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RESEARCH PAPER

A Solid Buffer Reagent for In Vitro Stepped-pH Dissolution Testing

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ABSTRACT

A novel method is described that simplifies in vitro dissolution tests of programmed-release drug dosage forms. It is based on a single solid reagent that affords immediate stepped-pH conditions using a single unitization to model the physiological gradient. Six pharmaceutical products were tested using two methods; the paddle method and the through flow cell method. The dissolution efficiencies obtained with the proposed reagent were identical to those obtained with classical buffers used in dissolution tests. Varying dissolution parameters (paddle rotation rate, flow rate in the through flow cell, use of surfactants) gave closely similar results for the two pH stepping methods but with the added advantage of a single medium.

INTRODUCTION

The dissolution test for oral slow-release dosage forms is described in the different Pharmacopoeias (1,2). To comply with regulatory requirements, sometimes this test uses specific methods to reproduce successive pH conditions met in the gastrointestinal tract. Numerous methods for measuring the drug dissolution have been proposed in recent years. Among the methods described, two (1,2) are most often used, namely paddle and through flow cell. However, in neither case are the volume and composition of the dissolution medium prescribed.

For oral slow-release dosage forms, which are in contact with different media of gradually increasing pH, from approximately 1.2 (stomach) to 7.5 (intestine),

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three ways of grading pH by changing the dissolution medium at different times can be used (1). The first two methods involve total change of medium, while the third requires changing only half the medium each time (half-change method) (3,4,5). The media used are generally buffered to control pH fluctuations (3,6). In the half-change method these buffers are obtained from two ionic solutions (2) corresponding to artificial gastric and intestinal media with pH values of 1.2 and 7.5, respectively (3,6). How changing the medium is to be carried out practically is not prescribed, probably owing to inherent difficulties.

Given the practicalities of trying to produce a pH gradient in this way (handling large volumes of reaction medium, risk of losing active material, turbulence, etc.) more convenient procedures have been proposed. These essentially involve avoiding successive emptyings, and reducing change volumes, while still offering a pH range from 1.2 to 7.5. Gaudy et al. (7) and Brossard (8) have proposed modifying the dissolution medium by adding a small amount of alkaline buffer. This is simpler than the half-change method, which requires replacing half the initial volume. Sallans et al. (9) have described a variable-volume method starting with a small initial volume (half the final volume), with no removal of medium. Compared with the half-change method, these methods are easier but still entail handing several different liquids, with modification of the reaction medium.

Overall, all the methods proposed to date have the following disadvantages:

1. They require preparing beforehand a series of buffer solutions corresponding to the different pH values needed.
2. They involve accurately mixing buffer solutions of different types to obtain the required pH values.
3. They require special apparatus for the accurate measurement of the quantities of medium to be added, displaced, or removed according to the method employed.
4. They require special apparatus for accurate pH measurement to monitor and regulate the pH obtained.
5. They entail applying a correcting factor to the results to allow for the changes in volume that have occurred.
6. The uncontrolled turbulence caused every time the pH is changed affects the dosage form and may cause loss of active material.
7. Automation of the procedure is awkward and requires special costly and space-consuming equipment.

This paper describes a simple, rapid, reliable, and inexpensive method, based on a single solid reagent that can be added in unitized doses to the initial acidic medium, to obtain immediate stepped pH conditions following the physiological gradient without any significant variation in volume.

MATERIALS AND METHODS

Reagent

The reagent used is a powder composed of a homogeneous mixture of 2.28 g (56.3%) tris-[hydroxymethyl]aminomethane (Tris buffer), and 1.77 g (43.7%) anhydrous sodium acetate (10).

The mixture is granulated by water before use to make it smooth flowing, for ease of handling during both processing and in the dissolution test when it is poured into the test apparatus. The mixture can be unitized in single-dose heat-sealed sachets or in bottles or vials. The unit dose is 4.05 g. This dose is used twice during the dissolution test to obtain two consecutive pH steps.

The pH increase starts from an initial dissolution medium of pH 1.5 $\pm$ 0.1, the exact composition of which is as follows: 1 N HCl: 31.6 ml; NaCl: 2 g; and H_2O: to 1 liter.

