1.2	acrylic
E	PTFE	5.0	polypropylene
F	Same as E	5.0	polyethylene
G	PTFE	1.0	polypropylene
H	Same as G except pretreat with 10 ml 3A alcohol and air flush	1.0	polypropylene
I	Nylon 66	0.45	polypropylene

were thus shared by the two apparatuses with fluid paths otherwise independent. Filters were placed in various locations on the system depending on the application. In the primary configuration (shown in Fig. 1) disposable filter cartridges or disposable membranes in reusable holders were downstream from the pumps. Some of the filters tested are listed in Table 1 (Millipore Corp., Bedford, MA; Gelman Sciences, Inc., Ann Arbor, MI; or Rainin Instrument Company, Inc., Emeryville, CA). The dead volume of the system with such cartridge filters installed was about 3 ml (15 s). Alternatively, filters were attached to the end of the sampling probes, e.g., a disposable sintered polypropylene filter with a 10–20 μm pore size (Technicon Instruments Corp., Tarrytown, NY). In some cases a small, fine-mesh screen was needed on the tip of the probe to keep larger particles in the flask and avoid plugging of the probe entrance. The lengths of the flow paths for each cell were equal, and the dead volume of the system was measured with filters in place. This value was entered into the controller and used to obtain timing accuracy.

At the beginning of the sampling sequence (Fig. 2), the lines were flushed with a selected volume of sample directed to waste either through a slot in the fraction collector tray or by 3-way valves at the collection manifold (V3 in Fig. 1, Neptune Research). Valves V3 served to double the capacity of the fraction collector. Samples were then directed to collection vials by switching V3 or

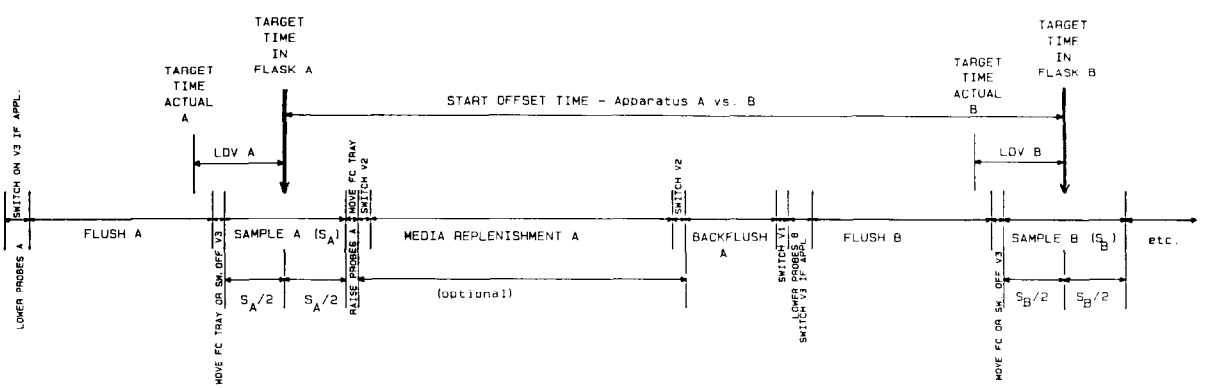


Fig. 2. Schematic of sampling time sequence: LDV, line dead volume, including filters, measured experimentally; FC, fraction collector; V1, V2, V3, see Fig. 1.

turning the tray. The controller calculated when each sample cycle should begin based on the dead volume and the flushing and sampling volumes so that half of the collected sample was drawn from the flask just before and half just after the target sampling time (see Fig. 2). This calculation allowed sampling as early as 1 min after the start of the test with reasonable accuracy. As an option the media volume removed by sampling could be accurately replenished by switching valves V2 (Neptune Research) and reversing the pumps. The required volume was calculated automatically by the program. Although the lines could then be completely cleared, this was usually not done unless the filter pore size was larger than 10 μm and the filter was bidirectional. It was however always necessary to include a short (1–3-s = 0.2–0.6 ml) pump reversal to prevent "vapor lock" at higher pressures. This short backflush also served to remove fluid from the small dead volume (5–10 μl) downstream from switching valves V3. If both dissolution apparatuses were to be sampled, valves V1 were then switched, and the sampling sequence was initiated for the second apparatus.

