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Accelerated Dissolution Testing for Controlled Release Microspheres Using the Flow-Through Dissolution Apparatus

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Theophylline controlled release capsules (THEO-24 CR) were used as a model system to evaluate accelerated dissolution tests for process and quality control and formulation development of controlled release formulations. Dissolution test acceleration was provided by increasing temperature, pH, flow rate, or adding surfactant. Electron microscope studies on the theophylline microspheres subsequent to each experiment showed that at pH values of 6.6 and 7.6 the microspheres remained intact, but at pH 8.6 they showed deterioration. As temperature was increased from 37–57°C, no change in microsphere integrity was noted. Increased flow rate also showed no detrimental effect on integrity. The effect of increased temperature was determined to be the statistically significant variable.

Keywords accelerated flow through dissolution, pH, temperature, flow rate, theophylline

INTRODUCTION

The objective was to develop an accelerated dissolution test for controlled release formulations for quality and process control, and formulation development. Reproducible dissolution testing has long been recognized as extremely important for quality control.

In this study, theophylline controlled release capsules (THEO-24 CR) were used as a model drug system. The variables of temperature, pH, and flow rate for aqueous dissolution medium were examined; the effect of adding surfactant (sodium lauryl sulfate) to the medium was also evaluated using the flow-through cell method (USP Apparatus IV). The test criteria were that it be substantially shorter than the USP eight-hour test, and that it correlated with USP specifications for products labeled for dosing every 24 h as described in USP Monograph test 6 for products labeled for dosing every 24 h.^[1] The specific goal was to attain USP acceptable theophylline release in less than three hours in order to make the accelerated test a viable alternative for process and quality control, and formulation development. THEO-24 CR was chosen as the model drug since as an extended release product it is a suitable dosage form for flow through dissolution testing and therefore, to evaluate accelerated flow through dissolution.^[2–9] Moreover, the flow through test, initially developed in FDA laboratories 40 years ago, has been reported to offer the best discrimination due to manufacture or product composition changes.^[2–4]

Two studies report on accelerated dissolution testing for process and quality control and formulation development of controlled release formulations. The goals were to substantially reduce testing time compared to USP requirements. The first used increased temperature to accelerate dissolution from a controlled release Roxiam formulation in a USP IV flow through apparatus. Analytical times were reported to be less than 30 min for one assay.^[10] The second study used increased temperature, solvent and stirring to accelerate dissolution of USP salicylic acid calibrator tablets in a USP II apparatus. Dissolution times could be accelerated by a factor of 5 which meant that a single analysis could be performed in less

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than one hour.^[11] Both of these ground-breaking studies showed that substantial time could be saved using the 'Accelerated Dissolution Rate Analysis' (ACDRA) method with no loss in correlation compared to the standard USP test. Further discussions on the possibility of using increased temperature to accelerate the dissolution process, and the use of surfactants and hydroalcoholic media have also been reported.^[12,13]

MATERIALS AND METHODS

Materials

The following materials were obtained from the commercial suppliers below and used without further purification. Theophylline (mw 180.16) as THEO-24 Controlled Release capsules (100 mg) from Wal-Mart Pharmacy, Athens, GA, USA; acetonitrile, ammonium hydroxide, sodium acetate trihydrate, from Sigma-Aldrich Chemicals, St Louis, MO, USA; glacial acetic acid, potassium phosphate monobasic, hydrochloric acid, and sodium hydroxide from Aldrich Chemicals, Milwaukee, WI, USA; methanol and sodium lauryl sulfate (SLS; powder/NF/FCC) from Fisher Chemicals, Fairlawn, NJ, USA; Theophylline RS Anhydrous (USP) from Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA.

Instruments

Spectronic 2000 Spectrophotometer, Bausch and Lomb, NJ; Thermo Spectronic Helios Aquamate, Rochester, NY; Haake E-52 Water Bath, Haake Instruments, Inc., Saddle Brook, NJ; Sigma Motor Peristaltic Pump Model T8, Middleport, NY, USA.

