Comparative In Vitro Dissolution Studies between Different Types of Acetaminophen Suppository Dosage Forms Using a Novel Modified Basket Method

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ABSTRACT

Objective: Diverse studies have demonstrated that no single method of dissolution testing is suitable for different types of suppository dosage forms by either current USP and Ph.Eur methodologies. The objective of the research work was to overcome the methodological problem and limitations by a novel modified basket method.

Materials and Methods: USP dissolution apparatus-I was modified by placing a dialysis membrane of specific molecular weight cut off to prevent any kind of clogging which may give irreproducible, inconsistent results. Physical properties like melting range, liquefaction and solidification time, disintegration time and mechanical strength were studied as per Ph.Eur III.

Results: The amount of drug released from water soluble bases were fast with 94.5 ± 1.8 %, 96.8 ± 2.2% during 1h and slow with lipophilic bases with 91.6±2.3% and 92.7±3.7% respectively during 6h. There was no significant difference between the dissolution profiles by flow through cell and modified basket method (P>0.005). Average lag time was 8.2, 9.8 min for water soluble suppositories and 14.4 and 11.8 min for lipophilic suppositories respectively. Release kinetics showed first order release rate for water miscible suppositories and zero order release profiles for lipophilic suppositories till 3 h and first order release after this time interval. Disintegration time of water soluble suppositories was 12-15 min but lipophilic suppositories demonstrated an extended disintegration time of 20-22 min Determination of plastic viscosity versus temperature indicated higher yield value for fatty bases compared to water soluble bases.

Conclusion: Based on the data, it was concluded that proposed method could be used as a substitute for flow through cell of Ph.Eur. We further hypothesized that change in viscoelastic behavior due to the variation in temperature and aging may be responsible for the differences in the dissolution behavior between different suppositories bases.

Keywords: Suppository, paracetamol, modified basket method, plastic viscosity, dissolution test
INTRODUCTION

Dissolution is an important tool for controlling batch-to-batch uniformity and to understand drug release mechanisms in *in vivo* environment. Over the past two decades, efforts towards harmonization between pharmacopoeias have led to more reliable and uniform method for *in vitro* dissolution of conventional drug products.

Suppositories are usually prescribed for systemic and local action in pediatric population and patients who have dysphagia due to varied reasons. Rectal and vaginal delivery provides immediate drug release but very erratic absorption. Absorption is always preceded by dissolution of the drug in the body fluid at application site and this technique is usually carried out *in vitro*. Currently limited *in vitro-* *in vivo* correlation data are available with suppositories dosage forms compared to solid dosage forms\(^1\)\(^-\)\(^3\). Dissolution technique development would be beneficial during the initial formulation development, physical stability as well as routine quality control.

Dissolution testing of suppositories is an appropriate tool to test for polymorphic transition and solidification of drugs and suppository bases. Unlike conventional solid dosage forms, there are few official dissolution methods for suppositories. Several methods have been used for the investigation of *in vitro* dissolution rate from suppositories until lately European Pharmacopoeia (Ph.Eur.III) and British Pharmacopoeia (BP) have brought in membrane-less dissolution apparatus for suppositories. Prominent methods used for *in vitro* dissolution testing are: basket, membrane diffusion, dialysis and flow-through apparatus\(^4\)\(^-\)\(^6\). Existence of diffusion layer or membrane could cause variation among those techniques mentioned above.

*In vitro* drug release from suppositories due to melting, deformation and dispersion in the dissolution medium have always being problematic. Various studies have demonstrated that any single dissolution method is unsuitable for all types and formulations of suppositories. For example, the paddle method showed fatty base floating immediately to the surface of the medium. With the basket method, fat droplets that were dispersed into the fluid might sometimes block the mesh. The flow-through method was much slower, probably due of the slowed spreading of the melted base in the dissolution apparatus. In addition, the contact surface area with the dissolution medium is smaller than that can be achieved with either the paddle or the basket method. Literature review have revealed many methodological problems regarding the dissolution in Ph.Eur apparatus like re-warming the medium, precipitation and blocking due to suppository bases etc\(^7\)\(^-\)\(^8\). The major disadvantages of current USP and Ph.Eur are the precipitation and clogging of water and lipid extractable constituents of a suppository base in basket mesh and lag time. This might be due to the different pharmaceutical factors including the types of bases and manufacturing techniques used in the preparation of the different generic products\(^9\)\(^-\)\(^14\).

It is evident from the literature review that presently no single method of dissolution testing is suitable for different types and formulations of suppositories\(^15\)\(^-\)\(^16\). The objective of this proposed research work is to overcome these methodological problems and limitations by using USP basket apparatus having a dialysis membrane with a specific molecular weight cut off. The dialysis membrane placed inside the basket will prevent any kind of clogging which may give unacceptable, irreproducible results since membrane allows only dissolved drug to dialyze out of the basket.

