The Comparison of In Vitro Release Methods for the Evaluation of Oxytocin Release from Pluronic® F127 Parenteral Formulations

e-mail: R.B.Walker@ru.ac.za

F.A. Chaibva and R.B. Walker[1]

Department of Pharmaceutics, Rhodes University, Grahamstown, South Africa, 6140

ABSTRACT

The objective of these studies was to develop a discriminatory in vitro release test for assessing formulation factors that may affect oxytocin (OT) release during formulation development studies of a Pluronic® F127 OT in situ gel-forming parenteral dosage form. An appropriate release assessment method should be able to discriminate between the performance of different formulation compositions (*1*, *2*), and this was the primary criterion used for selection of an appropriate test procedure during the test method development process. ANOVA and the difference (f_1) and similarity (f_2) factors were used to evaluate the discriminatory behavior of different test methods that were investigated in these studies. The in vitro release tests that were investigated included the use of USP Apparatus 1, 2, and 3; a dialysis bag in USP Apparatus 2; and a membrane-less diffusion method. It was concluded that the use of USP Apparatus 3 was best able to discriminate between OT release for the different formulations tested. USP Apparatus 3 was thus considered the most suitable in vitro release test apparatus for studying formulation factors affecting OT release during the development of a parenteral dosage form prepared using Pluronic® F127.

INTRODUCTION

Post partum hemorrhage is one of the leading causes of maternal mortality in both the developed and developing world (*3*, *4*). Post partum hemorrhage is caused by the loss of blood from the uterus following labor due to decreased uterine tone, retained placenta or placental fragments, as well as lower genital tract trauma (*5*). Routine management of post partum hemorrhage involves the use of parenteral oxytocin (OT) to increase uterine tone and reduce bleeding and is administered via the intramuscular or intravenous route. However, OT is rapidly metabolized in the liver and cleared from the body via the kidney (*6*). The use of a long-acting parenteral preparation of OT to maintain uterine tone would be useful as a means of reducing maternal mortality by preventing post partum hemorrhage.

Pluronic® 127 (PF-127) is an amphiphilic block copolymer that, when dissolved in water, exists as a viscous liquid at low temperatures (2–8 °C) and forms a stiff gel when warmed (*7*, *8*). The thermo-gelling behavior of PF-127 means that at low temperatures, the sample is free-flowing, allowing relatively easy administration of a PF-127 based formulation using a syringe and a needle. The stiff gel that is formed in situ at body temperatures would produce a potential depot delivery vehicle for the sustained delivery of drugs such as proteins and peptides.

In vitro drug dissolution and release testing is used to generate vital information primarily for quality control purposes, batch uniformity assessment, and evaluating batch-to-batch variability. In the context of pharmaceutical research and development, prediction of in vivo behavior of dosage forms and assessment of the impact of formulation changes or method of manufacture on overall dosage form performance in vivo may be inferred from dissolution profiles (*9*).

The intrinsic variability of in vitro release testing warrants careful method development for a test to reflect the true drug release characteristics from any drug delivery system. The type of drug and apparatus that are used must be carefully considered, since these factors can influence the rate and extent of drug release obtained during in vitro release testing (*10*). The selection of an in vitro release method and dissolution medium must allow for the prediction of an in vitro–in vivo correlation for the ultimate method of choice, if at all possible (*11*).

There are no accepted compendial guidelines for in vitro testing to assess drug release for controlled and sustained release parenteral formulations, such as those made from PF-127, for example. The United States Pharmacopeia (*12*) provides guidance for dissolution testing of oral and transdermal dosage forms but not for assessing the in vitro release of an active ingredient from controlled release parenteral preparations. Guidelines for the evaluation of novel delivery systems such as orally disintegrating and chewable tablets have been reported, although recommendations for controlled release parenteral formulations have not yet been established. However, the use of compendial and modified flow-thorough cell apparatus for assessment of sustained

[1]*Corresponding author.*

release parenteral formulations has been applied with some success (*2*). The selection of an appropriate apparatus for in vitro release testing of controlled release parenteral systems, including microparticulate, nanoparticulate, hydrogel, and liposomal dosage forms, has been the subject of several conferences and publications (*1*, *2*, *11*, *13*, *14*). Mechanistic models for the release of active pharmaceutical ingredients (API) from semi-solid dosage forms such as those manufactured using PF-127 (*15*) and gelatin-alginate matrices (*16*) have been reported. API release followed near zero-order kinetics for PF-127 systems containing methotrexate (*15*), whereas when gelatin-alginate matrices were used, first-order release kinetics were observed (*16*). These reports are useful for the analysis of API release mechanisms from such systems; however, the impact of different test methods and the use of statistical comparisons in the selection of an appropriate in vitro test method were not investigated.

The comparison of in vitro release profiles can be achieved by the use of mathematical methods of analysis. Mathematical analysis for the assessment of in vitro release profiles can be divided into 3 types: model-dependent (curve fitting) methods, model-independent methods, and statistical analysis (*17*).

Statistical methods have been used for the evaluation and comparison of dissolution profiles following in vitro dissolution testing (*18*, *19*) and for development and optimization of in vitro dissolution tests (*20–22*). Statistical methods can be used to compare different formulations tested under the same experimental conditions and to compare dissolution tests for the same formulation tested under different test conditions (*17*, *20*, *22*).

Analysis of variance methods can be differentiated as univariate or one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). ANOVA tests may be used to assess the difference between the means of two dissolution or drug release data sets in single time point dissolution tests, and MANOVA analysis is useful for the evaluation of multiple time point dissolution tests (*17*). It has been reported that ANOVA analysis is more informative and easier to interpret than MANOVA analysis, which requires data transformation on repeated measures and makes interpretation of results difficult (*19*).