Addition of the first dose of powder to 1 liter of the initial medium gives pH 4.5 $\pm$ 0.1; addition of the second dose gives pH 7.2 $\pm$ 0.1, as shown experimentally on the neutralization curve (Fig. 1) for the powder in the initial medium described above. The high solubility of the powder in the moving medium ensures that the target pH is reached rapidly (within 1 min).

Drug Dosage Forms Tested

Six marketed slow-release dosages containing two different drugs were tested. Their characteristics are given in Table 1.

In Vitro Dissolution Tests

The in vitro dissolution tests were designed according to the Pharmacopoeia monographs (1,2) for oral slow-release dosage forms: the paddle method and the through flow cell method. In the first method, the

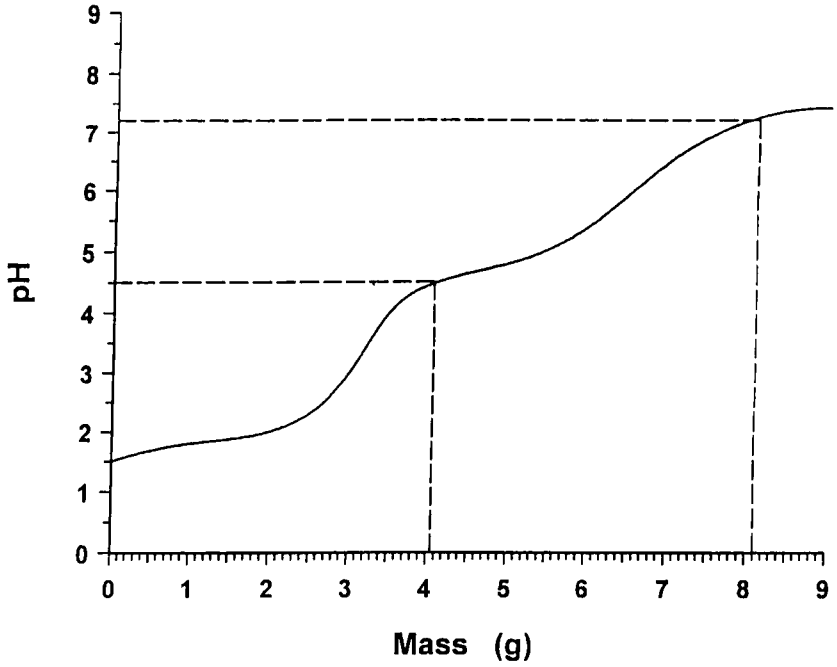

Figure 1. Neutralization curve of solid reagent in initial acid medium (pH 1.5).

paddle rotation rate was set at 100 rpm; in the second method the flow rate of the dissolution medium was set at 40 ml/min in a closed circuit. In both cases tests were run simultaneously in six vessels or cells.

For each of the two methods, and for each of the six dosage forms tested, using the solid reagent to change the pH was compared with the classical procedure using the succession of buffers prescribed in the Pharmacopoeias (1,2) or those usual in this test (3,6,9,11–15). The compositions of the media as well as the stepped progression used are given in Table 2.

The pH modifications were made after 1 hr for the intermediate pH 4.5 and after 3 hr for the final pH 7.2. Total test duration was 8 hr, corresponding to the standard dissolution profile of an oral slow-release dosage form.

Table 1

Characteristics of Drug Dosage Forms Tested

Product	Drug Dosage Form	Drug (Dose)	Batch Dosage Form	Number	Company
A	Théostat 100®	Theophylline (100 mg)	Hydrophilic matrix tablet	T215	Inava[a]
B	Théolair 100®	Theophylline (100 mg)	Inert matrix tablet	243012	3M[b]
C	Armophylline 100®	Theophylline (100 mg)	Microspheres in capsules	DJ1827	Rorer SA[c]
D	Diacor LP 120®	Diltiazem (120 mg)	Microspheres in capsules	EP21	Houde[d]
E	Mono-Tildiem 300®	Diltiazem (300 mg)	Microspheres in capsules	13042	Synthelabo[e]
F	Bi-Tildiem 120®	Diltiazem (120 mg)	Inert matrix tablet	13554	Synthelabo[e]

[a]45 place Abel Gance 92100, Boulogne, France.
[b]3 rue Danton 92445, Malakoff, France.
[c]16 rue Clisson 75013, Paris, France.
[d]1 terrasse Bellini 92800, Puteaux, France.
[e]22 avenue Galilée 92350, Le Plessis Robinson, France.