UV Spectrophotometric Studies

UV Spectrophotometric analyses for all samples were conducted at 271 nm, the uv maximum for theophylline using a 10 mm quartz at 25°C. Absorbance standard curves were constructed using solutions of reference standard Theophylline RS Anhydrous (USP) in either phosphate buffer dissolution medium (0.05M) at the indicated pH values; 0.05 M phosphate buffer at pH 1.2 and 7.6 with SLS at 25°C; theophylline samples were prepared at percent concentrations from 5–100%. The absorbance versus percentage concentration plots for Theophylline RS were linear over this concentration range and were used to determine the percent drug released in all dissolution samples at 25°C.

Dissolution Method

In vitro dissolution studies of THEO-24 CR capsules were conducted in triplicate; a sample was withdrawn at each time point in three separate experiments using the flow-through cell method (USP Apparatus IV). Samples of 4 mL were withdrawn every 30 min for 3 h; the 4 mL sample removed was immediately replaced with 4 mL of fresh dissolution medium (either phosphate buffer, or phosphate buffer with SLS). Dissolution profiles of Percent Released versus Time ($n = 3$) were constructed as shown in Figures 1–4. Medium temperature, pH and flow rate were changed to evaluate the effect on release rate.

One hundred milligrams of THEO-24 CR microspheres were introduced into the flow cell. Dissolution media (1000 mL) pH was altered depending on experiment requirements. Phosphate buffer 0.05 M was adjusted to the desired pH by adding small increments of 1M NaOH. Samples were withdrawn from the media reservoir at the predetermined time intervals stated above. The percent concentration of dissolved Theophylline in each sample was measured spectrophotometrically at 271 nm against a reference cell containing only the dissolution medium.

Flow-Through Dissolution

The flow-through cell, of transparent and inert material, was mounted vertically containing a filter system that prevented escape of undissolved particles from the top of the cell; standard cell diameter was 12 mm; the bottom cone was filled with small glass beads of about 1 mm diameter with one bead of about 5 mm positioned at the apex to protect the fluid entry tube.^[1] The flow-through cell used had three parts: the lower cone, the middle cylindrical portion, and the filter head on top. The cone is separated from the cylindrical portion by a #40 mesh screen and a microfiber filter; the filter head also holds a glass microfiber filter. The entire cell is immersed in a water bath with the temperature maintained at 37, 47, or 57 ± 0.5°C as per experiment requirements; and the dissolution medium is kept at the corresponding bath temperature for each experiment. All experiments were conducted in a closed loop setup. Flow rates of the dissolution medium through the cells were within USP specifications of 4, 8, and 16 mL/min.

Electron Microscope Study

Microspheres were dried 24 h at 50°C then placed in a dessicator for 48 h at room temperature before