To compare in vitro dissolution release profiles, a repeated measures design may be used, where the percent drug dissolved is the dependent variable and time the repeated factor. Univariate ANOVA analysis can be applied to each time point when comparing dissolution profiles to determine where differences, if any, exist between the dissolution profiles being compared (*19*).

Different post hoc tests including the least significant difference test, Tukey's multiple range test, Scheffé method, Newman-Keuls test, and Dunnett's test can be used to determine the exact points of difference between dissolution profiles of test products (*23*). ANOVA-based methods of analysis reveal information about differences in the shape and levels of different dissolution data sets that are being compared (*19*), although they have been criticized for being too discriminatory, showing statistical differences in dissolution profiles that may not necessarily reflect pharmaceutical differences (*18*).

The difference (f_1) and similarity (f_2) factors may be used for the comparison of dissolution profiles (*24*). The f_1 factor measures the percent error between dissolution profiles of a test and reference product at all time points. The percent error is zero when the in vitro release profiles of the test and reference product are identical, and this value increases as dissolution profiles become dissimilar. The f_2 factor is a logarithmic transformation of the sum-squared error of the differences between a test and reference product over all time points. The factor falls between 0 and 100, and a value of 100 indicates that the dissolution profiles are identical (*24*). The difference and similarity factors cannot be applied to the evaluation of the same formulation under different experimental conditions, and fit factors have been reported to be of use during the development of an appropriate in vitro dissolution release test (*21*, *25*).

The objective of these studies was to develop a discriminatory in vitro release method for an OT parenteral formulation prepared using Pluronic® F127 as a gel-forming matrix. Different compendial test apparatus and an in-house in vitro release method were used. ANOVA and the f_1 and f_2 factors were used to assess the discriminatory ability of each of the test methods that were assessed. Furthermore, the selection of an appropriate dissolution test method would also consider the apparent ease of use and the ability to further optimize the method by adjusting the pH and speed of rotation or agitation, where applicable.

MATERIALS AND METHODS

Materials

PF-127 was donated by BASF (Lot number WPIA571B) (Ludwigshafen, Germany) and used as received. Oxytocin (Lot number oxt 051210) was purchased from Inter-Chemical Hongkong Ltd (Shenzhen, China) and had a potency of 470 IU/mg. HPLC grade water was purified using a Milli-Ro®-15 water purification system (Millipore, Bedford, MA, USA). The water was filtered through a 0.22-µm Millipak® stack filter prior to use (Millipore, Bedford, MA, USA).

Preparation of the Dosage Form

The preparation of OT-containing PF-127 dosage forms was undertaken using a modification of the "cold method" as previously described by Schmolka (*7*). The solutions of PF-127 were prepared by weight so as to contain 20%, 25%, and 30% w/w of the polymer. Appropriate amounts of PF-127 flakes were weighed and added slowly to a previously cooled (5 °C) aqueous solution of OT (200 IU/mL) over a period of about 2–3 min with gentle stirring using a Labcon™ MSH 10 magnetic stirrer (Labmark, Maraisburg, South Africa). The solution was then

placed in a refrigerator for approximately 24 hours or until all the PF-127 had dissolved and the solution was clear on visual inspection. The solutions were assayed for OT content using a previously validated HPLC method (*26*) before use, and the gels were set in molds of dimensions 23.6 × 11.0 × 8.0 mm (L × W × D) such that only one surface of the gel was exposed to the dissolution test medium. An appropriate amount of gel was weighed such that each dosage unit to be tested contained approximately 300 IU of OT, and the gels were set in a Gallenkamp drying cabinet (Weiss Gallenkamp, Loughborough, United Kingdom) for 30 min at 37 °C prior to in vitro release testing. All release tests were performed on three dosage units (n = 3).

Dissolution Test Conditions

Preparation of the In Vitro Release Medium

The dissolution medium was a 0.1 M phosphate buffer (pH = 7.2). The buffer was prepared by dissolving 68 g of potassium dihydrogen phosphate and adjusting the pH to 7.2 using sodium hydroxide pellets. This medium was used for all experiments.

USP Apparatus 1 and 2

In vitro dissolution release testing with the basket (USP Apparatus 1) and paddle (USP Apparatus 2) apparatus was conducted using a fully automated Hanson Research SR 8 PLUS™ dissolution tester fitted with an Autoplus™, Multifill™, and Maximizer Syringe Fraction Collector (Hanson Research Cooperation, Chatsworth, CA, USA). The dissolution medium (500 mL) was maintained at 37 ± 0.5 °C. The baskets (40 mesh) and the paddles were rotated at 25 rpm for the duration of the experiments. A low rpm was selected to minimize the hydrodynamic agitation in the system. Low agitation rates are expected in vivo since the intended route of administration is by intramuscular injection. The gels, retained in the molds, were placed in either the basket or at the bottom of a dissolution vessel when using USP Apparatus 1 or 2, respectively. Sample aliquots (1.5 mL) were withdrawn for analysis at 0, 30, 60, 90, 120, 180, 240, 360, and 480 min, and an equivalent amount (1.5 mL) of fresh dissolution medium was replaced automatically.

USP Apparatus 3

The VanKel® Bio-Dis® dissolution tester (VanKel® industries, New Jersey, USA) was used. Temperature was maintained at 37 ± 0.5 °C using a model VK 750D digitally controlled water heater/circulator (VanKel® industries, New Jersey, USA). A dissolution media volume of 180 mL was used in these studies. A 177-µm pore size screen mesh was used to retain the dosage form in the inner tube, and a 5-dpm dip speed provided agitation. The dosage form was moved through the different rows of dissolution vessels at 30, 60, 120, 240, and 360 min, and testing was maintained in the final row until the end of the dissolution test at 480 min.

