A New Method for Dissolution Studies of Lipid-Filled Capsules Employing Nifedipine as a Model Drug

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INTRODUCTION

Considerable interest has been shown in the formulation of lipid-filled capsules for the enhancement of either *in vivo* dissolution rates or bioavailability of bioactive agents (1,2). Both softgel and hard shell capsules filled with vehicles which are capable of self-emulsification (due to their ability to form fine oil-in-water emulsions) offer great potential for the oral delivery of insoluble hydrophobic and poorly absorbable drugs. For example, Labrasol®, an oily water soluble caprylocaproyl macrogolglyceride and Gelucire 44/14®, a waxy water dispersible lauroyl macrogolglyceride either alone or in combination have been used to develop self-emulsifying drug delivery systems (3). No official dissolution method for lipid-based formulations as yet has been established and none of the above-cited articles have provided *in vitro* dissolution studies. This may be due to the relative difficulties associated with the evaluation methodology of lipid-based formulations. A greater challenge is presented when poorly soluble drugs in a lipid-based vehicle are presented as lipid-filled capsules for enhancement of solubility. Such matrices however are not soluble in commonly used aqueous dissolution media. In addition, neither the USP 23 nor other official compendia provide dissolution method(s) for lipid-filled gelatin capsules containing poorly soluble drugs but they recognize that "the liquid nature of capsule contents presents different technological problems than dry-filled capsules in regard to disintegration and dissolution testing." "The contact between hard or soft shell and its liquid content is more intimate than exists with dry-filled capsules, and this may enhance the chances for undesired interactions" (4). The identification of such drug-capsule shell interaction during preformulation stages, necessitates a dissolution method that is able to differentiate minor but meaningful changes in dissolution rates. With some conventional dissolution methods the use of surfactants (2–5) or hydro-alcoholic media (1,5) have been recommended. However, it is speculated that exposure of the gelatin shell to such media may induce physical and/ or chemical changes, arising either through complex formation or crosslinking reactions. Typically, sodium lauryl sulfate (SLS), an anionic surfactant, is often employed in dissolution media; however many researchers fail to recognize that SLS will bind to cationic charges of gelatin at gastric pH. These interactions will influence the solubility and disintegration time of the shell and/ or true release potential of the product. Therefore, difficulties that may be experienced include, but are not limited to exposure of gelatin shell to the organic phase, separation of poorly soluble drugs as metastable liquid crystals, lack of reproducibility in dissolution data, dosage form and lipid floatation in the dissolution vessel etc.

Therefore, the necessity of developing a new dissolution method for lipid-filled capsules is apparent. The method proposed in this article encompasses the development, design and use of a modified two-phase dissolution media system (6,7) by a novel approach for testing of either soft or hard shell lipid-filled gelatin capsules. Nifedipine was chosen as the model compound due to its water insoluble nature (<10 μg/ml at 25°C) and high octanol-water partition coefficient (10 000:1). The experimental design takes advantage of the inherent immiscibility of aqueous phosphate buffer and 1-octanol, as well as the ability to modulate dissolution hydrodynamics and position of the formulation in the aqueous phase within the vessel. Furthermore, the organic phase will act as a sink for drug removal from the aqueous phase in the dissolution vessels, a concept also recognized and pointed out in the early work of Gibaldi and Feldman (8) on the establishment of *in vitro* sink conditions in dissolution rate analysis and the merits of using a two-phase dissolution media system.

MATERIALS AND METHODS

Materials

Nifedipine powder was purchased from Sigma (St. Louis, MO). Spectrophotometric grade 1-octanol (Aldrich, WI) was obtained for modified dissolution testing. Gelucire 44/14® and Labrasol® were kindly donated by Gattefosse Corporation (Westerwood, NJ). All other reagents used were of analytical grade.

Preparation of Lipid-Filled Nifedipine Capsules

Required quantity of Gelucire 44/14® (melting point = 44°C) was heated to 2°C above its melting point. On attainment of the appropriate temperature, nifedipine was added to the melt under continuous stirring. In order to completely dissolve nifedipine, an appropriate quantity of Labrasol® was added to the drug-melt dispersion. The resultant mixture was manually filled at 38°C into the body of #00 hard gelatin capsules, after which the upper half was replaced. In order to maintain consistency in the viscosity of the formulation, a warm glass syringe (38°C) was used. A fill of 0.8 ml of the formulation per capsule was equivalent to 30 mg nifedipine. The solution inside the capsule solidified at room temperature (23 ± 0.1°C). All operations in preparation and formulation were conducted under dark conditions due to the light-sensitive nature of nifedipine.