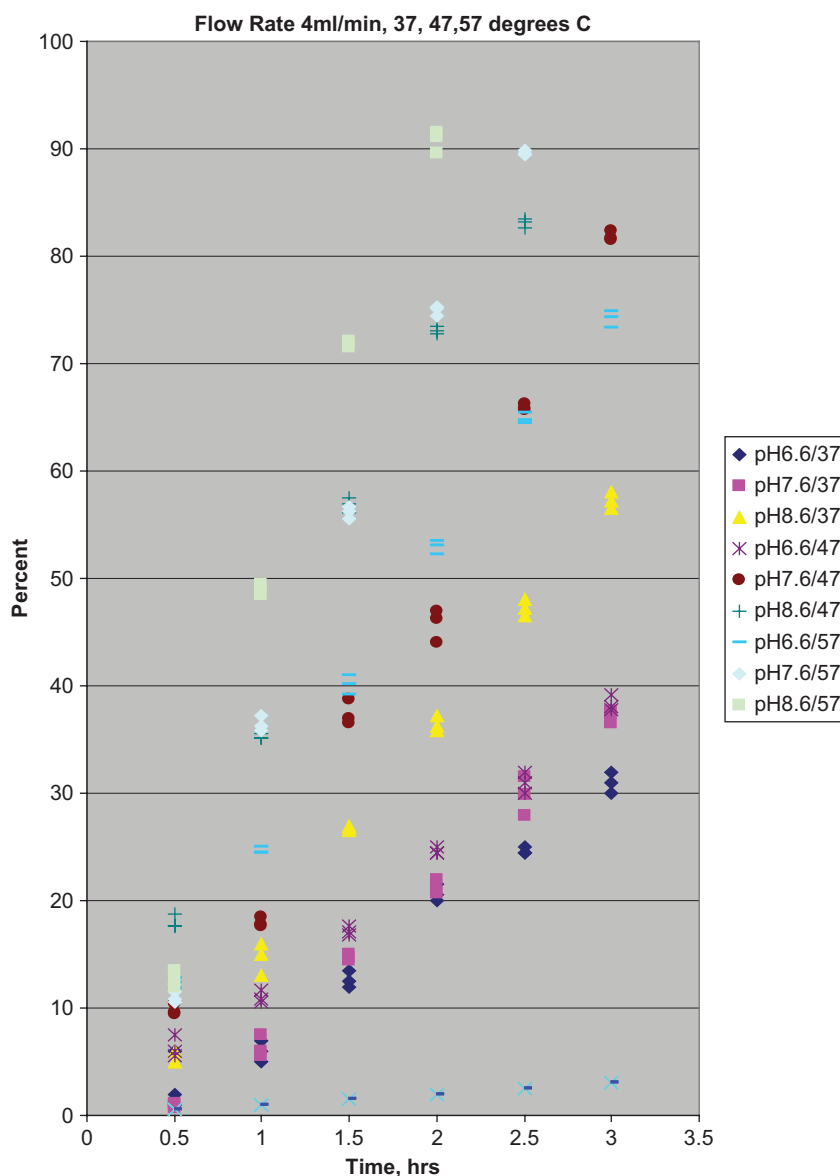


Figure 1. Dissolution profiles of Theo 24-CR in phosphate buffer 0.05M; flow rate 4 mL/min. Temperature (pH/r²): 37°C (6.6/0.9776, 7.6/0.9932, 8.6/0.9977); 47°C (6.6/0.9928, 7.6/0.9958, 8.6/0.9856); 57°C (6.6/0.9944, 7.6/0.9902, 8.6/0.9761); n = 3.

being submitted for electron microscopy imaging at the UGA Electron Microscopy Laboratory as shown in Figures 5–11.

Process Variables

To determine the influence of the process variables pH (A), temperature (B) and flow rate (C), a two-level

3 factor (2³) factorial design was selected. Standard runs were generated for times 0.5, 1.0, and 1.5 h, as shown in Table 1 for 0.5 h, but not for times 2.0–3.0 h since Theophylline release ended at about 1.5 h at higher temperatures. Pareto charts were constructed for experimental runs; the chart for 0.5 h is shown in Figure 12. The factorial design and analyses were generated using Stat-Ease Design-Expert Version 7.0.3 (Stat-Ease Inc., Minneapolis, MN, USA).