Dialysis Tubing Method

A 25-mm flat width dialysis tubing cellulose membrane (Sigma Aldrich, St Louis, MO, USA) was hydrated prior to use. One end of the dialysis tubing was tied, and an appropriate amount of OT-containing PF-127 gel was weighed and placed in the tube, such that there was approximately 300 IU in each dosage unit. The tube was then carefully tied at the other end. The gel was allowed to set in a convection oven (Weiss Gallenkamp, Loughborough, United Kingdom) at 37 °C for 30 min prior to commencing the test. The dialysis tubing containing the pre-set gel was placed in USP Apparatus 2 using 500 mL of dissolution medium and agitated at 50 rpm. The dissolution medium was used to assess drug release, and samples were harvested at 30, 60, 120, 240, 360, and 480 min.

Membrane-Less Diffusion System

Appropriate amounts of each gel of different polymer composition (equivalent to 300 IU) were weighed and placed into 5-mL test tubes. The gels were allowed to set in a Grant Instruments (Cambridge Ltd, Cambridge, UK) water bath maintained at 37 °C. Dissolution medium (500 µL) was carefully placed on the surface of the gel, and the tubes were placed in the water bath for the duration of the study. Figure 1 shows a schematic diagram of the dissolution method that was employed. At predetermined times of 0, 30, 60, 90, 120, 180, 240, 360, and 480 min, the receptor fluid was removed completely and replaced with fresh receptor fluid. The sample solution was diluted and analyzed by HPLC.

HPLC System

The analytical method used for the in vitro assessment of OT dosage forms was previously reported (*26*). The system consisted of a Model P100 dual-piston solvent delivery module (Thermo Separation Products, San Jose, CA, USA), a Model AS100 autosampler (Thermo Separation Products, San Jose, CA, USA) fitted with a Rheodyne® Model 7010 injector (Rheodyne, Reno, Nevada, USA) and a fixed-volume 20-µL loop, and a Model 1725 GASTIGHT®

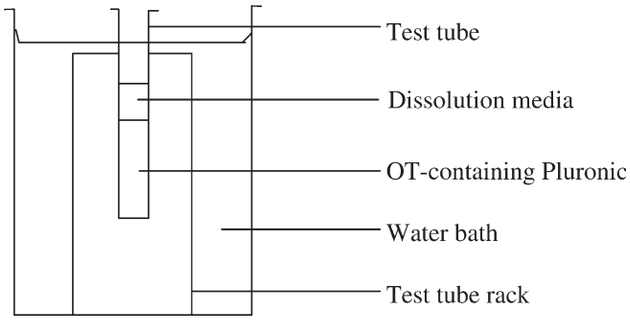

Figure 1. Schematic representation of the membrane-less diffusion system.

250-µL syringe (Hamilton Co., Reno, NV, USA). A Phenomenex Hypersil® column, 5-µm, 4.6 × 150 mm (Phenomenex, Torrance, CA, USA) was used at ambient temperature (22 °C). The separation was conducted under isocratic conditions using a mobile phase consisting of 20% v/v acetonitrile in 80 mM phosphate buffer at a flow rate of 1.5 mL/min with UV detection at 220 nm using a Linear UV/VIS-500 Model 6200-9060 detector (Linear Instrument Co., CA, USA). Data was collected using a Spectra Physics SP 4600 integrator (Thermo Separation Products, San Jose, CA, USA). The injection volume was 20 µL.

Mathematical Treatment of In Vitro Release Data

The different test methods that were used to evaluate OT release from PF-127 gels were assessed for potential discriminatory behavior using ANOVA analysis and model-independent methods. In addition, the similarity of dissolution profiles for OT release from PF-127 dosage forms containing different concentrations of gel former was also evaluated. Univariate ANOVA analysis was performed using GraphPad Prism software Version 4.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) to assess whether differences between each time point of the dissolution profiles existed for each of the formulations tested. Tukey's multiple range test was used as a post hoc test to assess which of the formulations were different at the individual time points evaluated during the dissolution tests. Model-independent approaches that were used to assess the similarity or difference between in vitro dissolution profiles were the difference (f_1) and similarity (f_2) factors. Formulations were considered different if the f_1 and f_2 factors were > 15 and < 50, respectively.

RESULTS AND DISCUSSION

In Vitro Release Profiles of OT from the Different Test Methods

The in vitro release profiles for OT from PF-127 gels using the different test methods are shown in Figure 2. As expected, the rate of OT release from the different formulations tested was dependent on the formulation concentration of PF-127, with lower concentrations producing the fastest rate of release. The trend was observed for all dissolution apparatus tested. Formulations with low gel content have a lower viscosity than formulations with higher gel content and undergo faster gel dissolution. This effect ultimately results in a faster rate of OT liberation, and these findings are supported by other studies that have shown that the rate of release of a drug from PF-127 systems is controlled by the rate of dissolution of the gel (*27*, *28*).

ANOVA of the Different Test Methods

To establish which of the test methods, if any, were discriminatory, analysis of variance (ANOVA) was used to compare the release profiles of OT from the different formulations using different methods. The primary advantage of using ANOVA for this purpose is that it allows for the detection of differences at individual time points. Furthermore, ANOVA allows for careful monitoring of the profiles to determine if there are any changes in the discriminatory pattern of a test system as testing proceeds. The ANOVA results comparing the in vitro release profiles for each apparatus and formulation are summarized in Tables 1–5.