Table 2

Composition of the Buffered Dissolution Media Used

pH	Standard Method	Solid Reagent Method
Initial (pH 1.5)	1 N HCl 31.6 ml; NaCl 2 g; H_2O to 1 liter	
Intermediate (pH 4.5)	Standing in for Citric acid: 11.55 g Na_2HPO_4: 11.96 g H_2O: to 1 liter	Addition of the first dose of 4.05 g
Final (pH 7.2)	Standing in for KH_2PO_4: 2.69 g Na_2HPO_4: 8.35 g H_2O: to 1 liter	Addition of the second dose of 4.05 g

Analytical Methodology and Interpretation

For each dissolution test carried out, 1 ml of medium was withdrawn and filtered on a Whatman filter (model GF/D ref 1823025 of porosity 2.9 μm). This lost volume of medium was not made up. Nine samples were taken between 0 and 8 hr for each run (0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 7 hr, 8 hr). The amount of the dissolved drug was evaluated by HPLC with UV detection (validated methods).

The results obtained are expressed in terms of dissolution efficiency (DE) (16–18) representing the area under the dissolution curve at time t as a percentage of the area of the rectangle formed by the ordinate 100% and the abscissa t. This parameter (DE%) was calculated for all the tests over an 8-hr run. The values given are averages (± standard deviations) obtained on the six vessels.

RESULTS

Comparison of the Methods

The dissolution profiles obtained with the paddle or the through flow cell methods are shown in Figs. 2 and 3. The values of dissolution efficiency are shown in Fig. 4 for the paddle and through flow cell methods.

Influence of Buffered Media Composition

Preliminary test were performed to establish the range of values for dissolution results obtained with each drug using different media. The French Pharmacopoeia (1) makes no prescription concerning the nature or composition of the buffers to be used. Hence it is important to assess these differences to define a confidence limit for the results for the two methods used. This test series was carried out on product A (Table 1) using different types of media (Table 3). Arbitrarily chosen combinations gave the pH progression 1.5 → 4.5 → 7.2.

The results in terms of dissolution efficiencies obtained after 8 hr with the paddle method are given in Table 4.

Influence of Paddle Rotation Rate or Medium Flow Rate

Paddle rotation rates range classically from 50 to 120 rpm (3). Lowering the rotation rate is liable to reduce the dissolution rate of the solid reagent, which can then collect at the bottom of the flask, producing a zone of high alkalinity that may affect the drug being tested. It is therefore important to make sure that under these conditions the results obtained with the solid buffer are comparable to those obtained with the classical procedure in which the buffer is dissolved beforehand. A test run was carried out on the same product A (Table 1) with the paddle, using speeds of 50 and 100 rpm with the two buffers.

Flow rates used in the through flow cell range classically from 15 to 50 ml/min (3). In contrast to the paddle method, the solid buffer does not come into contact with the drug being tested, since it is dissolved