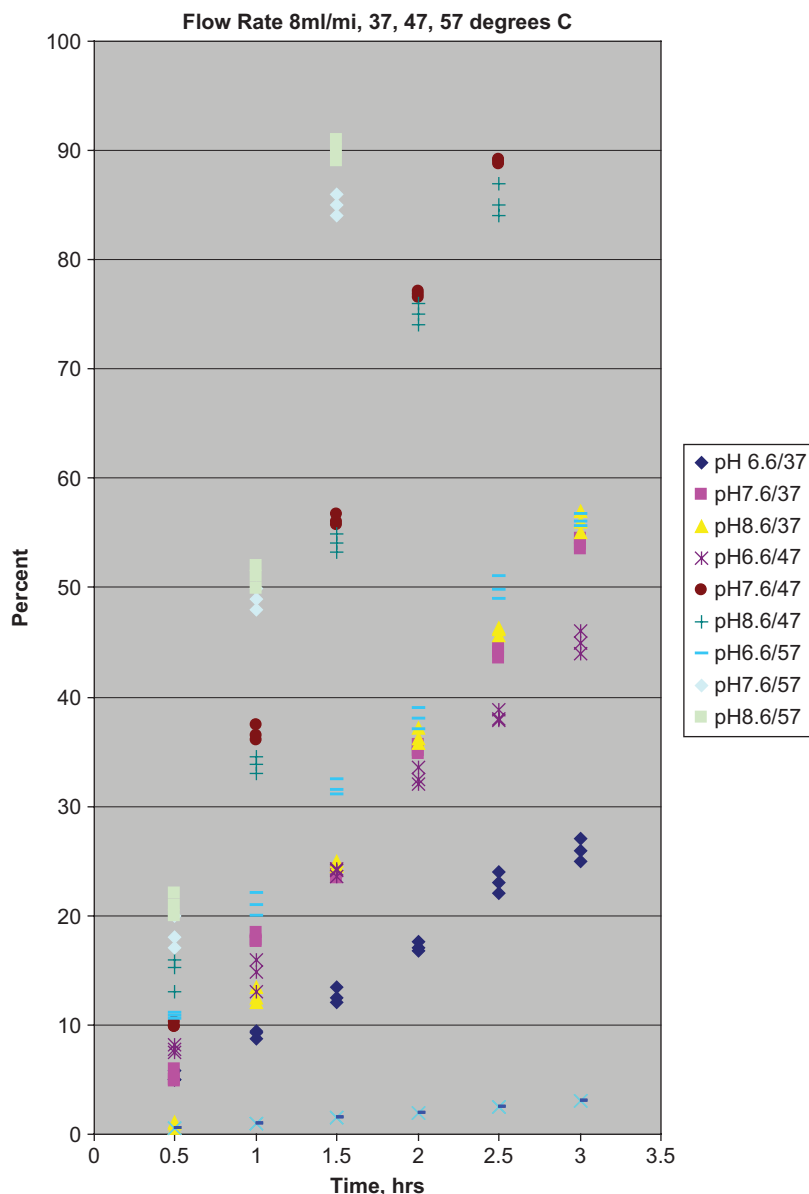


Figure 2. Dissolution profiles of Theo 24-CR in phosphate buffer 0.05M; flow rate 8 mL/min. Temperature (pH/ r^2): 37°C (6.6/0.9871, 7.6/0.9971, 8.6/0.9948); 47°C (6.6/0.9847, 7.6/0.9901, 8.6/0.9811); 57°C (6.6/0.9921, 7.6/0.9935, 8.6/0.9835); n = 3.

RESULTS AND DISCUSSION

Dissolution Profiles

The process variables associated with flow through dissolution testing: temperature, pH, and flow rate were manipulated to assess the potential for accelerated dissolution testing. In this study, the drug release of commercially available theophylline microspheres (THEO-24 CR) was

determined using a flow-through cell apparatus (USP Apparatus IV) at three different flow rates, 4, 8, and 16 mL/min; three different pH values, 6.6, 7.6, and 8.6; and three temperatures, 37, 47, and 57°C. Figures 1–4 show the linear dissolution profiles and regression coefficients upon changing these variables, which closely resemble those published by Macheras et al. for Theo-Dur under comparable conditions.^[6,14] The most benign parameters for the current study were pH 7.6,