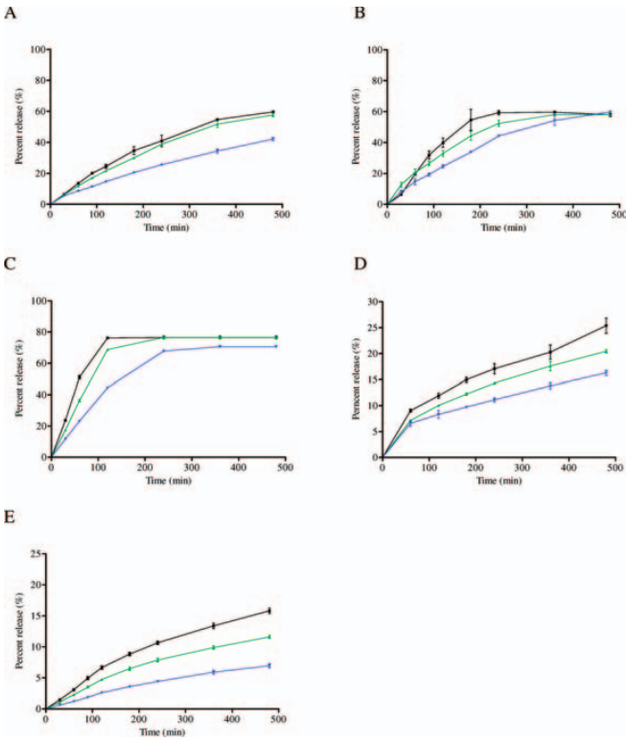

Figure 2. The extent of OT release (n = 3) from PF-127 gels using (A) USP Apparatus 1, (B) USP Apparatus 2, (C) USP Apparatus 3, (D) Dialysis bag in USP Apparatus 2, and (E) Membrane-less diffusion methods. ---- 20% w/w PF-127, ---- 25% w/w PF-125 and ---- 30% w/w PF-127.

ANOVA results for data generated using USP Apparatus 1 are summarized in Table 1. For the initial 30 min of the dissolution test, there was no discrimination between the 25% PF-127 formulation and both the 20% and 30% formulations, although discrimination was observed between the extremes of gel concentration (i.e., between the 20% and 30% gels). However, between 60 and 120 min, it can be seen from ANOVA results and visual inspection of the dissolution profiles (Figure 2A) that the three curves corresponding to the different concentrations of PF-127 are separated from one other. In the later stage dissolution testing using USP Apparatus 1, the ANOVA results indicate that there were no significant differences between the in vitro release profiles that were obtained from the 20% and 25% formulations, although the 30% formulation can be visually discriminated from both the lower and intermediate PF-127 formulations.

Table 1. ANOVA Results and Post Hoc Summary for In Vitro Release Profiles Generated Using USP Apparatus 1.

			95% Confidence interval to mean difference			
Time (min)	Comparison	Mean difference	Lower limit	Upper limit	*P* value	Summary
30	20% vs. 25%	0.3021	–0.5800	1.184	$P > 0.05$	Not sig.
	20% vs. 30%	0.9569	0.07475	1.839	$P < 0.05$	Sig.
	25% vs. 30%	0.6548	–0.2274	1.537	$P > 0.05$	Not sig.
60	20% vs. 25%	1.665	0.2874	3.043	$P < 0.05$	Sig.
	20% vs. 30%	4.939	3.561	6.317	$P < 0.01$	Sig.
	25% vs. 30%	3.274	1.896	4.652	$P < 0.01$	Sig.
90	20% vs. 25%	2.961	1.734	4.189	$P < 0.01$	Sig.
	20% vs. 30%	8.594	7.367	4.189	$P < 0.001$	Sig.
	25% vs. 30%	8.594	4.406	6.860	$P < 0.001$	Sig.
120	20% vs. 25%	2.903	–1.890	7.696	$P > 0.05$	Not sig.
	20% vs. 30%	9.846	5.053	14.64	$P < 0.01$	Sig.
	25% vs. 30%	6.943	2.150	11.74	$P < 0.05$	Sig.
180	20% vs. 25%	4.612	–4.439	13.66	$P > 0.05$	Not sig.
	20% vs. 30%	14.15	5.098	23.20	$P < 0.05$	Sig.
	25% vs. 30%	9.537	0.4866	18.59	$P < 0.05$	Sig.
240	20% vs. 25%	2.146	–10.40	14.69	$P > 0.05$	Not sig.
	20% vs. 30%	15.37	2.822	27.91	$P < 0.05$	Sig.
	25% vs. 30%	13.22	0.6752	25.77	$P < 0.05$	Sig.
360	20% vs. 25%	2.983	–5.357	11.32	$P > 0.05$	Not sig.
	20% vs. 30%	20.31	11.97	28.65	$P < 0.01$	Sig.
	25% vs. 30%	17.33	8.987	25.67	$P < 0.01$	Sig.
480	20% vs. 25%	2.058	–3.278	7.394	$P > 0.05$	Not sig.
	20% vs. 30%	17.57	12.24	22.91	$P < 0.01$	Sig.
	25% vs. 30%	15.52	10.18	20.85	$P < 0.01$	Sig.

ANOVA results reveal that the use of USP Apparatus 2 (Table 2) does not show discrimination between the formulations tested and that no significant differences exist between the dissolution profiles when using these data. Visual inspection of Figure 2B clearly shows that the early and late segments of the in vitro release profiles are very close together. However, ANOVA results show differences between the 20% and 30% PF-127 formulations at the early and late sample time points in the release profiles and no discrimination between the 25% formulation and the 20% and 30% formulations.

ANOVA results for the comparison of the release profiles generated using USP Apparatus 3 are reported in Table 3. The data indicate that significant differences exist in the percent OT dissolved at different time points in the early stages of test procedure, specifically between 0 and 240 min. Thereafter, a plateau is reached for the 20% and 25% formulations, and no significant differences are observed between the OT release profiles.