Differential Scanning Calorimetry Studies (DSC)

The thermal properties of the vehicles and formulation were determined, using a Perkin-Elmer Differential Scanning

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Calorimeter (DSC 7). Accurately weighed samples were sealed in aluminum pans by crimping, and scans were recorded at a heating rate of 5°C/min from −10 to 200°C.

Textural Analysis Studies

Physical consistency profiles of the vehicles and formulation were generated by use of the Texture Analyzer (TA-XT2*i*, Stable Micro Systems, England) equipped with a perspex 60° angle conical probe and appropriate adapter. Accurately weighed samples were evenly spread in the conical adapter, after which forces in compression and tension were measured. Upon attainment of maximum force in compression (4.9 N) a hold time of 15 seconds was employed. Data was collected at a rate of 200 points per second (pps) and computed with aid of the Texture Expert software for Windows Version 1.17. All studies were conducted at room temperature (23 ± 0.1°C).

Dissolution Studies

All dissolution studies were conducted in a fully calibrated dissolution apparatus under dark conditions (Vankel Dissolution Apparatus, model VK 7000) using either the rotating basket or paddle or modified paddle method with media maintained at 37 ± 0.1°C. Details regarding the nature and volume of dissolution media varied for each test are described in the following section. In a modified paddle method, a designed ring/ mesh stainless steel device fitted with a 16-mesh screen, which fits precisely under the paddle of the standard dissolution vessel, was employed to prevent flotation of the capsule. More detailed description of the above device and its utility can be found elsewhere (7). Drug transfer was measured spectrophotometrically at 238 nm under dark conditions (HP Diode Array UV Spectrophotometer, model 8452A). The term "drug transfer" instead of "drug release" is employed, since drug transport would have to occur from the lower aqueous phase to the upper organic phase from which samples will be analyzed. All dissolution studies were performed in triplicate.

Nifedipine Release Measurements

Nifedipine, a practically water insoluble drug (<10 μg/ml at 25°C) is incorporated in a lipophilic fill material, which upon dispersion in aqueous dissolution medium forms a fine microemulsion. Employment of the standard aqueous dissolution media (usually of acidic pH), does not enable direct analysis of drug release due to the presence of an oily microglobular opaque mixture. This consequently necessitates dilution of samples with organic solvent and fine membrane filtration (usually ≤0.45 μm membrane filter). However, such processing of samples may lead to drug loss and yield false drug release rates. In addition, nifedipine may precipitate from its solid solution phase and aggregate due to its low water solubility. In view of the inherent solubility limitation, floatation of the dosage form, lipoidal nature of the emulsified formulation and analytical difficulties, we therefore modified the current USP 23 Apparatus II dissolution method by employing a two-phase solvent system and ring/mesh assembly.

As illustrated in the Schematic I, four dissolution designs were adopted to determine the optimum hydrodynamic and drug transfer conditions. In general, the dissolution medium consisted of a lower phase of simulated gastric fluid (without enzymes) and an upper phase of 1-octanol. The choice of 1-octanol over other organic solvents has been identified in our previous work (7). In summary, the propensity for octanol volatilization/evaporation is low and the octanol-water partition coefficient for nifedipine is 10 000:1, favoring the "drag" of drug molecules into the organic phase from the aqueous phase and hence maintaining sink conditions. In order to avoid exposure of the gelatin capsule shell to the organic phase, this phase was gently added to the vessel immediately after the introduction of the capsule to the aqueous phase (i.e., lower phase).

In each dissolution study 5 ml samples were manually withdrawn from the 1-octanol phase at specific time intervals using 10 ml glass syringes over a period of 6 hours and immediately analyzed. An equal volume of fresh, drug-free 1-octanol was replaced in each vessel.