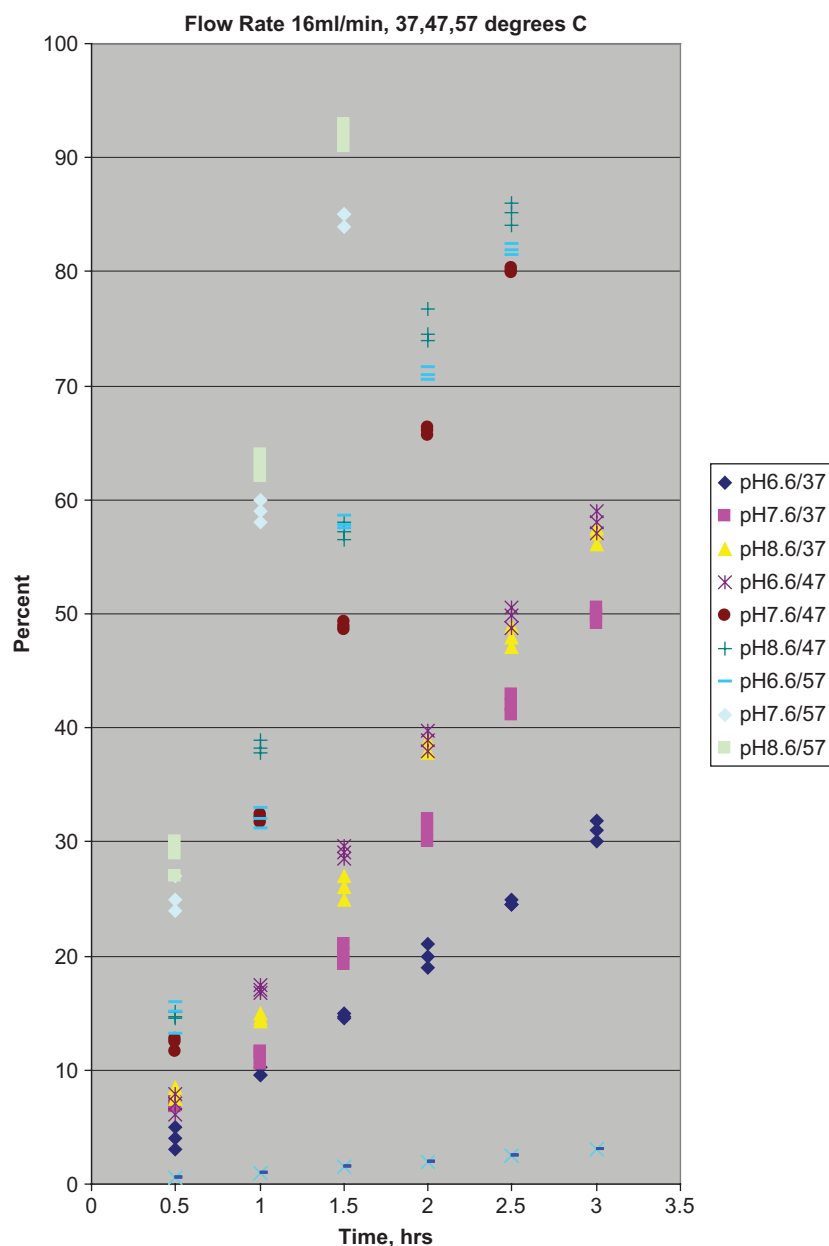


Figure 3. Dissolution profiles of Theo 24-CR in phosphate buffer 0.05M; flow rate 16 mL/min. Temperature (pH/ r^2): 37°C (6.6/0.9938, 7.6/0.9851, 8.6/0.9943); 47°C (6.6/0.9808, 7.6/0.9966, 8.6/0.9965); 57°C (6.6/0.9755, 7.6/0.9926, 8.6/0.9962); n = 3.

37°C, and flow rate 8 mL/min showing 55% linear drug release in 3 h (Figure 2). The addition of 0.1% SLS to the same solution increased drug release from 55–65% in 3 h (Figure 4). Dissolution under these conditions correlates with USP test 6 which provides for 55–75% release of theophylline within 8 h for products labeled for dosing every 24 h. Fastest drug release occurred at 57°C at flow rates of 8 and 16 mL/min at which point 85% of the theophylline was linearly released in 1.5 h from solutions at pH 7.6 and 8.6, also meeting the USP

requirements (Figures 2 and 3). These results compare with those of Zackrisson et al., where a flow through apparatus was employed using increased medium temperature to accelerate dissolution rate of roxipride microcapsules.^[10] Their results indicate that the time needed for one analysis of Roxiam takes less than 5% of the time required for the USP method; estimated to be about one hour. They report a direct correspondence between their data recorded at 85°C with USP data recorded at 37°C with no mention of microsphere

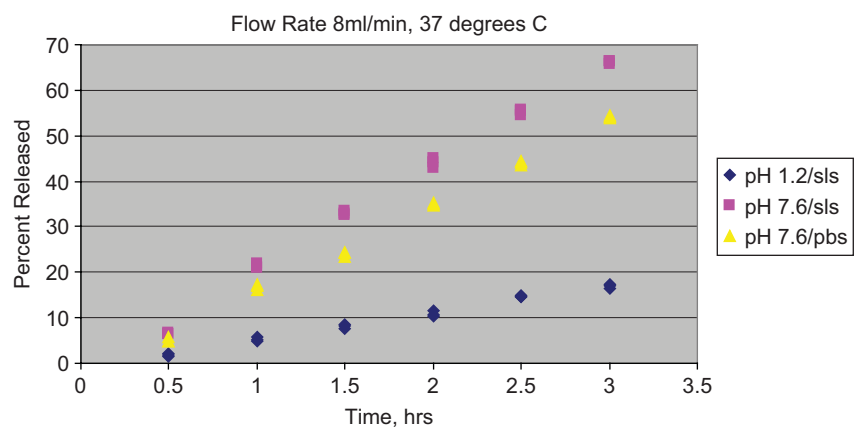


Figure 4. Dissolution profiles of Theo 24-CR at 37°C, in phosphate buffer at pH 1.2 (r^2 : 0.9924); and 7.6 (r^2 : 0.9973) with SLS (0.1%) compared to pH 7.6 (r^2 : 0.9965), in buffer alone, $n=3$.