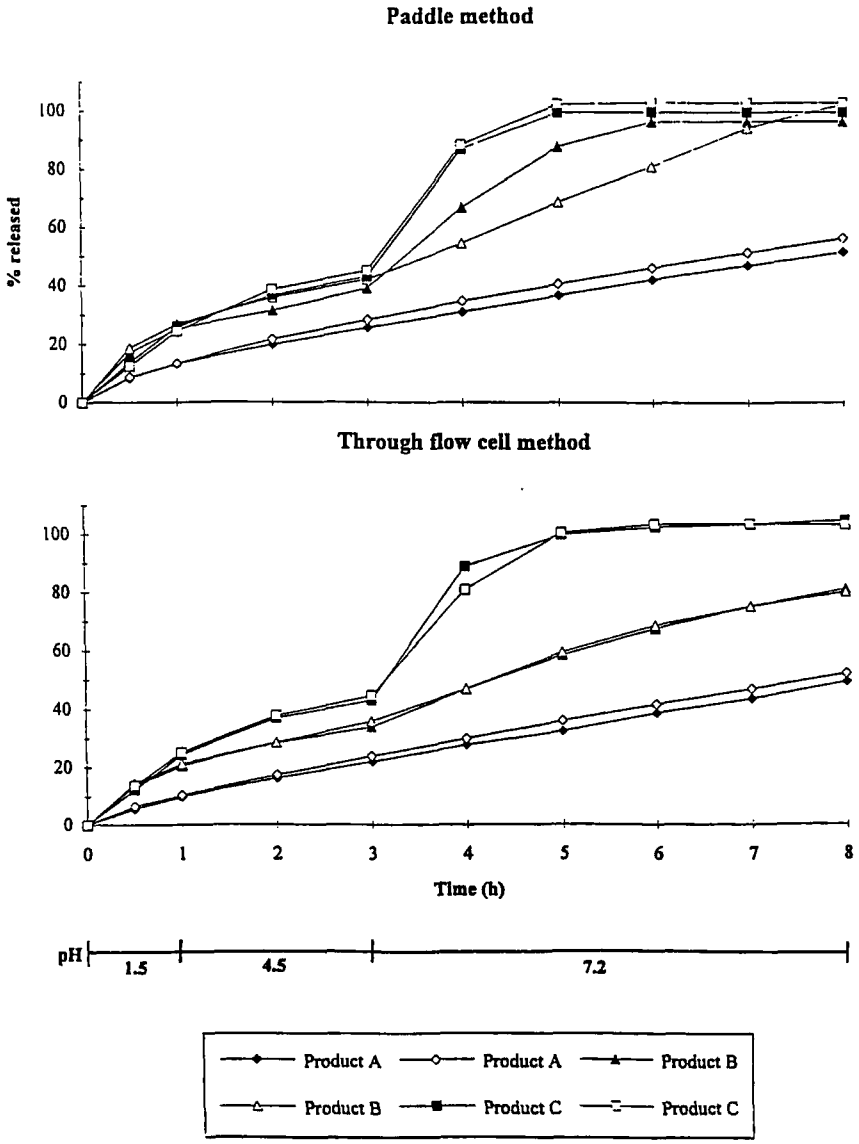

Figure 2. Dissolution profiles of theophylline products (A, B, C) with paddle and with through flow cell (black symbols = classical method, white symbols = solid reagent method).

in a separate vessel before the medium enters the cell. A test was performed on the same product A (Table 1) with the through flow cell with flow rates of 15 and 40 ml/min with both buffers.

The results obtained in terms of dissolution efficiencies after 8 hr are given in Fig. 5. The results show no difference between the two methods.

Influence of the Presence of Surfactants in the Dissolution Medium

The addition of surfactants to the medium allows dissolution tests to be carried out on products that are sparingly soluble in water (19). A test was carried out on the same product A (Table 1) with the paddle using