Treatment of Dissolution Data

From the overall analysis of dissolution data, we have shown that the application of the "similarity factor, f_2" (7), recently proposed by the CDER at the FDA (9), is superior to other time-point methods used in dissolution data treatment (viz. t_x% and mean dissolution time). To this end the guidelines and specific published work (10) describe the mathematical treatment of dissolution data by comparing dissolution profiles using the "similarity factor, f_2" which may be defined as follows:

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

where n is the number of pull points (no more than one measurement after 85% of dissolution of both products), w_t is an optional weight factor, R_t is the reference assay at time point t and T_t is the test assay at time point t. In the present study, the "reference" and "test" products are identical (same batch was used for each experiment), however evaluated under modified dissolution conditions. The f_2 value between 50 and 100 suggests that the dissolution profiles are similar. The f_2 value of 100 suggests that the test and reference profiles are identical and as the value becomes smaller, the dissimilarity between release profiles increases.

In addition, Moore and Flanner (10) in their recent work also describe an f_1 fit factor or "difference factor" as follows:

$$f_1 = \left\{\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t}\right\} \times 100\% \quad (2)$$

where f_1 describes the relative error between two dissolution profiles. "It approximates the percent error between two curves. The percent error is zero when the test and reference profiles are identical and increases proportionally with the dissimilarity between the two profiles." An acceptable range of 0–15 has been identified for the f_1 value (9). Where appropriate, data was subjected to statistical analysis (t-test) at a 95% confidence level.

RESULTS AND DISCUSSION

Generally in lipid-based formulations, thermal, viscosity and textural properties of the excipient used can influence the

overall physicochemical characteristics of the dosage form. Consequently the following procedures adopted in our laboratory for the development of lipid-based systems were performed.

Evaluation of Thermal Properties of the Vehicles and Formulation

The representative thermograms of the vehicles and formulation are provided in Figure 1a. Gelucire 44/14® has a melting peak at 44°C (thermogram A) while nifedipine typically demonstrates a melting peak at 175°C (thermogram B). The melting endotherm of Gelucire 44/14® is lowered to 36°C when Labrasol® and drug are included (thermogram C). Lowering of the endotherm is due to a change in the consistency of the material. Based on this study, a temperature of 38°C was selected for encapsulating the drug solution mixture. No endotherm corresponding to the melting point of nifedipine was observed in the formulation thermogram (i.e. thermogram C) since the drug was completely solubilized.

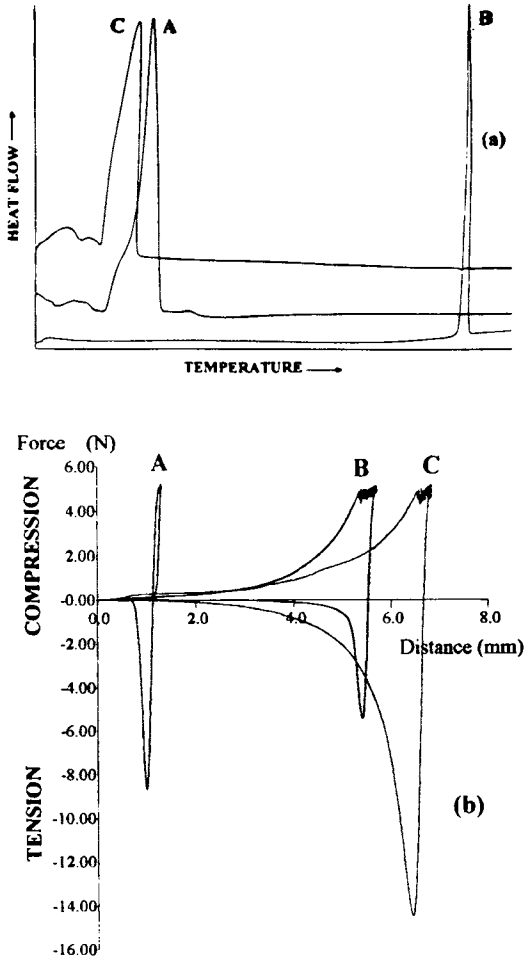


Fig. 1. DSC and textural profiles of the formulation employed. (a) DSC thermograms of (A) Gelucire 44/14®, melting peak at 44°C; (B) Nifedipine powder, melting peak at 175°C; and (C) Formulation i.e. Gelucire 44/14®/Labrasol®/Nifedipine solid solution, melting peak at 36°C. (b) Relationship between probe displacement and force for (A) Labrasol®; (B) Gelucire 44/14®; and (C) Formulation, i.e., Gelucire 44/14®/Labrasol®/Nifedipine solid solution.