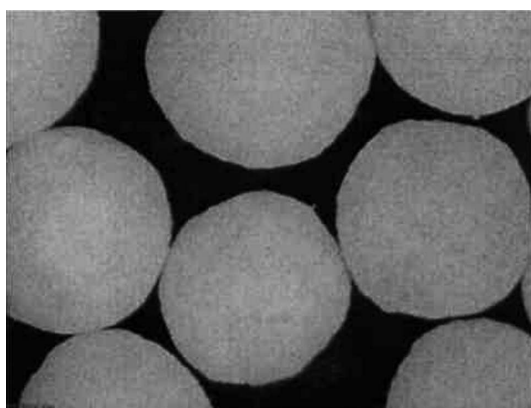


Figure 5. Electron micrograph of intact theophylline microspheres before dissolution.

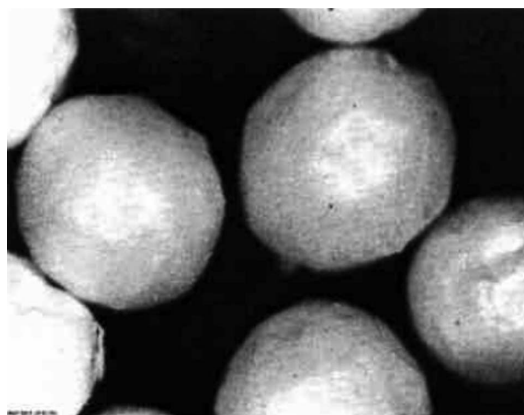


Figure 7. Electron micrograph of microspheres after dissolution at 37°C, pH 7.6, 8 mL/min flow rate.

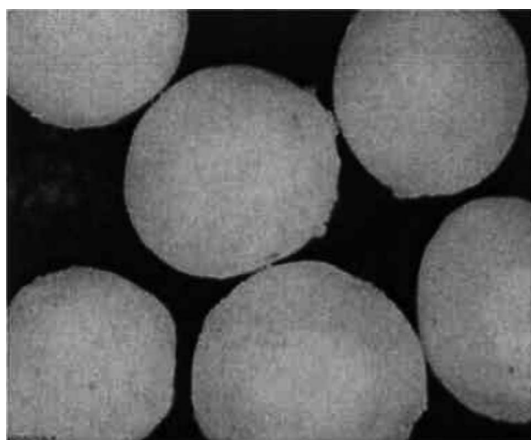


Figure 6. Electron micrograph of microspheres after dissolution at 37°C, pH 6.6, 8 mL/min flow rate.



Figure 8. Electron micrograph of microspheres after dissolution at 37°C, pH 8.6, 8 mL/min flow rate.

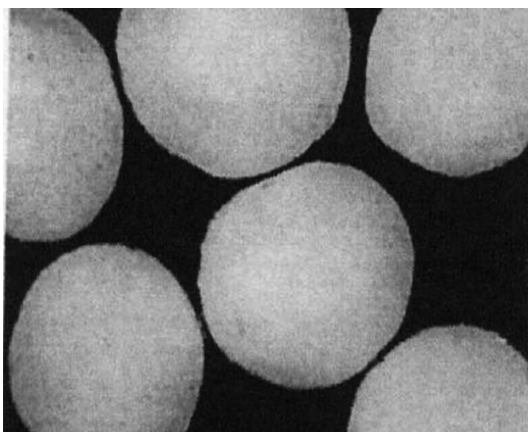


Figure 9. Electron micrograph of microspheres after dissolution at 47°C, pH 6.6, 8 mL/min flow rate.

