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Research paper

Evaluation of paracetamol suppositories by a pharmacopoeial dissolution test – comments on methodology

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Abstract

Ph.Eur. and BP have introduced a dissolution apparatus for suppositories. Suitability of the apparatus for quality control of paracetamol suppositories was evaluated and the effect of experimental conditions on dissolution profiles was studied. Paracetamol suppositories containing 80–500 mg of the drug, on fatty base, were obtained from four manufacturers (A, B, C, D). The diffusion cell was modified by incorporation of an in-built thermoprobe and large difference (up to 1.7°C) between temperature in the water-bath and in the dissolution chamber was observed. This effect was avoided by increasing the length of tubing immersed in the thermostat at the inlet of the cell. The most reproducible results were observed for A and C suppositories, however from suppository C the total dose of paracetamol was released after 3.5–4.5 h while the release from suppository A was slow with only 40–87% of the total dose liberated during 6 h. Suppositories B did not melt at 37°C and less than 5% of the drug was released. Fast release was observed after melting when the temperature was elevated to 39.5°C. The results demonstrate clearly that essentially complete melting of a suppository in the dissolution chamber is required for an appropriate dissolution of paracetamol in vitro. Disintegration time, softening time, drop point and particle size of the suspended drug were measured and the relevance of these parameters for dissolution behaviour of the preparations was discussed.

Keywords: Suppository; Paracetamol; Dissolution test; Melting temperature

1. Introduction

Suppository dosage form is not popular in USA or Great Britain but extensively employed in many other countries. Suppositories are indicated for systemic action in paediatric patients and in patients who cannot take or tolerate oral medication due to variety of reasons. Rectal delivery ensures relatively fast but also very unpredictable absorption [1–3]. Absorption must be always proceeded by dissolution of a drug in a fluid available in site of application and this process is usually tested in vitro. Suppositories have not been investigated to as great an extent as the oral dosage forms regarding correlation between in vitro dissolution and in vivo absorption. Despite some studies showing good correlation [4–8] up to now dissolution test for suppositories can not be implemented in the assessment of bioavailability. The continued interest in suppositories has led to recognition that dissolution test would be helpful during the initial phase of dosage form design as well as for routine control. In addition, such test would provide valuable information on physical stability of suppositories [3,9].

Testing for the rate of in vitro release of drugs from suppositories has always posed a difficult problem, owing to melting, deformation and dispersion in the dissolution medium. Several techniques have been employed for study of in vitro drug release from suppositories but none of them, until recently, was recommended as a standard method. The most popular are: beaker method, basket method, membrane diffusion method, dialysis method, continuous flow method or flow-through bead-bed apparatus [3,9]. Use of membranes or various techniques to eliminate a boundary diffusion layer make difference among those methods.

Only recently European Pharmacopoeia III (Ph.Eur. III) and British Pharmacopoeia 1998 have introduced a dissolution apparatus for suppositories (Fig. 1). This is a modified apparatus described in a literature [3] and it is recommended especially regarding reproducibility of the results. It is a membrane-free system consisting of two adjacent cham-