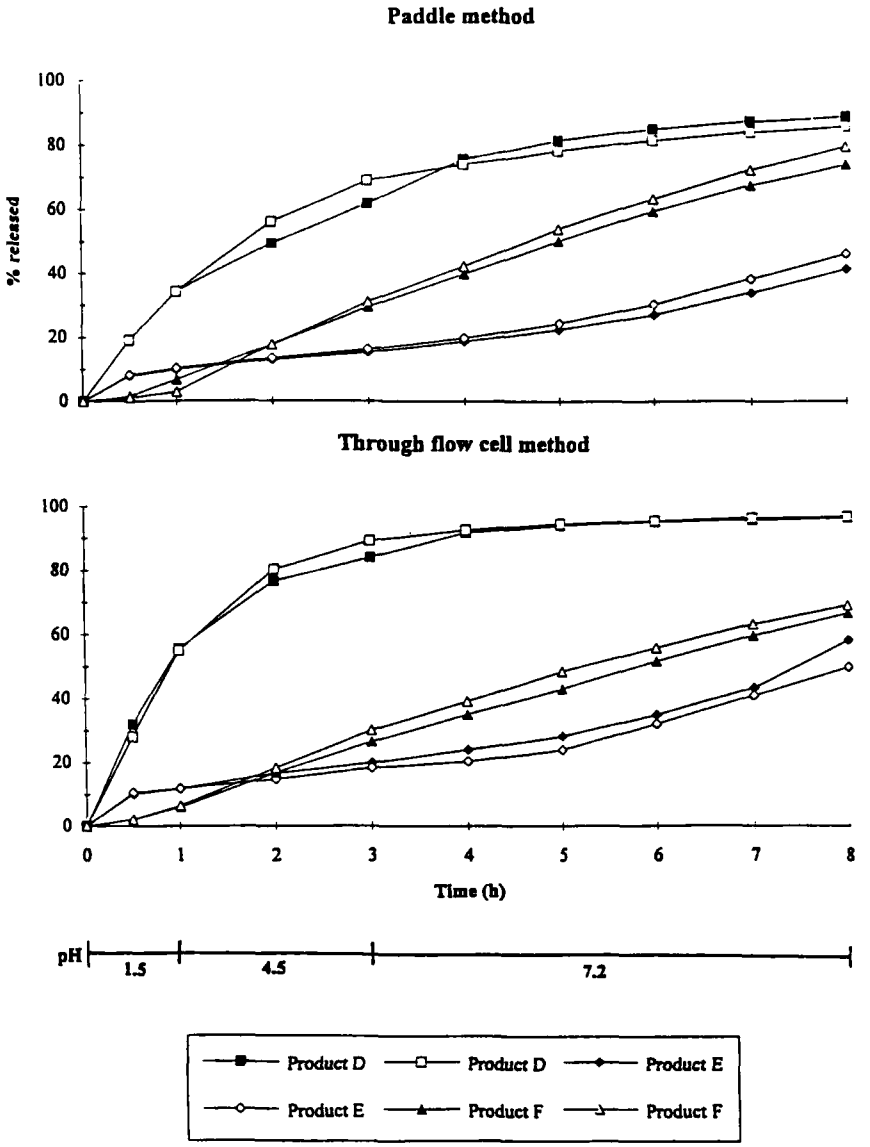

Figure 3. Dissolution profiles of diltiazem products (D, E, F) with paddle and with through flow cell (black symbols = classical method, white symbols = solid reagent method).

buffer containing either 1% sodium lauryl sulfate (SLS) (anionic surfactant) or 1% polysorbate 80 (PS 80) (nonionic surfactant). The results obtained in terms of dissolution efficiencies after 8 hr are given in Fig. 6. They show no difference between the two methods.

DISCUSSION

To evaluate the pH stepping method using a new powdered buffer, a comparison of the dissolution efficiencies obtained with the classical buffers most widely used in dissolution testing has been made. A difference of 10% observed overall between extreme dissolution efficiencies was chosen arbitrarily as an acceptable limit of variation between the two methods. The results collected in Fig. 4 show that the difference between the dissolution efficiencies obtained with the two pH-stepping procedures was always below 10%. These two procedures may therefore be considered equivalent, especially since they gave dissolution profiles that are the same overall for any one of the pharmaceutical products

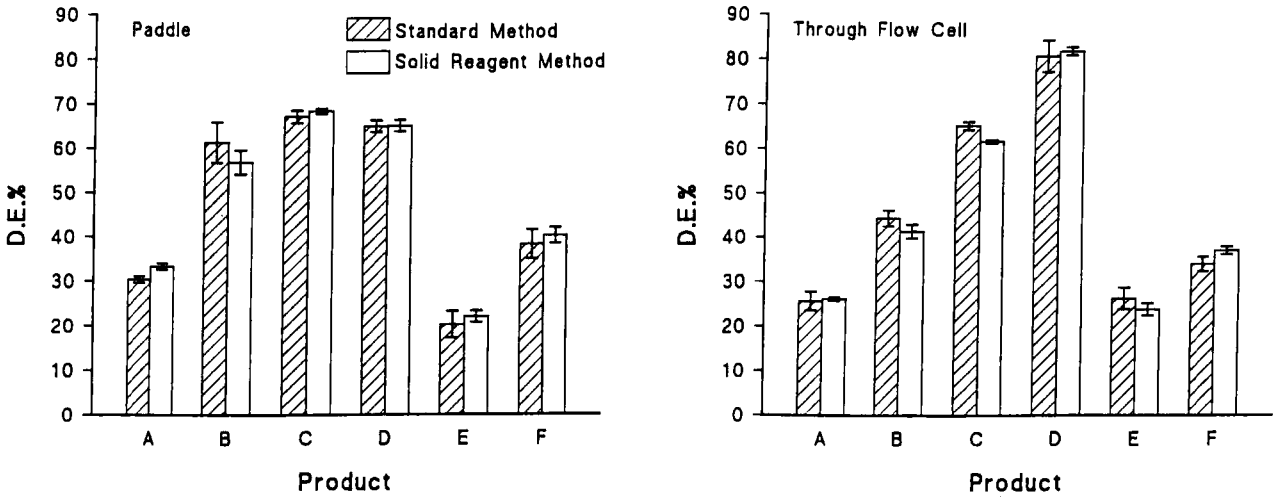

Figure 4. Compared values of dissolution efficiencies (DE) after 8 hr, obtained by the paddle (100 rpm) and the through flow cell methods (40 ml/min).