The dialysis method results indicate that there are no significant differences between the 25% and both the 20% and 30% formulations for the majority of the time points tested; the data are summarized in Table 4. However, discrimination was achieved between the 20% and 30% formulations for all time points using this release method.

The results from membrane-less diffusion indicate that the in vitro release profiles are different for the majority of time points tested. Significant differences between the means were observed according to the ANOVA performed and are summarized in Table 5. Visual inspection of the dissolution profiles shown in Figure 2E confirm these observations since the release profiles for the different

Table 2. ANOVA Results and Post Hoc Summary for In Vitro Release Profiles Generated Using USP Apparatus 2.

			95% Confidence interval to mean difference			
Time (min)	Comparison	Mean difference	Lower limit	Upper limit	*P* value	Summary
30	20% vs. 25%	–6.316	–13.41	0.7769	$P > 0.05$	Not sig.
	20% vs. 30%	–1.612	–8.705	5.482	$P > 0.05$	Not sig.
	25% vs. 30%	4.705	–2.389	11.80	$P > 0.05$	Not sig.
60	20% vs. 25%	–0.9969	–11.05	9.052	$P > 0.05$	Not sig.
	20% vs. 30%	5.052	–4.997	15.10	$P > 0.05$	Not sig.
	25% vs. 30%	6.049	–4.000	16.10	$P > 0.05$	Not sig.
90	20% vs. 25%	5.433	–5.343	16.21	$P > 0.05$	Not sig.
	20% vs. 30%	12.62	1.849	23.40	$P < 0.05$	Sig.
	25% vs. 30%	7.192	–3.584	17.97	$P > 0.05$	Not sig.
120	20% vs. 25%	7.014	–5.647	19.68	$P > 0.05$	Not sig.
	20% vs. 30%	15.42	2.758	28.08	$P < 0.05$	Sig.
	25% vs. 30%	8.404	–4.256	21.07	$P > 0.05$	Not sig.
180	20% vs. 25%	10.27	–14.96	35.50	$P > 0.05$	Not sig.
	20% vs. 30%	20.82	–4.411	46.05	$P > 0.05$	Not sig.
	25% vs. 30%	10.55	–14.68	35.78	$P > 0.05$	Not sig.
240	20% vs. 25%	6.988	–1.476	15.45	$P > 0.05$	Not sig.
	20% vs. 30%	15.15	6.688	23.62	$P < 0.05$	Sig.
	25% vs. 30%	8.164	–0.2997	16.63	$P > 0.05$	Not sig.
360	20% vs. 25%	1.631	–9.472	12.74	$P > 0.05$	Not sig.
	20% vs. 30%	5.443	–5.661	16.55	$P > 0.05$	Not sig.
	25% vs. 30%	3.812	–7.292	14.92	$P > 0.05$	Not sig.
480	20% vs. 25%	–0.1106	–4.397	4.176	$P > 0.05$	Not sig.
	20% vs. 30%	–1.956	–6.242	2.331	$P > 0.05$	Not sig.
	25% vs. 30%	–1.845	–6.132	2.441	$P > 0.05$	Not sig.

formulations are clearly well distinguished from each other.

The f_1 and f_2 Difference and Similarity Factors

The f_1 and f_2 factors were used to compare the release profiles generated for OT from PF-127 gels of different concentrations. To assess the discriminatory behavior of the release tests evaluated, the 25% formulation was used as the reference product, and the results of these analyses are summarized in Table 6.

USP Apparatus 1 and 2 were not able to discriminate between the dissolution profiles generated for the 20% and 25% formulations since the f_1 factors were < 15 for these comparisons. The f_2 factor for the comparison of the 20% and 25% formulations indicates that the profiles are similar, and therefore, it may be concluded that both USP Apparatus 1 and 2 are unable to produce data that differentiate between formulations. However, the f_1 for the comparison of the 25% and 30% formulations indicates that discrimination is achieved ($f_1 > 15$) for both USP Apparatus 1 and 2. However, f_2 for the profiles generated using USP Apparatus 2 ($f_2 = 66.6$) indicates similarity between the release profiles, but the test indicates that discrimination is achieved for profiles generated using USP Apparatus 1 ($f_2 = 49.1$).

Data generated using USP Apparatus 3 show that the use of this apparatus is able to discriminate between different formulations since $f_1 > 15$ and $f_2 < 50$ in all cases, indicating that differences were detected when the 25% formulation was compared to the 20% and 30% formulations. The use of a dialysis bag with the paddle apparatus and the membrane-less diffusion system did not allow for

Table 3. ANOVA Results and Post Hoc Summary for In Vitro Release Profiles Generated Using USP Apparatus 3.

			95% Confidence interval to mean difference			
Time (min)	Comparison	Mean difference	Lower limit	Upper limit	*P* value	Summary
30	20% vs. 25%	6.236	4.620	7.852	$P < 0.01$	Sig.
	20% vs. 30%	11.93	10.32	13.55	$P < 0.001$	Sig.
	25% vs. 30%	5.699	4.083	7.314	$P < 0.01$	Sig.
60	20% vs. 25%	14.80	9.749	19.85	$P < 0.01$	Sig.
	20% vs. 30%	27.93	22.88	32.97	$P < 0.001$	Sig.
	25% vs. 30%	13.13	8.080	18.18	$P < 0.01$	Sig.
120	20% vs. 25%	7.502	5.617	9.387	$P < 0.001$	Sig.
	20% vs. 30%	31.77	29.88	33.65	$P < 0.001$	Sig.
	25% vs. 30%	24.26	22.38	26.15	$P < 0.001$	Sig.
240	20% vs. 25%	–0.09111	–4.318	4.136	$P > 0.05$	Not sig.
	20% vs. 30%	8.688	4.461	12.91	$P < 0.01$	Sig.
	25% vs. 30%	8.779	4.552	13.01	$P < 0.01$	Sig.
360	20% vs. 25%	–0.09111	–4.798	4.615	$P > 0.05$	Not sig.
	20% vs. 30%	5.892	1.186	10.60	$P < 0.05$	Sig
	25% vs. 30%	5.984	1.277	10.69	$P < 0.05$	Sig.
480	20% vs. 25%	–0.09111	–4.798	4.615	$P > 0.05$	Not sig.
	20% vs. 30%	5.892	1.186	10.60	$P < 0.05$	Sig.
	25% vs. 30%	5.984	1.277	10.69	$P < 0.05$	Sig.