Evaluation of Material Textural Properties

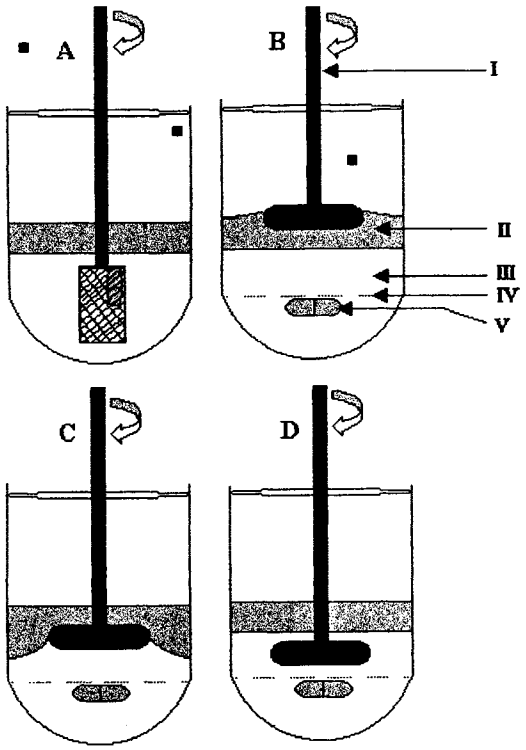
Figure 1b depicts the consistency profiles for Labrasol® (A), Gelucire 44/14® (B) and formulation with drug (C). Forces above and below zero are denoted as forces in compression and tension respectively.

Based on textural analysis, as expected, it is evident that the formulation is more firm and rigid (profile C) as opposed to the consistency profile of Gelucire 44/14® (profile B). Labrasol®, on the other hand, depicts sharp compression and tension peaks due to its lower viscosity. To achieve the pre-set maximum force in compression of 4.9N in the formulation, the probe had to travel a distance 6.61 mm. For equivalent force in the cases of Labrasol® and Gelucire 44/14®, distances of 1.21 and 5.33 mm was recorded, indicating the varied nature in material softness and spreadibility. It is believed that the formulation viscosity/ consistency has a significant effect on release characteristics of lipid-filled capsules. Therefore in dissolution studies, the inter-relationship between the textural properties and dissolution profiles should be viewed collectively. The consistency profiles depicted in Figure 1b are typical of those found in lipid-filled soft or hard gelatin capsules.

Evaluation of the Rate and Extent of Drug Liberation Derived from Different Dissolution Designs

Exposure of the formulation to the aqueous phase after approximately 10–15 minutes resulted in the formation of a fine microemulsion. The intensity of opaqueness of the microemulsion progressively increased and attained a maximum within half an hour. Gelucire and Labrosol alone are soluble in 1-octanol, however, it was noted that the emulsion inherently confined itself to the aqueous phase, i.e., it did not exhibit partitioning into the organic phase. The reason for this lies in the fact that the polarity of the fatty acid chains are changed and hence the system demonstrates a partitioning property in accordance with that new polarity attained, which also controls the rate of drug liberation. An ultraviolet spectrophotometric scan of pure octanol and octanol samples obtained from the described two-phase dissolution set-up containing blank formulations (i.e., no drug), showed identical spectrums. On the other hand, during typical dissolution runs, an increasing yellow discoloration could be observed in the 1-octanol phase, indicating the gradual transfer of solubilized nifedipine, which was spectrophotometrically analyzed. This observation is consistent with previous results (7) showing a similar "nifedipine drag effect" of 1-octanol in a two-phase dissolution media system.

As depicted in Schematic I, the position of the rotating basket was always in the aqueous phase in order to prevent exposure of the capsule shell to the organic phase (Schematic I, Design A). Different position for the paddle was selected to determine most favorable hydrodynamic condition to facilitate complete drug transport from the lower aqueous phase to upper organic phase. Furthermore, in order to establish closer contact and fluid flow between the two phases at the interface, the volume of the aqueous phase was appropriately reduced to 200 ml, unless otherwise stated. Figures 2a–e depicts the typical drug transfer profiles obtained by application of the rotating basket and different positionings of the rotating paddle within the two-phase organic/aqueous dissolution media system (see Schematic I, Designs A–D).