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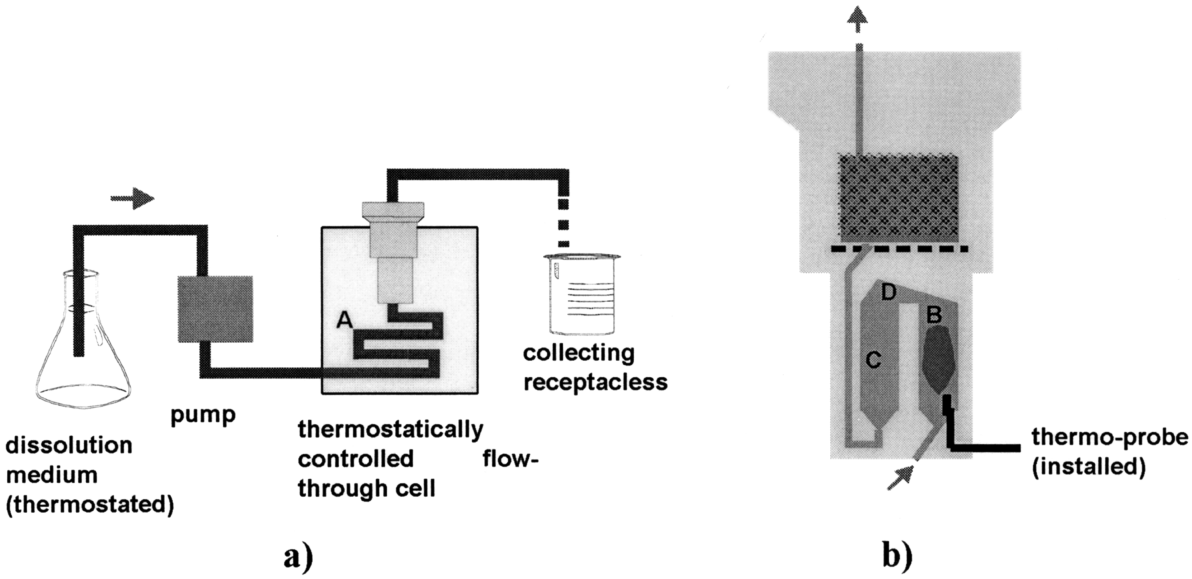

Fig. 1. Ph.Eur. III flow-through apparatus (a) and dissolution cell (b) for testing drug release from suppositories: A, a coil of tubing for re-warming of dissolution medium; B, dissolution chamber; C, adjacent chamber; D, a niche retaining melted lipids. A thermoprobe was installed in the dissolution chamber as indicated.

bers; in one of them (chamber B in Fig. 1b) a suppository is placed. Especially designed niche (D in Fig. 1b) between two chambers retains melted suppository mass preventing further expansion and restricting area exposed to the dissolution medium.

Majority of dissolution studies using other techniques concerns fast release formulations but according to the Ph.Eur. general monograph dissolution test is required only for preparations intended for modified release or prolonged local actions. No data is available on use of the pharmacopoeial apparatus for non-modified suppositories although it is more advanced in design than other systems. The aim of our study was to evaluate suitability of the Ph.Eur. dissolution system for testing suppositories intended for non-modified drug release. Suppositories containing 125–500 mg of paracetamol were investigated. They were marketed products of four different manufacturers and comparison of the release profiles was also a goal of the study.

Ph.Eur. provides only limited and very general information concerning test conditions. Such parameters like type of dissolution medium, flow-rate, duration of the test, sampling time or temperature are expected to be adjusted for each preparation individually. In our study standard experimental conditions in respect to temperature (37°C) and dissolution medium (phosphate buffer pH 7.4) were employed. Other parameters were also adjusted and suitability of the experimental parameters was evaluated. A thermoprobe was installed in the flow-through chamber to establish appropriate test conditions and identify some methodological problems.

Disintegration time and softening time are two other pharmacopoeial tests prescribed for suppositories. They are performed as separate tests but in our study correlation between dissolution rates and these two values was also discussed. Moreover two other parameters, namely drop point and particle size of the suspended drug were measured and taken into consideration.

2. Materials and methods

2.1. Materials

Commercially available paracetamol suppositories from four different manufacturers (A, B, C and D) were investigated. Doses of paracetamol and information on the vehicle composition are given in Table 1. At least two batches of each preparation were tested. The Ph.Eur. flow-through cells were purchased from Pharma Test (Hainburg, Germany). The cells were modified by installing of an in-built thermoprobe (Fig. 1). Silastic tubing 0.5 mm in diameter were employed for pumping dissolution medium through the cells.

Table 1
Suppositories under investigation

Suppository	Paracetamol dose (mg)	Excipients
A	125, 250, 500	Soya-bean lecithin, Witepsol, Estaram
B	80, 150, 300	Fatty base[a]
C	125, 250, 500	Lecithin, Witepsol
D	50, 125, 250, 500	Fatty base[a]

[a] The composition is not disclosed by a manufacturer.