discrimination between all formulations tested since f_2 values that were calculated from the relevant release profiles were greater than 50, although values greater than 15 were obtained for some of the f_1 factors.

Although the three tests that were used to assess the discriminatory power of the dissolution methods evaluated in these studies produced some conflicting results, there is also consensus among the tests. The results of these comparisons are summarized in Tables 7 and 8.

A comparison of the different mathematical tools used to assess the similarity and difference of the dissolution profiles generated using different apparatus indicates that there is some consensus among the data. Consensus in the results can be observed in Tables 7 and 8, which show that the different methods for comparison yield the same conclusions for USP Apparatus 1 and 2 in Table 7 and for USP Apparatus 1 and 3 in Table 8. The difference in sensitivity of the dissolution methods to assess discrimination between dissolution profiles is evident when comparing the conflicting results obtained for the f_1 and f_2, factors generated for the evaluation of the dialysis tube and the membrane-less diffusion methods. Specifically, the f_1 test results indicate that a difference between the release profiles exists, whereas the f_2 factor indicates that the release profiles are similar. This is in part due to the insensitivity of this particular model-independent method when comparing dosage forms with a relatively low percent API release from the dosage unit. The f_2 factor was designed for the comparison of dissolution profiles that have near complete release of an API and only indicates non-similarity between dosage forms when the percent released differs by 10% or more. Consequently, methods that generate a low percent release would be affected by the insensitivity of this model, thereby allowing inappropriate conclusions to be drawn when less than 10% difference exists between API release profiles generated using test methods such as the dialysis and membrane-less diffusion methods.

Although the use of ANOVA has been deemed too discriminatory for the comparison of release profiles, these studies indicate that there is a general consensus between the data generated using ANOVA and those generated using the other comparison techniques. Although the use of the f_2 similarity factor for the membrane-less diffusion system indicates that there were no differences between the formulation variants tested, the use of ANOVA

Table 4. ANOVA Results and Post Hoc Summary for In Vitro Release Profiles Generated Using the Dialysis Method.

			95% Confidence interval to mean difference			
Time (min)	Comparison	Mean difference	Lower limit	Upper limit	*P* value	Summary
60	20% vs. 25%	1.893	–0.2980	4.085	$P > 0.05$	Not sig.
	20% vs. 30%	2.464	0.2730	4.656	$P < 0.05$	Sig.
	25% vs. 30%	0.5710	–1.620	2.762	$P > 0.05$	Not sig.
120	20% vs. 25%	1.872	–1.215	4.958	$P > 0.05$	Not sig.
	20% vs. 30%	3.586	0.4989	6.673	$P < 0.05$	Sig.
	25% vs. 30%	1.714	–1.373	4.801	$P > 0.05$	Not sig.
180	20% vs. 25%	2.755	0.8885	4.621	$P < 0.05$	Sig.
	20% vs. 30%	5.244	3.378	7.111	$P < 0.01$	Sig.
	25% vs. 30%	2.489	0.6228	4.356	$P < 0.05$	Sig.
240	20% vs. 25%	2.731	–0.9598	6.422	$P > 0.05$	Not sig.
	20% vs. 30%	5.921	2.230	9.612	$P < 0.05$	Sig.
	25% vs. 30%	3.190	–0.5006	6.881	$P > 0.05$	Not sig.
360	20% vs. 25%	2.695	–3.338	8.728	$P > 0.05$	Not sig.
	20% vs. 30%	6.457	0.4245	12.49	$P < 0.05$	Sig.
	25% vs. 30%	3.762	–2.271	9.795	$P > 0.05$	Not sig.
480	20% vs. 25%	4.949	–0.4982	10.40	$P > 0.05$	Not sig.
	20% vs. 30%	9.107	3.660	14.55	$P < 0.05$	Sig.
	25% vs. 30%	4.158	–1.289	9.605	$P > 0.05$	Not sig.

indicates that there are differences. The f_2 factor is based on the difference between the relatively low percent released, and since the data are close together, the tool fails to distinguish between the dissolution profiles for the different formulations tested, despite the difference in the mean amount released.

CONCLUSIONS

The selection of the appropriate dissolution method for use from the different methods evaluated was based on the ability of the method to discriminate between formulations, ease of use of the method, and possibility for optimization of the release test method.