Schematic I. An illustration of the four dissolution designs employed for the induction of different hydrodynamic conditions. Key: I = Position of either rotating basket or paddle with hydrodynamic arrangements as follows: Design A—Centrally positioned in aqueous phase between boundaries of organic phase and bottom of vessel; Design B—Halfway at air/organic phase interface; Design C—Halfway at organic/aqueous phase interface; Design D—Centrally positioned in aqueous phase between boundaries of organic phase and ring/mesh assembly. Stirring rate of 75 rpm was used in all designs with exception of Design D where in addition 100 rpm was also tested. II = organic phase, i.e., 100 ml 1-octanol. III = aqueous phase, i.e., phosphate buffer: 400 ml for Design A, 200 ml for Designs B and C, 300 ml for Design D. Note that 400 ml and 300 ml of phosphate buffer was employed in Designs A and D to ensure that basket and paddle are fully immersed in aqueous phase. IV = ring/mesh assembly. V = Position of capsule either within basket or below ring/mesh assembly.

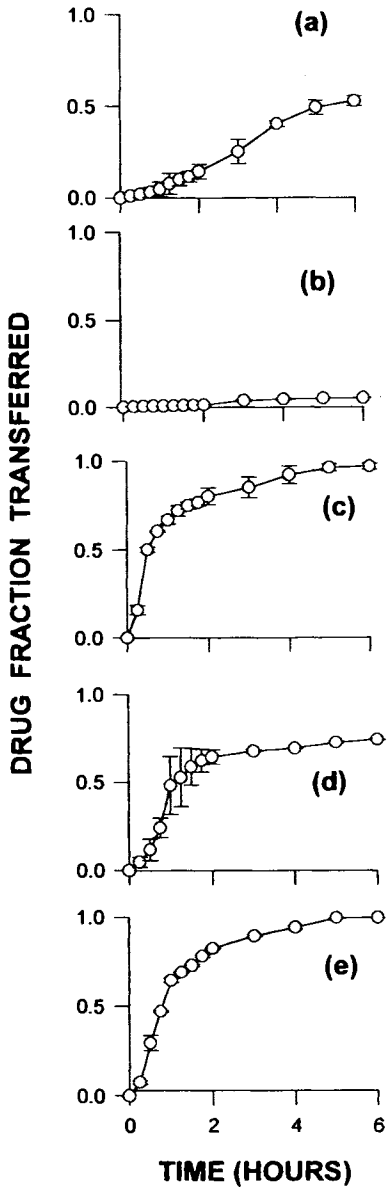


Fig. 2. Transfer profile of lipid-based nifedipine capsule preparation derived under different hydrodynamic conditions and designs as shown in Schematic I: (a) Profile obtained using the USP 23 rotating basket method at 75 rpm (Dissolution Design A); (b) Profile obtained using paddle over ring/mesh assembly halfway at air/organic interface at 75 rpm (Dissolution Design B); (c) Profile obtained using paddle over ring/mesh assembly halfway at organic/aqueous interface at 75 rpm (Dissolution Design C); (d) Profile obtained using paddle over ring/mesh assembly in aqueous phase at 75 rpm (Dissolution Design D); (e) Profile obtained using paddle over ring/mesh assembly in aqueous phase at 100 rpm (Dissolution Design D).

Various agitation rates (50, 75, and 100 rpm) were initially studied to optimize the fluid flow patterns. Application of 100 rpm in Design A (rotating basket apparatus) markedly induced interference in sampling and analysis due to generation of air bubbles in the organic phase; while 75 rpm did not allow complete drug transfer to the aqueous phase (Figure 2a). In fact, after 6 hours of dissolution, most of the viscous oily vehicle still remained entrapped within the basket; hence failure to release drug into the aqueous phase. It appears that the standard dissolution basket pores (40-mesh) and lack of appropriate hydrodynamic conditions within the basket have a significant limiting effect on drug release from the oleaginous formulation. As a result, this particular option for dissolution evaluation was not further investigated.