The investigation will also focus on kinetics of dissolution behavior among different types of acetaminophen suppository dosage forms and other pharmaceutical parameters like disintegration time, particle size, drop point, hardness, viscosity and concentration of excipients.
MATERIALS AND METHODS

Materials
Commercially available Paracetamol suppositories dosage forms of different dose strength and bases from four different manufacturers (A, B, C, D) were explored. Details were given in Table 1. Minimum 6 batches of each preparation were evaluated. Sigma membrane used has a molecular weight cut off 3000-12000 Dalton.

In vitro release study

USP dissolution apparatus-I (rotating basket; Lab India DS-8000)) was modified by enclosing both upper and lower side the basket with sigma membrane (molecular weight cut off 12000 Dalton). Both upper and lower segment inside the basket were covered by sigma membrane. This ensures that dissolution process through modified basket will take place only by dialysis process. Schematic diagram of the modified basket method was given in Figure 1. All dialyzing membrane was soaked in phosphate buffers pH 8.0 overnight before use. Dissolution test was performed using 900ml of medium, rotational speed at 50 rpm while temperature was set at 37±0.5°C (Figure 1).

We have compared the proposed dissolution method with a method recommended by Ph.Eur.III. The experimental conditions established for the dissolution method was phosphate buffer pH 8.0, flow rate 150 ml/min and temperature set at 37± 0.5°C. Aliquots of the dissolution medium were collected every half an hour up to 6 h and the amount of released paracetamol was analyzed.

Analysis of paracetamol

Samples were diluted suitably and analyzed spectrophotometrically (T80-PG Instruments, UK) at analytical wavelength of 244 nm (A1%=715) at λmax 244 nm. The content uniformity test was done for all suppositories at the dose 250 mg or 325 mg.

Physical properties of suppositories

Melting range, melting and solidification time, disintegration time, mechanical strength and liquefaction time were studied as per the procedure described in Ph.Eur III (10).

Particle size of the paracetamol particles and droplets of the suppository base was quantified by microscopic observation (light microscope; Olympus, Japan) after melting and subsequent cooling on a glass slide.
The liquefaction test used was Krowczynski’s method, which determines the time needed for a suppository to liquefy under pressures similar to those found in the rectum (approximately 30 g) in the presence of dissolution buffer pH 8 at 37±0.5°C (17-18).

A suppository penetration test was used to measure the temperature at which the suppository becomes sufficiently soft for a penetrating rod to bore across its length. Mechanical/crushing strength was assessed by a laboratory set up where addition of increasing weights were placed on it until it loses its structure. Brittle and elastic nature of the suppositories can be evaluated by this method.

**RESULTS**

Dissolution profiles of two types of suppositories i.e., water soluble and lipid soluble from four different types of suppositories were evaluated. Figure 2 presents the release profiles of suppositories A and B containing 250 of paracetamol from modified basket. The faster release was observed for suppositories A and B with modified basket method, however slower release of paracetamol was observed for suppositories C and D (Figure 3). Maximum amount of drug released from suppository A and B was 94.5 % and 96.8% respectively during 1 hour. The amount released from suppository C and D was 91.6 and 92.7% during 6 hours. The plateauing trend showed between 5-6 hours and none of the suppositories displayed cent present drug release. Average lag time was found to be 8.2 minutes and 9.8 minutes for suppositories A and B and 14.4 and 11.8 minutes for suppositories C and D respectively. Drug release from hydrophilic suppositories of A and B showed similar trend in the dissolution curve. Both have demonstrated initial slower release (25-30.6% in 30 min) followed by faster release. In contrast, the suppositories C and D exhibited much slower release profile till 3 hours and much faster release profile until 6 hours. Drug release from C and D showed significant differences between the batches (ANOVA test, P>0.05). Surprisingly similar trend of dissolution profiles was observed between suppositories A, B, C and D by flow through cell (ANOVA test, P>0.05).
Figure 2: Cumulative amount of paracetamol released from individual suppositories A and B by modified basket method.

![Modified Basket Method](image)

Figure 3: Cumulative amount of paracetamol released from individual suppositories A and B by flow through cell (Ph.Eur)

Mean release rate constant from different batches of suppositories C and D demonstrated that the release rate is dose independent (ANOVA test, p<0.05). We found no influence of temperature range between 36.5-38°C for suppositories A and B. Significant changes in release profile between different batches of suppositories C and D above 38.5°C.

All different types of suppositories complied with the USP and Ph.Eu.III for content uniformity tests (±15%). The paracetamol content in suppositories A and C was in the range of ± 6.75% of the label claim, while suppositories C and D were in the range of ± 4.5%.