2.2. In vitro release study

The test was performed following general recommendations given in Ph.Eur. III. The following experimental conditions were set up: 0.2 M phosphate buffer pH 7.4 was used as dissolution medium, flow rate was 100 ml/min and thermostat temperature was 37 ± 0.5°C. In order to study flexibility of the system the flow rate was changed in the range 100–400 ml/h. Fractions of the dissolution medium were collected every 30 min up to 6 h and the amount of released paracetamol was analysed.

2.3. Analysis of paracetamol

Concentration of paracetamol in the collected fractions of dissolution medium was analysed spectrophotometrically (Ultrospec III spectrophotometer, Pharmacia LKB) at the analytical wavelength 245 nm. Standard solutions for calibration curve were prepared in water or in an aqueous extract from a standard suppository base composed of 2.0 g of Witepsol (Contensio, Witten, Germany) and 0.2 g egg lecithin (Lipoid E80; Lipoid, Ludvigshafen, Germany). The melted base was extracted with 100 ml of water.

The uniformity of content test was performed for all studied preparations at the dose 250 or 300 mg. Paracetamol was extracted with hexane and analysed by HPLC according to the method described in USP 24 for Acetaminophen suppositories.

2.4. Physical properties of suppositories

Disintegration time, softening time and drop point were studied using procedures and apparatus described in Ph.Eur. III. Size of the suspended paracetamol particles was estimated by microscopic observation (light microscope; PZO Studar, Poland) of the suppository mass after melting it on a slide glass in a thin layer and subsequent cooling.

3. Results

The initial length of the tubing for pumping dissolution medium from a thermostated reservoir to the thermostated flow-through cell was approximately 150 cm. The length exposed to the air was approximately 40 cm and the length immersed in water bath at the inlet to the diffusion cell was approximately 30 cm. Difference between the temperature recorded by a thermoprobe in the dissolution chamber and temperature in thermostat (exactly 37.0°C) was observed: approximately 0.4°C at the flow rate 100 ml/h and up to 1.7°C at 400 ml/h. Such effect was eliminated, however, when the tubing were immersed in the thermostated bath at length at least 100 cm before entering the cell (a coil A in Fig. 1a).

Fig. 2 presents the release profiles of paracetamol from four different suppositories containing 250 or 300 mg of paracetamol. The fastest release was observed for suppositories C and D, however release of paracetamol from suppository D was non-reproducible. Some suppositories D, from the same batch, did not melt and in those cases not more than 5% of the drug was released during 6 h. That was observed for each dose studied. The mean amount of paracetamol released from suppository C during 6 h was 94.5% (the range 80–111% for all doses) and the plateauing portion of the curve presented in Fig. 2c and Fig. 3 demonstrate that the total amount was released after 4.5–5.5 h. In contrast, the profiles observed for suppository A do not show plateau – the release rate is constant and the amount of paracetamol released during 6 h is in the range 40–87% for all investigated doses. Only 5% or less of the total drug dose was liberated from all suppositories B that did not melt or deformed under experimental conditions. Changes in temperature in the range 36.5–38.5°C did not influence dissolution profiles. The suppository B, however, melted in temperature above 39°C and released 70–85% of the total dose within 2.5 h.

The mean release profiles for all studied doses of suppositories A and C are presented in Fig. 3. It is not possible to present statistically proven evidence that the release rate is dose-dependent (ANOVA test, $P < 0.05$), although, when mean profiles are compared faster release was observed for the smallest doses i.e. 125 mg. Lag time 30 min or longer was observed for all preparations.

All studied suppositories complied with the Ph.Eur. uniformity of content limits (±15%). The paracetamol content in suppositories C was in the range ±12% of the declared dose, while in all other preparations was in the range maximum ±5%.

The suppositories A and C differ from two other preparations (Student's t-test, $P < 0.05$) by shorter disintegration and softening times what is shown in Table 2. Moreover, these suppositories melted in temperature 37°C in a disintegration test, while suppositories B and D only deformed, without a complete melting. Drop point measurements revealed that all suppositories but suppository B melted at 36°C or below.