In all dissolution designs it was observed that an agitation rate of 50 rpm produced minimal laminar interfacial fluid overlap; while 100 rpm created turbulent flow and subsequent mixing of the two phases in the case of Designs B and C. Notably, 75 rpm ideally resulted in lateral fluid dilatation of the organic phase around the paddle and shaft in Design B (i.e., absence of vortex creation but induction of situation similar to the Weissenberg effect). Similar behavior was also observed in the case of Design C. This fluid flow pattern was postulated as desirable in terms of "pulling out" drug molecules from the aqueous phase as a result of greater surface exposure to the organic phase. Furthermore, this pattern in Designs B and C appeared to also enhance laminar fluid overlap at the interface

Table 1. Statistical Analysis of Dissolution Data Derived from Designs C (75 rpm) and D (100 rpm)

Data comparisons made from[a]	Statistical factors		
	f_1[b]	f_2[c]	t-test[d]
Dissolution vessel 1	11.79	54.25	$p > 0.05$
Dissolution vessel 2	12.75	54.05	$p > 0.05$
Dissolution vessel 3	9.87	57.99	$p > 0.05$
Mean	10.68	56.18	$p > 0.05$

[a] Reference and test products are identical. Data obtained from positioning paddle in aqueous phase at 100 rpm was reference, while data generated from position of paddle at organic/aqueous interface was test. A one-to-one comparison of individual data was individually performed to ensure uniformity and correctness of data interpretation, prior to using the mean values.
[b] Difference factor.
[c] Similarity factor.
[d] No statistical significance was noted (i.e., $p > 0.05$).

(greater boundary layer). Figure 2b depicts negligible drug transfer when the paddle is positioned at the air/organic interface (5.26% in 6 hours), despite the induction of lateral fluid dilatation (Schematic I, Design B). Induction of similar fluid dilatation at the organic/aqueous interface (Schematic I, Design C), proved to be effective in encouraging rapid dissolution of the capsule shell and subsequent self-emulsification of the formulation. This essentially enabled complete drug transfer in 6 hours (96.84%) (Figure 2c). Manipulation of the hydrodynamic conditions in the case of Design D proved crucial in determining the rate of drug transfer and reproducibility of such a process (Figures 2d at 75 rpm and 2e at 100 rpm). At 75 rpm marked tailing is evident in the transfer profile (Figure 2d), which may be attributed to possible aggregation of precipitated drug particles as a result of inadequate mixing. As a statistical marker of the reproducibility in drug transfer rates, comparison of the coefficient of variation (%CV) produced a maximum of 5.55% in the case of 75 rpm (Figure 2d) as opposed to 1.37% at 100 rpm (Figure 2e).

Complete dissolution and reproducible drug transfer profiles were only associated with Designs C and D at 75 and 100 rpm respectively. In order to validate the close resemblance of the drug transfer profiles, data was evaluated using the "f_1 and f_2" fit factor pairwise approach i.e. the respective difference and similarity factors. Based on the similarity factor, f_2 (mean eq; 56.18, Table 1), it is apparent that the two drug release/dissolution profiles are similar (i.e. f_2 between 50–100). However, based on the reproducibility of the transfer process (lower standard deviation and %CV), dissolution Design D appears to be the method of choice for lipid-filled products.

CONCLUSIONS

In order to ascertain that drug is completely delivered from its formulation over an appropriate time period and is able to reach and cross the gut wall, an aqueous environment similar to the gut luminal fluid and a sink resembling the lipoidal nature of the gastrointestinal mucosa becomes a necessary condition for the development of a prognostic *in vitro* test method. In this work, the two-phase media system together with appropriate mixing procedure and dosage form positioning within the standard dissolution vessel has illustrated the importance of the relevant test conditions for *in vitro* dissolution performance of lipid-filled capsule products. When dealing with viscous and oleaginous systems, the limiting effects of mesh openings, absence of appropriate hydrodynamics and encountered difficulties such as flotation associated with the USP 23 rotating basket and paddle methods can be prevented by adopting the ring/ mesh assembly fitted with a 16-mesh screen. Of the various dissolution designs described in this paper, Design D (as shown in Schematic I) provides excellent conditions for the evaluation of lipid-filled capsule products. Whilst some pharmaceutical scientists may advocate an additional consideration to the inclusion of gastric lipases, pancreatic lipases and co-lipases as well as bile salts in the aqueous phase of the dissolution medium, our experience shows that the present dissolution design does not require such modifications. The results of this work in relation to the routine difficulties experienced in *in vitro* dissolution studies of lipid-filled capsules suggest that a consideration of this or similar methods may be of value to formulation scientists engaged in research and product development. Such efforts will complement one of the underlying objectives of the USP in its aim to develop rational alternative dissolution methods.

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