Table 3

Buffered Media Used with Product A

pH 4.5	pH 7.2
1. Citric acid: 11.55 g Na_2HPO_4: 11.96 g H_2O: to 1 liter	3. KH_2PO_4: 2.69 g Na_2HPO_4: 8.35 g H_2O: to 1 liter
2. KH_2PO_4: 13.61 g H_2O: 750 ml pH is adjusted by 0.1 N NaOH or 0.1 N HCl Complete with 1 liter water	4. KH_2PO_4: 6.81 g NaOH: 1.4 g H_2O: to 1 liter
	5. NaCl: 8 g KCl: 0.2 g $CaCl_2$ anhydrous: 0.1 g $MgCl_2$, $6H_2O$: 0.1 g Na_2HPO_4, $12H_2O$: 3.18 g KH_2PO_4: 0.2 g H_2O: to 1 liter

(Figs. 2 and 3). Furthermore, the standard deviations obtained with the proposed solid reagent were always lower than those obtained with the classical pH stepping method. This implies that the proposed method is more reproducible. The effects of varying the dissolution parameters (paddle rotation rate, medium flow rate, surfactants) were evaluated and no difference was obtained (Table 5).

Table 4

Values of Dissolution Efficiencies (DE) After 8 hr with Different Types of Buffered Media Tested with the Paddle on Product A

pH 4.5	pH 7.2	DE% ± Standard Deviation
Buffer 1	Buffer 3	30.4 ± 0.7
Buffer 2	Buffer 4	33.3 ± 0.6
Buffer 2	Buffer 5	32.6 ± 0.7

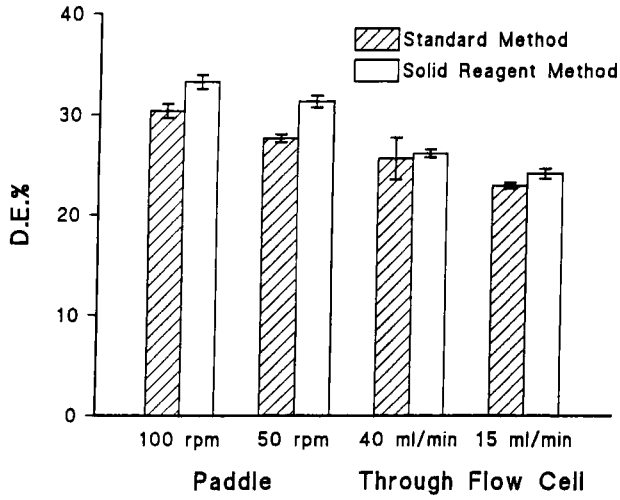

Figure 5. Influence of paddle rotation rate or flow rate on product A dissolution efficiencies (DE) after 8 hr.

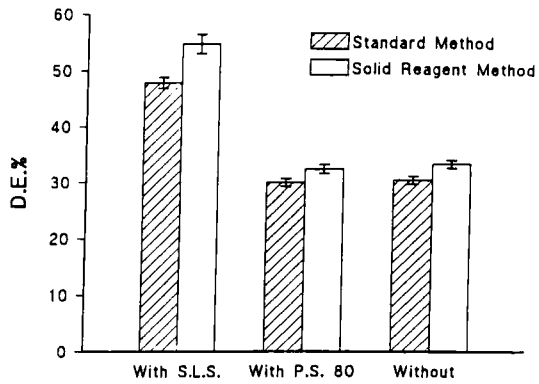

Figure 6. Influence of presence of 1% sodium laurylsulfate (SLS) or 1% polysorbate 80 (PS 80) in the dissolution medium on product A dissolution efficiencies (DE) after 8 hr.