Drug particle analysis showed some differences between the preparations what is presented in Table 2. The largest particles were observed in suppository C where more than 10% of all particles were in sizes 120–170 μm. On the other hand no particles larger than 65 μm were detected in suppository B and very rare particles larger than 60 μm were detected in suppository A.

All presented release profiles were calculated using calibration curve obtained for the aqueous standard solutions. The linear regression equation was $A = 0.0634c - 0.001$ (where A is absorbance and c concentration in μg/ml). In the presence of water extractable components of a standard base higher absorbances were measured and the equation was $A = 0.0638c + 0.047$. Due to such effect the real concentrations of paracetamol in the sampled dissolution fluid may be lower by 5–10% than calculated.

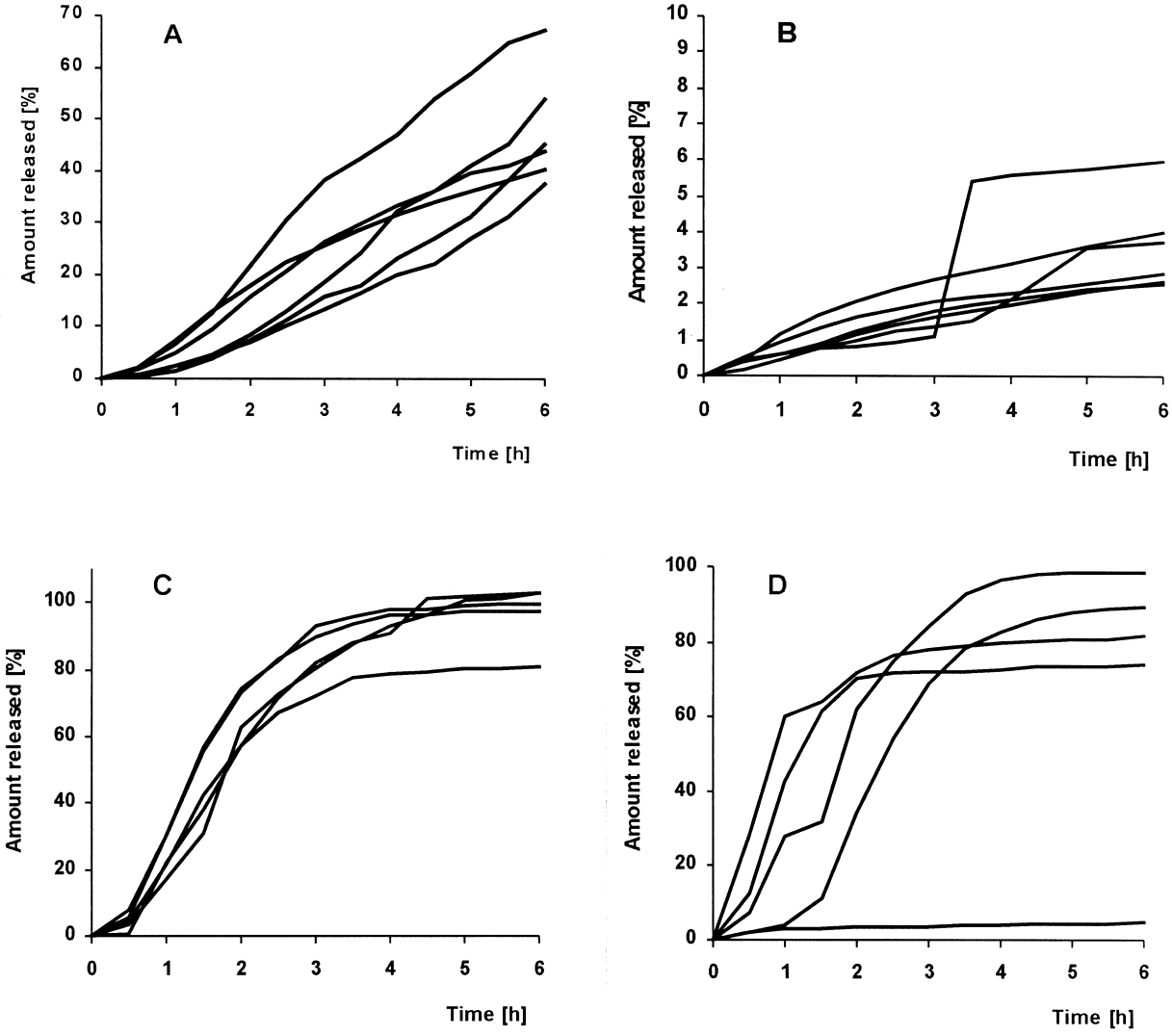

Fig. 2. Cumulative amounts of paracetamol released from individual suppositories A, C, D (total dose 250 mg) and B (total dose 300 mg).

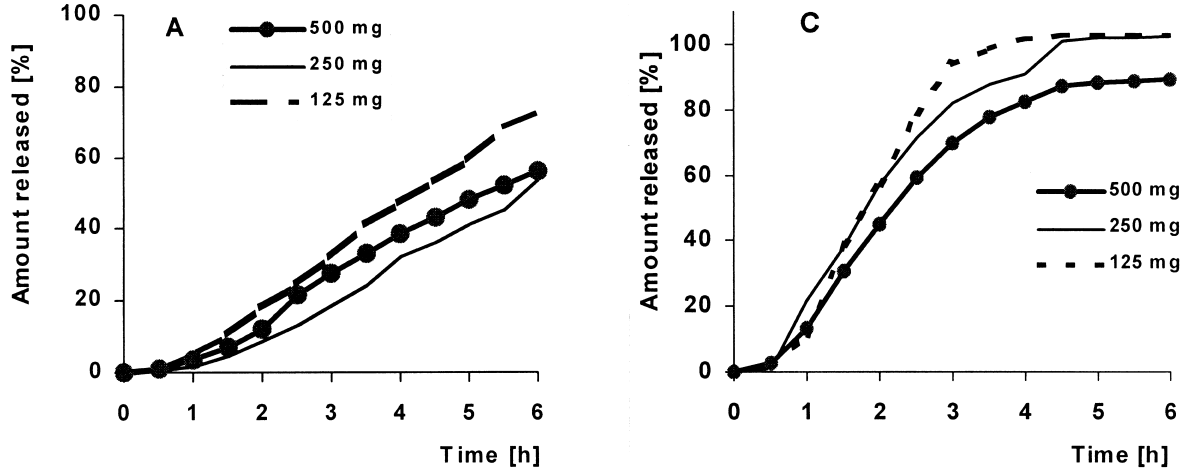

Fig. 3. The effect of paracetamol content on the release from suppositories A and C (mean values).

Table 2
Physical characteristics of the investigated suppositories

	A	B	C	D
Softening time (min)	4–7	5–12	4–5.5	6–13
Disintegration time (min)	11 (melting)	25 (softening)	16 (melting)	19 (softening)
Drop point (°C)	35.0–36.0	35.5–37.0	35.5–36.0	35.5–36.0
Particle size (μm)				
80%	5–30	10–50	20–60	5–50
Maximum[a]	60 (120)	65	170 (250)	100

[a] In parentheses size of very rare largest particles is given.