Liquefaction time, disintegration time, drop point and particle size was given in Table 2. The suppositories A and B showed liquefaction time between 8-10 minutes while suppositories C and D demonstrated softening time between 12-15 minutes. Drop point experiments revealed that hydrophilic suppositories (A and B) melted, dispersed and dissolved between temperature 35-36.5°C while lipophilic suppositories B and D only deformed, with incomplete melting between 36.5-37.5°C. Disintegration experiment showed that all water soluble suppositories disintegrates around 12-15 minutes but suppository B and D demonstrated extended disintegration time of 20-22 minutes.

Particle size analysis of all suppositories batches by optical microscopy showed size range between 60-90 µm. There was no significant difference between different batches of suppositories (ANOVA test, p<0.05).

We found that extractable components in the dissolution medium affect the actual release rate calculation. Therefore, separate linear equation was used for suppositories A and B containing water soluble extract in pH 8 phosphate buffer was $Y = 0.0629c - 0.001$ (where $Y$ is absorbance, $c$ concentration in µg/mL and $r^2=0.9998$). The calibration linear equation used for suppositories C and D was $Y = 0.0632c - 0.001$ ($r^2=0.9994$) in phosphate buffer pH.8 in presence of lipid soluble extracts.
DISCUSSION

All tested suppositories were purchased from reputed manufacturers. Two of the suppositories (A and B) contain water soluble bases and remaining two (C and D) contain fatty bases. Details of the compositions of various suppositories were given in Table 1. Significant difference in dissolution profiles was observed for hydrophilic as well as lipophilic suppositories (ANOVA test, p<0.05). This might be due to two probable reasons either due to the faster solubility of bases compared to the drug or rheological behavior among different types of suppositories. Although according to Gjellan and Graffener, the melted fat may leave the gap between the basket and holder, but in modified basket there was no such type of leakage observed. The dialyzing membrane covering the entire surface area of the basket allows only dissolved drug to dialyze out of the basket.

Table 1: Types of suppositories

<table>
<thead>
<tr>
<th>Suppository</th>
<th>Paracetamol dose (mg)</th>
<th>Types of bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>250 mg</td>
<td>water soluble bases</td>
</tr>
<tr>
<td>B</td>
<td>250 mg</td>
<td>water soluble bases</td>
</tr>
<tr>
<td>C</td>
<td>250 mg</td>
<td>Fatty base</td>
</tr>
<tr>
<td>D</td>
<td>250 mg</td>
<td>Fatty base</td>
</tr>
</tbody>
</table>

Hydrophilic suppositories melt, disperse and dissolve in the dissolution medium while lipophilic bases deform or solidify in the dissolution buffer. This was evident from the liquefaction time, melting and solidification time and disintegration time as shown in Table 2. Mechanical/crushing strength further supports this hypothesis since hydrophilic have elastic and lipophilic suppositories have brittle structure. As shown in the Figure 2 and Figure 4, faster dissolution profiles were observed for suppositories A and B and slower dissolution profiles for C and D by modified basket method. Surprisingly similar trend of dissolution profiles were observed between suppositories A, B, C and D by flow through cell (ANOVA test, P>0.05) (Figure 3 and Figure 5). Liquefaction testing provides an insight into the behavior of suppositories at 37°C, a shorter liquefaction time for hydrophilic suppositories and longer liquefaction time for lipophilic suppositories was observed (Table 2). This showed that, a correlation exists between liquefaction time and release of drug from the suppository. Modification of the structure during temperature variation in the vessel might be responsible for difference among different batches of same suppository (data not shown). Since the drug is dissolved or dispersed in the suppository bases, changes in the polymorphic transition of base and drug might be affecting drug release.
Figure 4: Cumulative amount of paracetamol released from individual suppositories C and D by modified basket method

Figure 5: Cumulative amount of paracetamol released from individual suppositories C and D by flow through cell (Ph.Eur)
Table 2: Physical characteristics of the investigated suppositories

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting range (°C)</td>
<td>35.5-36.5</td>
<td>35.3-36.2</td>
<td>36.1-36.5</td>
<td>36.1-37.1</td>
</tr>
<tr>
<td>Melting and solidification time (min)</td>
<td>4-7</td>
<td>4.7-8</td>
<td>6-12</td>
<td>7-14</td>
</tr>
<tr>
<td>Disintegration time (min)</td>
<td>9.4</td>
<td>12.1</td>
<td>17.3</td>
<td>20.5</td>
</tr>
<tr>
<td>Mechanical/Crushing strength (kg/cm²)</td>
<td>1.7</td>
<td>1.9</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Liquefaction time</td>
<td>5.5</td>
<td>6.4</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Penetration test</td>
<td>2.5±0.3</td>
<td>2.7±0.7</td>
<td>5.4±0.9</td>
<td>6.2±0.2</td>
</tr>
<tr>
<td>Particle size (µm) d₉₀</td>
<td>10-30</td>
<td>20-60</td>
<td>20-60</td>
<td>10-50</td>
</tr>
</tbody>
</table>