Audible cues at the start of the test signaled the correct times for dosages to be introduced to each of the two apparatuses. The control program also ensured that intervals between requested time points were sufficient for the length of time needed to sample and for sampling from both apparatuses if time points were coincident. The program logic was designed to accommodate two such sampling systems so that sampling from 24 test vessels would be possible.

To validate the automated system drug concentrations in samples withdrawn automatically were compared to those from simultaneous manual sampling. Additional experiments measured carry-over for large concentration changes. Flush volumes were optimized to provide acceptably low adsorption and carry-over while minimizing the amount of sample withdrawn. Generally filter validation studies were indicative of the behavior of the automated system: the optimum flush volume could usually be approximated as the sum of the required filter flush volume and the system dead volume.

TABLE 2

Flurbiprofen adsorption biases in 0.05 M pH 7.2 phosphate buffer

Control $n = 4$, ±95% CI	% Dissolved (200 mg)	
	Result	Rel % bias
5.34 ± 0.08	5.11	−4.3
51.9 ± 0.1	51.3	−1.2
103 ± 0	102	−1
54.1	53.2 *	−1.7 *

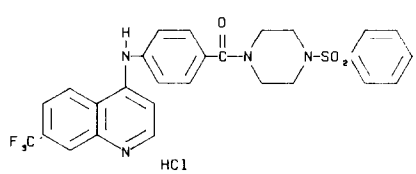
% Dissolved is filtered through sintered polypropylene, 10–20 μm pore size.

* Through 2 m of PVC tubing.

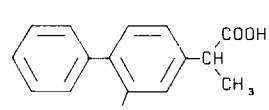
Results

The unique capabilities of the sampling apparatus were demonstrated with drug formulations for which filtration problems were encountered. These problems were drug adsorption, incomplete filtration, or filter plugging.

A common problem is illustrated with flurbiprofen, which was adsorbed by a sintered polypropylene filter and by PVC tubing (Table 2). These results were somewhat surprising because flurbiprofen (structure Fig. 3) is "completely" ionized and quite soluble (15 mg/ml) at pH 7.2. This shows the importance of using inert pumps, fluid lines, valves, and filters. A test for flurbiprofen was easily automated using filter E or F



Losulazine



Flurbiprofen

Fig. 3. Structures of losulazine hydrochloride and flurbiprofen.

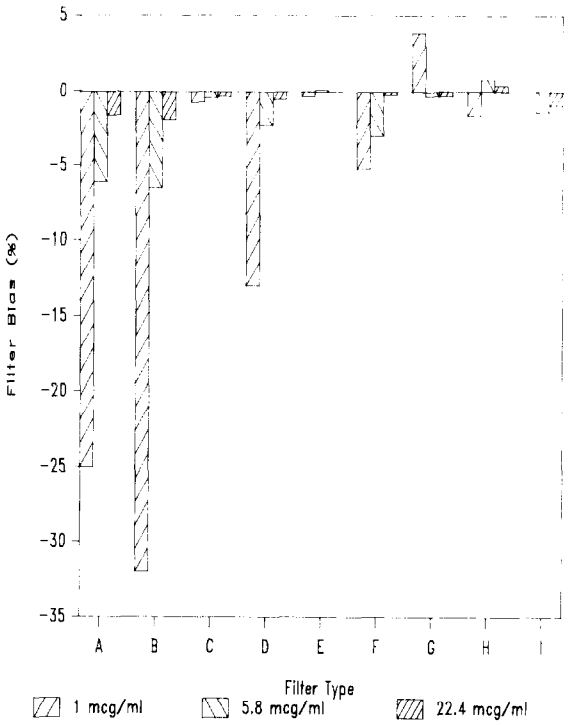
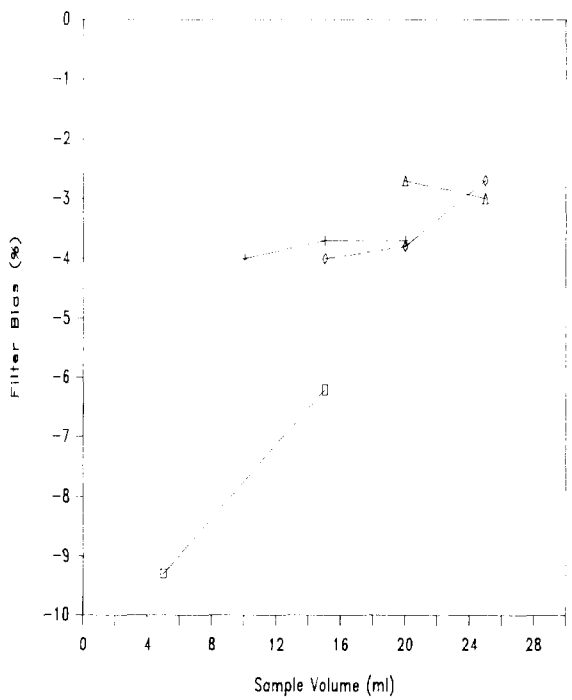