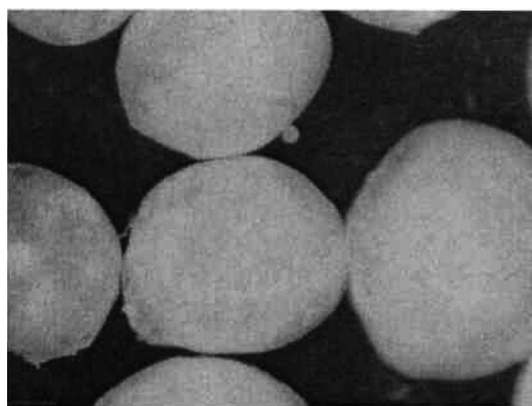


Figure 10. Electron micrograph of microspheres after dissolution at 57°C, pH 7.6, 8 mL/min flow rate.



Figure 11. Electron micrograph of microspheres after dissolution at 37°C, pH 7.6, 8 mL/min flow rate and containing 0.1% sodium lauryl sulfate.

Table 1

Standard 2^3 factorial run at time 0.5 hours for the three factors pH, temperature, and flow rate at two levels

Std	Run	Factor 1 A: pH	Factor 2 B: Temp	Factor 3 Flow rate	Response % Theo released
3	1	6.6	57	4	12
5	2	6.6	37	16	4
1	3	6.6	37	4	3
4	4	8.6	57	4	13
6	5	8.6	37	16	8
2	6	8.6	37	4	5
7	7	6.6	57	16	14
8	8	8.6	57	16	29

integrity. However, the reported correspondence with USP data strongly indicates little or no loss in integrity. The data we present here convincingly show that increased drug release at 57°C, causes no apparent change in microsphere integrity as shown in the electron micrograph, Figure 10, at pH 7.6, or observed as change in linearity of release in Figures 2 and 3. Based on these results, the results of Zackrisson et al., and those of Quist and Ostling, increasing temperature should be considered first as a method for accelerating drugs release from microspheres constructed with polymers responsive to temperature.^[10,11,15,16] Further, the Pareto chart in Figure 12 indicates temperature as the significant variable for theophylline release in the current study. The Pareto Charts were generated from data obtained from three standard 2^3 factorial runs at 0.5, 1.0, and 1.5 hours; Figure 12 is for the 0.5 h time point. They were statistically analyzed to determine the significance of the process variables pH (A), temperature (B), and flow rate (C) on theophylline release.^[17] The chart in Figure 12 presents as a vertical bar graph in which the height of the bars are proportional to the value of the estimated effects of the three variables, temperature, pH or flow rate, as well the effects of interacting variables AB, AC, or ABC. Bars above the first dotted line (T-Value Limit) are most likely significant. Bars below it are not likely to be significant. Bars above the Bonferroni line are almost certainly significant but must be confirmed by ANOVA. For timepoints 0.5, 1.0, and 1.5, ANOVA calculates *P*-values of 0.0057, 0.169, and 0.0045, respectively, indicating the significance of the variable temperature on theophylline released from the microspheres. The effects of variables pH and flow rate ranked 2, and 3, respectively, were not statistically significant as were the effects of the interactive variables, even though flow rate and pH appear to increase drug release as seen in Figures 1–4.