4. Discussion

Four tested preparations are products of different manufacturers – suppositories A and B are produced by world-recognised companies and suppositories C and D by local Polish manufacturers. All suppositories are prepared on fatty bases (Table 1). Dissolution profiles shown in Figs. 2 and 3 indicate that significant differences were observed for commercial paracetamol suppositories. Type of the base is the most probable reason for such difference. The results demonstrate clearly that essentially complete melting of a suppository in the dissolution chamber is required for an appropriate dissolution of paracetamol in vitro. Such conclusion was already drawn by Bergren et al. [10] who studied dissolution from vaginal suppositories using USP basket method. When this step does not occur, drug release is extremely slow as it was observed for suppository B, and in some cases for suppository D. Lag time was at least 30 min and indicates that a certain time is required for melting, which approximately equals disintegration time. Suppository B melted in temperature above 39°C and only then fast dissolution was possible. Such observation brings a question about a temperature suitable for the test. Description of the dissolution method in Ph.Eur. III do not contain precise temperature of the medium although for other drug formulations 37°C is required. The requirement for suppositories is more general: 'Heat the dissolution medium to an appropriate temperature taking the melting point into consideration'. According to such statement suppositories B could be tested in temperature 39.5°C but this is not an acceptable value if in vitro–in vivo correlation is considered.

The design of dissolution test should guarantee that any difference existing between formulations could be demonstrated. This aim is achieved in the present study since under the established experimental conditions two different dissolution profiles were obtained for suppositories A and C although both preparations melted completely. Release of paracetamol from suppository C is completed within 3.5–4.5 h, but suppository A behaves like an extended-release formulation with near zero-order release rate. However, the manufacturer does not indicate that such modification of the release rate is intended. This example should also give a warning that the dissolution test may not be a reliable tool to demonstrate extended release of a drug from suppository and in vivo studies are always necessary.

The most reproducible dissolution rates were obtained for suppositories A and C. However relatively large deviations were also noted for these preparations. Moreover, in the case of suppositories C not in all units 100% release was observed (Fig. 2c). There is no evidence that the method lacks precision since drug release from completely melted base may be erroneous only when a flow rate or temperature are not constant and that was not a case. Thus poor uniformity of drug content (in the case of suppository C, although still in the permitted limits) or non-uniform drug particles might be rather a reason for unsatisfactory reproducibility and recover.

The investigated suppositories complied with pharmacopoeial requirements regarding disintegration time (Ph.Eur. – 30 min) and softening time (Polish Pharmacopoeia – 15 min). Moreover shorter disintegration time with accompanying melting correlated with shorter softening time (Table 2). It has to be noted that the compliance with pharmacopoeial requirements regarding disintegration or softening times does not guarantee melting and drug release during dissolution test. Further studies should be carried on in order to investigate whether it has to be required that suppositories on fatty base should melt, not only soften, in temperature 37°C under conditions of dissolution and disintegration tests.

More information about melting behaviour may be obtained during disintegration test rather than by drop point measurement. The temperature required for melting suppositories B and D during disintegration or dissolution test was higher than 37°C what does not agree with the measured drop point. The need to remelt the mass in order to place it in a cup of the apparatus for drop point measurement may be a reason of such discrepancy.

Particle size of the suspended drug influences the release rate in vivo and in vitro [3]. Faster release of paracetamol from formulation C than A may also result from larger drug particles in suppository C (Table 2). Such correlation was already reported by some authors [3].

Summarising the above observations one has to conclude that in vitro–in vivo correlation may not exist since the observed dissolution profiles show lag time longer than 30 min and slow release is maintained even during 6 h,

although the products are intended as fast acting preparations.

The experiments revealed some methodological problems regarding the dissolution test. It has been demonstrated that temperature in the dissolution chamber may deviate from temperature of the water bath where dissolution cell is located. A care must be taken to re-warm medium by immersing the tubing at an appropriate length in thermostat at the inlet to a dissolution chamber as indicated by a coil in a scheme in Ph.Eur. (Fig. 1). On the other hand, the outlet tubing, exposed to the air, should be as short as applicable, otherwise precipitation of water extractable components of a suppository base may occur and the tubing may be blocked.

Water extractable components of a suppository base may interfere with the method chosen for quantitative analysis of a drug, especially when spectrophotometrical analysis is performed. The amount of the interfering components in dissolution medium varies depending on dissolution rate of the base components, which may be influenced by a flow-rate of dissolution medium or physical status of a suppository mass. Possibility of overestimation of the amount of the drug released even by 10% should be realised, unless derivative spectrophotometry or HPLC analysis is employed.

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