Fig. 4. Filter bias for 3 concentrations of losulazine hydrochloride in 0.05 M, pH 2 phosphate buffer for 15 ml samples with a 10-ml flush volume (using various filters) (see Table 1 for description of filters).



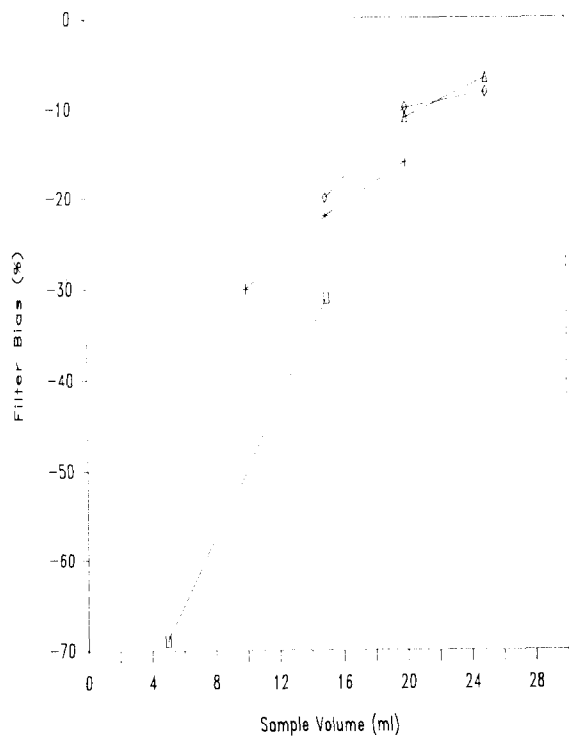


Fig. 6. Effect of sample and flush volume on filter bias using a nylon filter with solutions of losulazine in 0.063 M pH 2 HCl/KCl USP buffer (flush volume: □ = 0 ml, + = 5 ml, ◇ = 10 ml, △ = 15 ml).

(Table 1), and representative results are shown in Table 3.

Previous workers have reported adsorption problems with membrane filters (Liu et al., 1977). For that reason selection of a filter for losulazine hydrochloride (structure Fig. 3) was particularly difficult. The drug has a low (< 4 μg/ml) intrinsic water solubility, hence adsorption problems might be anticipated. Additionally, a small-pore filter was needed to remove the micronized drug particles. The filters listed in Table 1 were evaluated at 3 drug concentrations in 0.05 M pH 2 phosphate buffer (solubility > 90 μg/ml). Adsorptive loss was a severe problem for most filters (Fig. 4). Adsorption of drug by the membrane decreased in

Fig. 5. Effect of sample and flush volume on filter bias using a polysulfone filter with solutions of losulazine in pH 2 phosphate buffer (flush volume: □ = 0 ml, + = 5 ml, ◇ = 10 ml and △ = 15 ml).