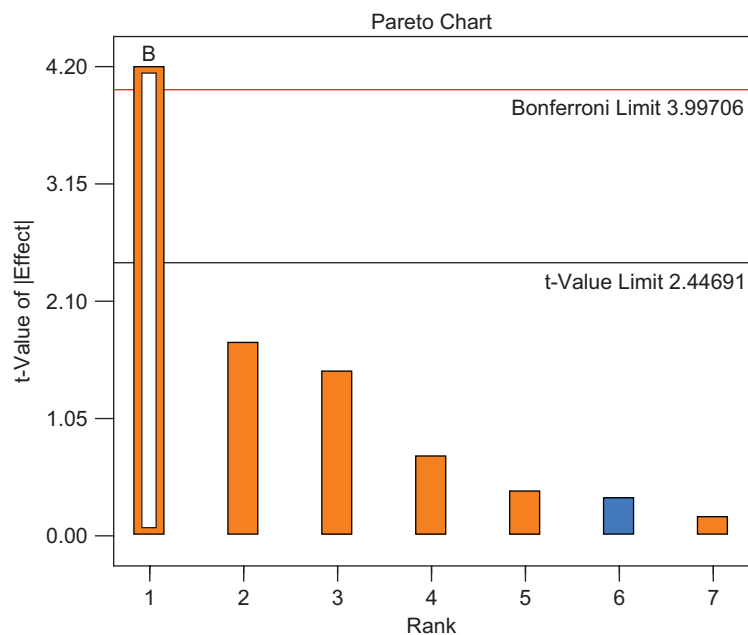


Figure 12. Pareto chart: t-Value of [Effect] versus Rank, at time point 0.5 hours for percentage release of theophylline for the effect of variables ranked according to significance: B, Temperature (rank 1); pH, A (rank 2); Flow Rate C, (rank 3); and interactions AB (rank 4); AC (rank 5); BC (rank 6); ABC (rank 7).

Electron Micrographs

Figures 5–11 show representative electron micrographs of Theo-24 CR microspheres after dissolution testing and how their physical appearance is altered after changing the variables, pH, temperature, and surfactant. Figure 5 shows intact microspheres before dissolution which are similar to microspheres after dissolution at pH 6.6, 37°C and 8 mL/min flow rate (Figure 6). Microspheres at pH 8.6 appear degraded compared to those at pH 6.6 and 7.6 (Figures 6, 7, 8). However, the apparent degradation did not adversely affect drug release or cause a dose dumping effect since theophylline release remained linear and zero order (Figures 1–3). The effect of pH on increasing drug release is also shown in Figures 1–3; even though not statistically significant the increase may reflect changes in theophylline ionization upon changing the medium from pH 6.6–8.6. In aqueous media of pH 8.6 theophylline (pKa 8.77) is almost 50% ionized to the conjugate base suggesting that a relatively minor pH related solubility effect may be causing increased drug release. This view supported by the results shown in the Pareto charts where pH is ranked second in effects but is not significant. It is clear that increased temperature does not alter microsphere integrity upon comparing Figures 7, 9, and 10 as previously discussed.

Surfactant

Adding sodium lauryl sulfate (0.1%) to the buffer medium at 37°C, pH 7.6, and flow rate 8 mL/min increased drug release by roughly 20% at the three hour mark as shown in Figure 4 with no loss of microsphere integrity as seen in Figure 11. Moreover, when compared to data in Figure 4, addition of surfactant results in even greater drug release than elevating medium pH to 8.6. A recent study showed that 1% sodium lauryl sulfate increased carbamazepine from controlled release tablets fourteen fold.^[18]

Upon review of this article it was noted that potassium salts (from the potassium phosphate buffer) precipitate SLS by forming the relatively insoluble potassium lauryl sulfate (PLS; solubility at room temperature about 0.02%).^[19,20] This is a dynamic equilibrium and the effect of PLS formation is nullified by increasing concentrations of SLS.^[21] PLS solubility has been reported to be temperature dependent such that at 37°C its solubility is about 0.11%.^[20] The current study used SLS at 0.1% concentration and potassium ion concentration was about 0.6% from the buffer solution. Therefore PLS could only form to the extent of its solubility at 37°C, and for this reason PLS solubility or precipitation was not a factor in the results presented in Figure 4 of the current study.

CONCLUSIONS

Accelerated dissolution testing of theophylline-24 CR capsules using USP Apparatus IV may be attained by increasing medium temperature, pH, increasing flow rate, and adding a surfactant; however only the effect of temperature is significant. Increasing dissolution medium above pH 7.6–8.6 caused a loss of microsphere integrity. The most benign parameters for the current study were pH 7.6, 37°C, and flow rate 8 mL/min showing 55% linear drug release in 3 h. Addition of the surfactant sodium lauryl sulfate increased the maximum drug released in 3 h to 65%. Dissolution under both of these conditions correlates with USP test 6 which provides for 55–75% release of theophylline in 8 h for products labeled for dosing every 24 h. Fastest drug release (85%/1.5 h) occurred at 57°C, pH 7.6 or 8.6, and flow rate either 8 or 16 mL/min. A combination of increased medium temperature and added surfactant may prove to be the optimal conditions for accelerated drug release of controlled release formulations while maintaining microsphere integrity.

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