The basket and paddle apparatus are automated, easy to use, and have the potential for modification of API release patterns by a use of different pH and rotation speeds, yet based on statistical evaluation, are unable to discriminate between the dissolution profiles of the formulation compositions tested in these studies. The dialysis method that was tested in the study provided conflicting results with respect to the ability to discriminate and therefore was not suitable for evaluating OT release from these dosage units. Furthermore, since the dialysis method resulted in a low percent API release from the dosage form, conflicting results between the f_1 and f_2 factors were observed. ANOVA of the membrane-less diffusion method results indicates that this method is able to discriminate between the formulation compositions tested, although the low percent API release resulted in conflicting difference and similarity factors. A further disadvantage of this method is that it is non-automated, making it very tedious to perform and impractical to use when testing many formulations. USP Apparatus 3 permits discrimination between the formulation compositions evaluated. Since the system is automated, convenient, and easy to use, multiple formulation compositions can be evaluated for their impact on drug release. Furthermore, the use of USP Apparatus 3 permits further optimization of an in vitro dissolution test method by allowing for changes in pH and other in vivo conditions to be mimicked over time as API release is monitored. In addition, the system allows for an assessment of whether such changes in pH or other conditions affect the release kinetics of OT from PF-127 gel formulations.

The success of a formulation depends on several factors including the knowledge and experience of a formulator and the judicious selection of an in vitro release test method. Inference of in vivo behavior from an in vitro

Table 5. ANOVA Results and Post Hoc Summary for In Vitro Release Profiles Generated Using the Membrane-Less Diffusion Method.

			95% Confidence interval for mean difference			
Time (min)	Comparison	Mean difference	Lower limit	Upper limit	*P* value	Summary
30	20% vs. 25%	0.3581	–0. 1599	0.8762	$P > 0.05$	Not sig.
	20% vs. 30%	0.8357	0.3177	1.354	$P < 0.01$	Sig.
	25% vs. 30%	0.4776	–0.04045	0.9957	$P > 0.05$	Not sig.
60	20% vs. 25%	0.8112	0.2285	1.394	$P < 0.05$	Sig.
	20% vs. 30%	1.884	1.302	2.467	$P < 0.001$	Sig.
	25% vs. 30%	1.073	0.4904	1.656	$P < 0.01$	Sig.
90	20% vs. 25%	1.446	0.6220	2.269	$P < 0.01$	Sig.
	20% vs. 30%	3.085	2.261	3.909	$P < 0.001$	Sig.
	25% vs. 30%	1.639	0.8155	2.463	$P < 0.01$	Sig.
120	20% vs. 25%	1.922	1.066	2.778	$P < 0.01$	Sig.
	20% vs. 30%	4.049	3.192	4.905	$P < 0.001$	Sig.
	25% vs. 30%	2.127	1.271	2.983	$P < 0.001$	Sig.
180	20% vs. 25%	2.348	1.315	3.381	$P < 0.01$	Sig.
	20% vs. 30%	5.238	4.205	6.272	$P < 0.001$	Sig.
	25% vs. 30%	2.890	1.857	3.924	$P < 0.001$	Sig.
240	20% vs. 25%	2.783	1.711	3.854	$P < 0.001$	Sig.
	20% vs. 30%	6.233	5.162	7.305	$P < 0.001$	Sig.
	25% vs. 30%	3.451	2.379	4.522	$P < 0.001$	Sig.
360	20% vs. 25%	3.500	1.969	5.030	$P < 0.01$	Sig.
	20% vs. 30%	7.464	5.933	8.994	$P < 0.001$	Sig.
	25% vs. 30%	3.964	2.434	5.495	$P < 0.001$	Sig.
480	20% vs. 25%	4.211	2.738	5.684	$P < 0.001$	Sig.
	20% vs. 30%	8.839	7.366	10.31	$P < 0.001$	Sig.
	25% vs. 30%	4.627	3.154	6.100	$P < 0.001$	Sig.

Table 6. f_1 and f_2 Values for the Different Formulations Using the 25% w/w Formulation as the Reference (shaded areas indicate discrimination).

	20% w/w PF-127		30% w/w PF-127	
In vitro release test	f_1	f_2	f_1	f_2
USP Apparatus 1	9.9	72.8	31.1	49.1
USP Apparatus 2	13.7	72.6	15.7	66.6
USP Apparatus 3	23.3	49.4	26.1	41.9
Dialysis tube	21.9	75.7	19.4	81.6
Membrane-less diffusion	36.6	81.4	43.2	78.9

Table 7. Summary of Comparison of the Mathematical Models Used to Compare the 20% vs. 25% Formulations.

	ANOVA	f_1	f_2
USP Apparatus 1	**X**	**X**	**X**
USP Apparatus 2	**X**	**X**	**X**
USP Apparatus 3	✓	✓	✓
Dialysis tube	**X**	✓	**X**
Membrane-less diffusion	✓	✓	**X**

✓ Indicates discriminatory behaviour and **X** indicates failure to discriminate

Table 8. Summary of Comparison of the Mathematical Models Used to Compare the 25% vs. 30% Formulations.

	ANOVA	f_1	f_2
USP Apparatus 1	✓	✓	✓
USP Apparatus 2	X	✓	X
USP Apparatus 3	✓	✓	✓
Dialysis tube	X	✓	X
Membrane-less diffusion	✓	✓	X

✓ Indicates discriminatory behaviour and **X** indicates failure to discriminate.

release pattern of a therapeutic agent must be confirmed by way of in vivo testing, and therefore, such studies are recommended in prospective research.