Table 5

Influence of Paddle Rotation (I), Flow Rate (II) and Presence of Surfactants in the Dissolution Medium (III and IV) on Product A Dissolution Efficiencies (DE%) After 8 hr

	DE Ratios	Standard Method	Solid Reagent Method
(I)	$\frac{\text{DE (100 rpm)}}{\text{DE (50 rpm)}}$	1.10	1.06
(II)	$\frac{\text{DE (40 ml/min)}}{\text{DE (10 ml/min)}}$	1.12	1.08
(III)	$\frac{\text{DE (with SLS)}}{\text{DE (without SLS)}}$	1.58	1.65
(IV)	$\frac{\text{DE (with PS 80)}}{\text{DE (without PS 80)}}$	0.99	0.97

Table 6

Influence of the Solid Reagent Mass Variation Against the Theorical Value on pH Obtained with Conditions Initially Established

One Single Dose Mass Variation (4.05 g)	pH	Two Single Doses Mass Variation (8.10 g)	pH
+4%	4.59	+8%	7.29
+3%	4.57	+6%	7.27
+2%	4.55	+4%	7.24
+1%	4.52	+2%	7.22
0%	4.51	0%	7.21
-1%	4.48	-2%	7.18
-2%	4.45	-4%	7.15
-3%	4.42	-6%	7.13
-4%	4.39	-8%	7.10

Table 7

Influence of the Variation in the Percentages of Two Constituents of the Solid Reagent (4.05 g) on pH Obtained with Conditions Initially Established

Tris Buffer (%)	Sodium Acetate (%)	pH
50.5	49.5	4.4
52.5	47.5	4.4
54.5	45.5	4.5
56.3	43.7	4.5
58.5	41.5	4.5
60.5	39.5	4.5
62.5	37.5	4.5

The solid buffer reagent contains a weak base, tris-[hydroxymethyl] aminomethane (Tris buffer) (20,21), designed to neutralize the acidity of the initial medium of set composition, and thereby raise the pH in the vessel. The pH of a 1 M aqueous solution of Tris at 20°C is 10.4. Tris is highly soluble in aqueous media (more than 80 g/100 ml water) and is chemically stable in such solutions. Tris does not bind to calcium, magnesium, or manganese and so does not cause precipitation. Its UV absorbance between 240 and 700 nm is not significant. However, to avoid any possible interference during direct UV monitoring, the test should be carried out using a reference. Sodium acetate is associated with the Tris buffer to optimize the neutralization of the initial acidic solution to meet the requirements set initially.

The use of this reagent as described implies preset pH steps (1.5 → 4.5 → 7.2). Although these are not the only steps specified in the Pharmacopoeias, this progression is commonly used in practice for variable-pH dissolution testing. In addition, use of this reagent is appropriate to all the dissolution test methods (flow cell, paddle, and basket). It avoids spurious turbulence when media have to be changed. Although these tests were carried out with volumes of 1 liter, any other volume can be used. Similarly, this reagent can be used without a pH check; tests showed that a variation of ±3% in the mass of a dose of reagent associated with variations of ±5% in the percentages of each of the constituents did not affect the pH (Tables 6–8).

The granulated form of the reagent was adopted because it affords a rapid dissolution (less than 3 min) in the medium. The powder flow out of the bag is smooth. Loss of reagent on the walls of the bag is about 0.3% of the mass of the dose, and has no effect on the performance of the reagent under the prescribed conditions of use.

CONCLUSION

As can be seen from the results, there is no significant difference between the classical buffer used with media renewal and the proposed powdered buffer added twice during a dissolution course. Easy use of the latter is a great advantage compared with the classical method.

In order to improve interest in the use of the proposed buffer, other classical buffers were tested with theophylline dosage forms. The results obtained failed to show any differences between the entire data set.

Similarly, the modification of the paddle dissolution rate, the flow rate in the through flow cell, and the addition of surfactants in the medium (SLS or PS 80), did not induce any differences between the data obtained with the classical buffers and the proposed buffers.

Table 8

Influence of the Variation of the Solid Reagent Mass and Percentages of Two Constituents on pH Obtained with Conditions Initially Established

One Single Dose Variation (4.05 g)				Two Single Doses Variation (8.10 g)			
Total Mass	Tris Buffer Mass	Sodium Acetate Mass	pH	Total Mass	Tris Buffer Mass	Sodium Acetate Mass	pH
-3%	+5%	-5%	4.4	-6%	+10%	-10%	7.1
	-5%	+5%	4.4		-10%	+10%	7.2
+3%	+5%	-5%	4.5	+6%	+10%	-10%	7.2
	-5%	+5%	4.6		-10%	+10%	7.3

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