Similar dissolution release profiles were demonstrated with flow through apparatus (Figure 3 and Figure 5) and modified basket method ([Figure 2 and Figure 4) (ANOVA test; P>0.05). There was no clogging or blocking of suppository extracts on basket mesh. This might be due the dialysis membrane which covers the entire surface area of the basket, where basket act as a container for melted or deformed base. In the proposed method, membrane act as a barrier to prevent clogging of the basket mesh as well as a more effective method to directly assay the drug. Probable drug partitioning between the melted base and the receptor fluid was avoided by maintaining proper sink conditions¹⁹-²⁰.

Suppositories C and D melted between 36.5 and -37.5°C and disintegrated between 20-22 minutes. Although temperature is not specified in the Ph.Eur III, 37±0.5°C should be maintained for in vitro in vivo correlation as given in literature¹. Although similar dissolution profiles obtained with flow through cells and modified basket method, significant difference observed between different batches of suppositories C and D. The requirement for suppositories is more general; ‘heat the dissolution medium to an appropriate temperature taking the melting point into considerations’. Temperature above 37.5°C had influenced strongly on melting, dispersion and dissolution of lipophilic suppositories. Lag time was approximately 30 min for fatty base suppositories and indicates that this is the time required for diffusion, percolation and disintegration of suppository bases. Lag time and disintegration was obviously longer for fatty base suppositories compared to water soluble bases.

The methodology of dissolution test should be robust enough to discriminate between different types of suppository formulations. This objective was achieved in the present study under the experimental conditions with different types of suppositories. There was no significant difference between the dissolution profiles with flow through cells and modified basket. The proposed method had avoided the many disadvantages like variability within the cell due to spreading agent in the formulation, reduction of contact area, interference due to extractable components from the bases etc. Release kinetics showed that first order release rate for suppositories A and B and Zero order release profiles for lipophilic suppositories till 3 hours and first order release after this time interval.
None of suppositories demonstrated 100% release either by flow through method or proposed basket method. This may be due to the adherence of drug particles to the discrete base, vessel wall, or dissolution assembly results in reproducibility and recovery issues. Dialysis membrane extracted with buffer ruled out any possibility of binding of drug. Content uniformity tests with all the suppositories complied Ph.Eur specifications.

We hypothesized that temperature on the viscoelastic behavior of semisolid suppositories might be responsible for the variation in dissolution profile. There are very few studies on the rheological behavior of the suppository mass in the literature on suppositories regardless of the fact that viscosity can influence the release and absorption of the drugs from these semisolid bases. Mild variation in temperature can change the consistency of the base from “softness” to “solidification” as demonstrated by melting and solidification temperature versus time (Table 2).

Determination of plastic viscosity versus temperature indicated higher yield value for fatty bases compared to water soluble bases (Figure 6. and Figure 7). Probably higher water absorbing capacity of hydrophilic bases as indicated by the higher water number may be responsible faster diffusion, percolation, dispersion and dissolution of compared to lipophilic bases. Further thixotropic index value versus temperature verified the same relationship that differentiates these two bases (Data not shown). The difference in viscoelastic nature might be due to waxy brittle gel matrix of fatty base which is broken down compared to elastic water soluble bases.

Figure 6: Temperature coefficient of plastic viscosity of water soluble bases (glycerol-gelatin) of suppositories A and water insoluble bases (fatty bases) of suppositories C.
Figure 7: Temperature coefficient of plastic viscosity of water soluble bases (glycerol-gelatin) of suppositories B and water insoluble bases (fatty bases) of suppositories D.

Particle size of the suspended drug influences the release rate *in vivo* and *in vitro*. Faster release of paracetamol from formulation B than A may also result from larger drug particles in the suppository (Table 2). Such correlation was already expressed in literature by Janicki et al.²

**CONCLUSION**

The experiments revealed some methodological problems regarding the dissolution test with current USP and Ph.Eur. Based on the data and curves, we recommend that proposed modified basket method could be used instead of flow through cell by Ph.Eur. Change in viscoelastic behavior of different types of bases due to the variation in temperature and aging may be responsible for the differences in the dissolution behavior between different types of suppositories and batches. Viscoelastic testing with rotational viscometer may be considered as a valuable tool in quality control testing of suppositories.

**REFERENCES**