REFERENCES

1. Burgess, D. J.; Hussain, A. S.; Ingallinera, T. S.; Chen, M. L.. Assuring quality and performance of sustained and controlled release parenterals: AAPS workshop report, co-sponsored by FDA and USP. *Pharm. Res*. **2002**, *19* (11), 1761–1768.
2. Siewert, M.; Dressman, J.; Brown, C. K.; Shah, V. P. FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms. *AAPS PharmSciTech* **2003**, *4*, Article 7.
3. Macphail, S.; Fitzgerald, J. Massive post-partum haemorrhage. *Curr. Opin. Obstet. Gynaecol*. **2001**, *11* (2), 108–114.
4. Thonneau, P. F.; Matsudai, T.; Alihonou, E.; De Souza, J.; Faye, O.; Moreau, J. C.; Djanhan, Y.; Welffens-Ekra, C.; Goyaux, N. Distribution and causes of maternal mortality during delivery and post-partum: results of an African multicentre hospital-based study. *Eur. J. Obstet. Gynecol. Reprod. Biol*. **2004**, *114* (2), 150–154.
5. Tamizian, O.; Arulkumaran, S. The surgical management of post-partum haemorrhage. *Best Pract. Res. Clin. Obstet. Gynaecol*. **2002**, *16*, 81–98.
6. Fjellestad-Paulsen, A.; Lundin, S. Metabolism of vasopressin, oxytocin and their analogues [Mpa^1,D-Arg^8]-vasopressin (dDAVP) and [Mpa^1,D-Tyr(Et)^2,Thr^4,Orn^8]-oxytocin (antocin) in human kidney and liver homogenates. *Regul. Pept*. **1996**, *67* (1), 27–32.
7. Schmolka, I. R. Artificial Skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns. *J. Biomed. Mater. Res*. **1972**, *6* (6), 571–582.
8. Alexandridis, P.; Hatton, T. A. Poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modelling. *Colloids Surf., A: Physicochem. Eng. Aspects* **1995**, *96*, 1–46.
9. Siewert, M.; Weinandy, L.; Whiteman, D.; Judkins, C. Typical variability and evaluation of sources of variability in drug dissolution testing. *Eur. J. Pharmaceut. Biopharmaceut*. **2002**, *53*, 9–14.
10. Qureshi, S. A.; McGilveray, I. J. Typical variability in drug dissolution testing: study with USP and FDA calibrator tablets and a marketed drug (glibenclamide) product. *Eur. J. Pharm. Sci*. **1999**, *7*, 249–258.
11. Burgess, D. J.; Crommelin, D. J. A.; Hussain, A. S.; Chen, M. L. EUFEPS Workshop Report. Assuring quality and performance of sustained and controlled release parenterals. *Eur. J. Pharm. Sci*. **2004**, *21* (5), 679–690.
12. *United States Pharmacopeia and National Formulary USP 28-NF 23*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2006; pp 1623, 2675–2693, 3050–3053, 3392–3394.
13. Burgess, D. J.; Hussain, A. S.; Ingallinera, T. S.; Chen, M. L. Assuring quality and performance of sustained and controlled release parenterals: Workshop report. *AAPS PharmSci* **2002**, *4*, Article 7.
14. D'Souza, S. S.; DeLuca, P. P. Methods to assess in vitro drug release from injectable polymeric particulate systems. *Pharm. Res*. **2006**, *23* (3), 460–474.
15. Pluta, J.; Karolewicz, B. In vitro studies of thermosensitive systems prepared on Pluronic-F127 as vehicles for methotraxate for delivery to solid tumours. *Polimery w Medycynie* **2006**, *36*, 37–53.
16. Pluta, J.; Haznar, D. Properties and active substance release kinetics from gelatin-alginate matrices. *Polimery w Medycynie* **2006**, *36*, 55–69.
17. Costa, P.; Lobo, J. M. S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci*. **2001**, *13*, 123–133.
18. Polli, J.; Rekhi, G. S.; Augsburger, L. L.; Shah, V. P.. Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. *J. Pharm. Sci*. **1997**, *86*, 690–700.
19. Yuksel, N.; Kanik, A. E.; Baykara, T. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. *Int. J. Pharmaceut*. **2000**, *209*, 57–67.
20. Carvalho-Silva, B.; Moreira-Campos, L. M.; Nunan, E. A.; Vianna-Soares, C. D.; Araujo-Alves, B. L.; Cesar, I. C.; Pianetti, G. A. Optimization and statistical evaluation of dissolution tests for indinavir sulfate capsules. *Il Farmaco* **2004**, *59*, 921–927.
21. Likar, M. D.; Mansour, H. L.; Harwood, J. W. Development and validation of a dissolution test for a once-a-day combination tablet of immediate-release cetirizine dihydrochloride and extended release pseudoephedrine hydrochloride. *J. Pharm. Biomed. Anal*. **2005**, *39*, 543–551.
22. Fernandes, C.; Junqueira, R. G.; Campos, L. M.M.; Pianetti, G. A. Dissolution test for lamivudine tablets:

Optimization and statistical analysis. *J. Pharm. Biomed. Anal.*, in press.
23. Bolton, S.; Bon, C. *Pharmaceutical Statistics: Practical and Clinical Applications*, 4th ed.; Drugs and the Pharmaceutical Sciences, Vol. 135; Marcel Dekker: New York, 2003.
24. Moore, J. W.; Flanner, H. H. Mathematical comparison of dissolution profiles. *Pharm. Technol*. **1996**, *20*, 64–74.
25. Missaghi, S.; Fassihi, R. Release characterization of dimenhydrinate from an eroding matrix: selection of appropriate dissolution apparatus. *Int. J. Pharmaceut*. **2005**, *293*, 35–42.
26. Chaibva, F. A.; Walker, R. B. Development and validation of a stability-indicating analytical method for the quantitation of oxytocin in pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.*, in press.
27. Moore, T.; Croy, S.; Mallapragada, S. K.; Pandit, N. K. Experimental investigation and mathematical modeling of Pluronic® F127 gel dissolution: drug release in stirred systems. *J. Controlled Release* **2000**, *67*, 191–202.
28. Zhang, L.; Parsons, D. L.; Navarre, C.; Kompella, U. B. Development and in-vitro evaluation of sustained release Poloxamer 407 (P407) gel formulations of ceftiofur. *J. Controlled Release* **2002**, *85*, 73–81.