TABLE 3

Comparison of individual results for automatically and manually drawn samples (% dissolved)

Time	Automated	Manual
Losulazine HCl 0.75 mg		
5 min	69.4	69.6
10 min	80.3	79.9
14 min	81.2	80.8
Aspirin capsules 450 mg		
5 min	42.7	42.7
15 min	88.6	87.3
30 min	95.4	94.5
Flurbiprofen 200 mg		
2 h	8.3	8.3
4 h	16.2	16.6
6 h	26.8	27.0
8 h	36.7	36.8
12 h	56.2	56.0
24 h	103	102
Codeine 60 mg		
10 min	72.3	74.4
20 min	95.5	95.5
30 min	96.4	95.1
45 min	97.9	97.6
Ibuprofen 400 mg		
10 min	52.8	54.8
20 min	88.5	89.1
30 min	92.4	92.4
45 min	98.1	98.1
60 min	98.4	98.6

the series: cellulose esters > acrylic on nylon > nylon > polysulfone = PTFE. Polypropylene and acrylic holders were better than polyethylene.

The technique of flushing some of the sample through the filter to saturate adsorptive sites prior to collecting the sample was investigated. These experiments were conducted in two different dissolution fluids, pH 2 phosphate and pH 2 HCl/KCl buffer (solubility about 25 μg/ml). Large reductions in the filter bias were obtained when a portion of the sample was used as a preflush (Table 4, Figs. 5, 6), and gains were larger in the buffer where solubility was lower (HCl/KCl). The drug exhibited unusual behavior when filtered with a PTFE filter: for a given volume flushed to waste, recoveries were lower when larger samples were collected (Table 4, filter E). We do not have an adequate explanation for this observation but we have been able to reproduce the data. Such results demonstrate the need for careful filter evaluation.

The test was automated using filter C, and results comparable to manual sampling were obtained even for low-strength tablets (Table 3).

An experimental ibuprofen salt suspension demonstrates the importance of providing a selection of filters with small pore sizes. A paper filter which was initially adequate gave cloudy filtrates and incomplete filtration (Table 5) as samples

TABLE 4

Losulazine HCl filter bias for dilute samples in phosphate and chloride buffers with selected filters and various sample and preflush volumes

Sample vol. (ml)	Flush vol. (ml)	Relative % bias for filter					
		0.05 M pH 2 phosphate (0.67 μg/ml)			0.063 M pH 2 chloride (HCl/KCl) (1.2 μg/ml)		
		E	C	I	E	C	I
5	0	−25%	−9.3%	−7.0%	−25%	−10%	−69%
15	0	−15	−6.2	−4.2	−17	−4.7	−30
10	5	−6.2	04.0	−3.0	−13	−3.0	−30
15	5	−8.2	−3.7	−4.0	−12	−1.6	−22
20	5	−12	−3.7	−2.7	−11	−1.6	−16
15	10	−4.3	−4.0	−2.7	−0.7 (n.s.)	−0.2 (n.s.)	−20
20	10	−5.3	−3.8	−2.8	−6.1	−1.9	−9.9
25	10	−6.8	−2.7	−1.7	−3.0	−1.4	−8.2
20	15	−1.0 (n.s.)	−2.7	−2.7	+0.7 (n.s.)	−1.9	−11
25	15	−4.8	−3.0	−2.7	−1.4	−1.6	−6.6

n.s., Bias not significantly different from the control sample at the 95% confidence level.

TABLE 5

Comparison of filtration methods for experimental suspension product

Age/temp.	Filtration method (% dissolved in 10 min)	
	Filter paper, gravity	0.45 μm cellulose acetate/nitrate ("true" result)
Initial	100	100
12 mo/amb	100	96
12 mo/30	83	76
1 mo/47	82	76

aged. A 0.45-μm filter (filter B, Table 1) provided clear filtrates but plugging produced high pressures which posed the danger of shattering glass syringes used for manual sampling. All filters with pores larger than 0.45 μm gave cloudy filtrates and plugging was more rapid with other filters (C and I, Table 1). Despite these difficulties at least two sequential 10-ml samples could be drawn with the automated system using filter B because of the low flow rate and high pumping pressure.

Representative validation data for the automated system are shown in Table 3. Samples drawn automatically gave results comparable to manual samples for a variety of drugs (complete validation would make use of several such data pairs at each sample time for statistical comparison). Carry-over studies sometimes revealed limitation on the difference in concentrations sampled from two apparatuses on a common sampler if media replenishment was not used (Table 6).

Discussion

For automated sampling the primary objective is to obtain results equivalent to those obtained by manual sampling (i.e. with a syringe). There are a number of problems evident with commercially available systems. Firstly, some systems use non-inert pumps and valves. Drugs are often sparingly soluble in the aqueous fluids used to simulate the in vivo environment and readily adsorb to many materials (e.g. those suitable for peristaltic applications). The advent of more potent drugs has led to smaller doses for which adsorptive problems are worse, since the relative bias depends on the concentration. Secondly, the types of filters which can be used are limited by the lack of a sufficient pressure differential for sample transport. Thirdly, the use of continuously resident probes may disturb the test hydrodynamics (Savage and Wells, 1982). Fourthly, the controllers are inflexible, lacking in facilities, or complicated to use. Finally, dedication of analytical instrumentation to the sampler is often unjustified, especially for extended release products where sampling is needed only every few hours.

Controller

Inexpensive control systems are readily available, but these systems tend to be inflexible, difficult to reconfigure, and not "user-friendly". These controllers cannot calculate accurate sampling for varying operating parameters (i.e. accurate sample timing requires compensation for flush volumes, dead volume etc.). A PC can run interac-

TABLE 6

Losulazine HCl in 0.05 M pH 2.0 phosphate buffer: carry-over between two dissolution apparatuses with common sampling and no media replenishment

Sample number	Time (min)	A 30 mg 30:1	B 1 mg 1:30	A 20 mg 4:1	B 5 mg 1:4	A 0.75 mg 1:1	A 0.75 mg 1:1
1	5% Rel. bias:	0	+24	−0.5	+2.5	−0.3	+1.9
2	10% Rel. bias:	0	+6	+0.4	+2	+0.5	+2.2
3	15% Rel. bias:	+0.6	+6	+0.4	+1	+0.5	−0.1

Apparatus A was sampled before apparatus B. Tablet strength and strength ratios are given for each sample. Relative bias = [(automated/manual) − 1] × 100.

tive software and additional facilities such as data acquisition, signal conditioning and high speed counting are readily added (see e.g. McQuaid, 1984). Report generation is useful for documentation of the test conditions. The PC also serves other purposes when not in use for dissolution studies. Such systems cost 5–10 times as much as simpler devices, but the added benefits and the ability to control several sampling systems outweighs the additional cost.

Filtration

The particles which arise in dissolution testing of pharmaceutical products are often as small as tenths of micrometers. The particle size of the bulk drug can sometimes be used as a rough guide for filter selection (for example micronized drugs typically average around 10 μm). Particles become smaller as the drug dissolves, however, and smaller particles may also be generated in a dosage form. Occasionally sophisticated techniques such as photon correlation spectroscopy were required to detect sub-visible drug particles in filtrates where assay values increased upon standing. In such cases membrane filters are the only means to effect rapid, complete filtration. The recent emphasis on filtering samples for HPLC analysis has provided a much larger selection of filters applicable for dissolution testing. A reasonable variety of membrane materials such as PTFE, nylon, cellulose esters, PVC, polysulfone, and acrylic-coated nylon are available in several diameters cast in disposable cartridges of fluoropolymer, polypropylene, polyethylene, PVC, and acrylic.

Several key considerations in the selection of a filter are: (i) adsorption of the analyte; (ii) leaching of materials into the dissolution media (a particular problem with UV analysis — see e.g. Cartwright, 1979 and Cooney, 1980); (iii) retention of undissolved drug particles; (iv) retention of excipient particles (a requirement in both UV and HPLC analysis, where particles scatter light or quickly plug HPLC columns). For an automated system additional constraints arise: (v) dead volume; (vi) bi-directional capability; (vii) filtering capacity; (viii) bubble point (the pressure required to force air through a wetted filter). The last two points affect the pressure differential needed for sample transport.

Adsorption of the analyte (i) and contaminant leaching (ii) depend on the filter composition and increase with increases in the effective surface area or filter depth. These problems are minimized by flushing the filter prior to collecting sample. Larger flush volumes are needed for filters with larger dead volumes (v). Dead volume also increases with increasing surface area and filter depth. Reducing the surface area or filter depth, however, reduces the amount of particulate material which can be filtered before transport pressures are insufficient (filter capacity (vii)). Pressure requirements can be minimized by using the largest pore size that will retain undissolved drug (iii) and excipients (iv). For most applications 25-mm diameter membrane filters provided the best compromise between the conflicting requirements for low dead volume, high filter capacity, and low analyte adsorption. Membrane filters place special demands on the sample transport system. As filters become plugged, more pressure is needed to force sample through the available pores at the same rate (filter capacity (vii)). The ability of a filter to pass air after wetting is critical for those systems that use vacuum to move samples or require air purges of the sample lines. The bubble points (viii) for 1.2, 0.45 and 0.22 μm filters are 10, 30 and 50 psi, respectively (Millipore Catalog, 1983). Since vacuum provides a maximum pressure differential of 15 psi (atmospheric pressure) drawing air through wetted filters or liquid through plugged filters with pores smaller than 1 μm is impossible, and in practical use 5 μm is about the lower limit. These pore sizes are too large for many applications.

Sample transport

There have been few reports of membrane filters placed downstream from sampling pumps (see however Embil et al., 1983). One reason is that a peristaltic pump only delivers limited pressures (at best about 50 psi). Another reason is the concern that collecting undissolved drug particles on a filter might cause contamination due to drug dissolving in the filter between samples or dissolving because of pumping turbulence during sampling. The use of moderate flow rates (< 15 ml/min)

coupled with an adequate flush volume (5–15 ml) eliminates significant biases from this source (note Table 3). The rather large (10–20 ml) samples required are a problem only if the volume removed significantly affects the hydrodynamics of the system. This was the reason for adding replenishment capability. The piston pumps accurately removed sample and replaced media due to their unique method of operation (FMI Catalog RP401-83A, 1982). The ceramic piston/fluorocarbon cylinder was found to be a rugged, inert assembly which pumped fluids high in particulates without damage, so that filters could be placed downstream. As these pumps contain no built-in filters, particulates are flushed through. The pumps are rated at -10 to $+100$ psi, and with slow pumping speeds (about 12 ml/min) filters with small (0.2 μm) pores could be used even when plugged. Suspensions with high particulate levels could be tested, although the number of sequential samples drawn with one filter was sometimes limited. The drive belt linking the 6 pump heads skipped on the gears if the pressure became too high, providing sufficient overpressure protection.

Conclusion

Release rate information was generated on every sample received for testing with minimal effort spent on filter selection and validation of the automation. Emphasis was placed on ease of set up, operation, and product change-over, and the system was designed to make efficient use of costly components such as the pumps and controller. The use of membrane filters with small pore sizes and of many types gives the system its unique performance characteristics. Although this filtering method should prove useful for planned on-line automation efforts, using "off-line" analytical endpoints increases flexibility and cost-effectiveness of analytical instruments. The system allows workers with minimal training to attain efficiencies competitive with more costly robotics systems; however, the device could be easily interfaced with robots to move collected samples, clean flasks, change filters and start new tests unattended, and should allow robots to achieve greater flexibility and speed.

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