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# Comparative Study of the Dissolution Profiles of a Commercial Theophylline Product after Storage

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The purpose of this work was to study the effect of storage time and temperature on the in vitro release kinetics of a commercial sustained-release dosage form of theophylline, at different pHs of the dissolution medium. The formulation was stored at 35°C for 16 months and at 45°C for 8 months, with a relative humidity of 60%. The in vitro release tests were performed at pHs 2, 4, 6 and 7.4. The mean values of the transport coefficient n, were close to 0.5 in all the conditions tested, which indicates that the transport system is not modified after storage of the formulation at 35°C and 45°C. The mean values of the dissolution rate constant ranged from 0.036 to 0.043 $min^{-n}$, under all the conditions tested. Significant differences ($\alpha$=0.05) were found between pHs 2, 4 and 6, 7.4 for all the model-independent parameters studied. When the formulation was kept at 35°C for 16 months, the mean percentage of drug dissolved at 8 hours was 25.61% (pHs 2, 4) and, 36.12% (pHs 6, 7.4), representing a 26% and 24% reduction, respectively. Similar results were obtained after storing the formulation at 45ºC for 8 months, corresponding to 33.3% (pHs 2, 4) and, 22.5% (pHs 6, 7.4) diminution, respectively.The values of the similarity factor, $f_2$, obtained were lower than 50, which indicates the lack of similarity among the dissolution profiles, after storing the formulation under the experimental conditions tested.

**Key words:** Theophylline, Dissolution, Sustained-release, Storage

## INTRODUCTION

The marketing of theophylline as sustained-release products has given renewed popularity to this drug. These modern drug formulations allow for the prophylactics and control of chronic asthma since the bronchodilatory effect, achieved within a narrow range of 10-20 μg/ml, can be easily maintained.

At least twenty theophylline prolonged release preparations have been introduced in the market recently, offering a variety of drug release mechanisms in order to obtain an adequate control of the release process. The knowledge of the drug release kinetics allows for the possibility to follow the performance of different systems as well as to predict in vivo availability.

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Several studies have demonstrated the influence of the experimental conditions on the in vitro release of theophylline from prolonged-release systems (De Bolas *et al.*, 1987; Junkman, 1989; Mohs, 1989; Peracchia *et al.*, 1992). In this respect, the aim of this work was to study the influence of storage conditions, such as time and temperature, on the dissolution behaviour of a commercial sustained-release theophylline product at different pHs. The formulation was stored, with a relative humidity of 60%, during 16 months at 35°C and, during 8 months at 45°C and, tested for dissolution at pHs 2, 4, 6 and 7.4 of the dissolution medium.

What we have tried to evaluate is the influence of certain storage temperatures, such as 35°C and 45°C, which are usually reached, during some months of the year, in the Mediterranean countries. Moreover, the selection of the relative humidity value of 60%, corresponds to that of the climatic zone II in which the said countries are included (Grimm, 1995).

Due to the narrow therapeutic index of theophylline,

with this study we pretended to determine if the experimental conditions assayed could have an influence on the in vivo levels attained by the drug.

## MATERIALS AND METHOS

### Materials

The sustained-release dosage form of theophylline studied was Theodur, Antibióticos Farma S.A., Madrid (Spain).

Theodur tablets contain a dose of 300 mg of anhydrous theophylline and the following excipients: sucrose, starch, lactose, HPMC, magnesium stearate, glyceril monostearate, white wax, cetyl alcohol, myristic alcohol, cellulose acetate phtalate and, diethylphtalate.

### Methods

#### Storage conditions

Theophylline tablets were kept in their commercial blisters under the following storage conditions: the formulation was paced in stability ovens at 35°C for 16 months and, at 45°C for 8 months, with a relative humidity of 60%.

#### *In vitro* release studies

In vitro release assays were performed at time zero and at 4, 8 and 16 months when the formulation was stored at 35°C and, at 4 and 8 months when kept at 45°C.

The product was tested for dissolution in Apparatus I of USP 24 (USP 24, 2000) (D.T. 6, Turu Grau S.A., Tarrasa, Spain), using 900 ml of dissolution medium at 37°C. The basket was rotated at 100 rpm. As dissolution media, the following buffered aqueous solutions were employed: 0.2M potassium chloride/0.2M hydrochloric acid (pH 2), 0.1M citric acid/0.2M dibasic sodium phosphate (pH 4) and, 0.067M monobasic potassium phosphate/0.067M monobasic sodium phosphate (pHs 6 and 7.4). Each buffer solution was prepared by dissolving the components in distilled water according to literature (Geigy Tables, 1984).

The content of theophylline in sampled and filtered solutions was determined spectrophotometrically (DU-6 spectrophotometer, Beckman, USA) at 271 nm. The validation of the analytical technique has been reported elsewhere (Villegas, 1995).

For each storage temperature and pH of the dissolution medium, a minimum of three replicates were assayed. The highest relative standard deviation obtained was 5%, in all the cases.

#### Analysis of *in vitro* release data

*Model-dependent analysis*:

The in vitro release kinetics of theophylline was studied based upon the following empirical exponential equation (Peppas, 1985):

$$M_t/M_\infty = K.t^n \qquad \text{Eq. 1}$$

in which a linear dependence is demonstrated between the fraction of drug released and the time power n. In the equation, $Mt/M_\infty$ is the fraction of drug dissolved at time t, K is the dissolution rate constant and, n is the transport coefficient, which gives indication of Fickian release kinetics (n=0.5) or zero-order release kinetics (n=1) or approaching that ($n\rightarrow1$). As indicated by Peppas (Peppas, 1985), in order to be able to estimate the exponent "n", one must only use the initial portion of the release curve ($Mt/M_\infty$ <0.6).

The ELSFIT computer program (Sheiner, 1981) was utilised to estimate the values of the parameters of the Peppas equation. The non-parametric Kruskal-Wallis test was then applied (Siegel, 1990), to reveal possible modifications in the coefficients caused by pH, storage time or temperature.

*Model-independent analysis*

The dissolution efficiency at 8 h ($DE_{8h}$) (Khan, 1975), and the percentage of drug dissolved at 8 h ($Qd_{8h}$), were estimated as model-independent parameters.

A two-way analysis of variance (ANOVA) and the Newman-Keuls test of multiple comparison (Winer, 1979) were used to determine the influence of pH, temperature and storage time on the model-independent parameters.

Moreover, in order to compare the in vitro theophylline dissolution profiles, the similarity factor, $f_2$, recently proposed (Moore *et al.*, 1996) was estimated. This factor is derived from the Minkowski difference (mean-squared difference).

## RESULTS AND DISCUSSION

### Theophylline release kinetics

#### Model-dependent parameters

As an example, Fig. 1 shows the profiles of the percentage of drug dissolved versus time for the tests performed at pH 7.4, without any controlled storage and, after 4 and 8 months when kept at 45°C.

The mean values of the dissolution rate transport coefficient (n) and of the dissolution rate constant (K) as well as their standard deviations, obtained at 35°C and 45°C storage temperatures and pHs 2, 4, 6 and 7.4 are summarised in Table I.

When the dissolution assays were carried out without any controlled storage conditions (time zero), the n exponents obtained were close to 0.5 (Table I). Theodur consists of a matrix of compressed theophylline crystals embedding coating theophylline granules (De Bolas *et al.*,

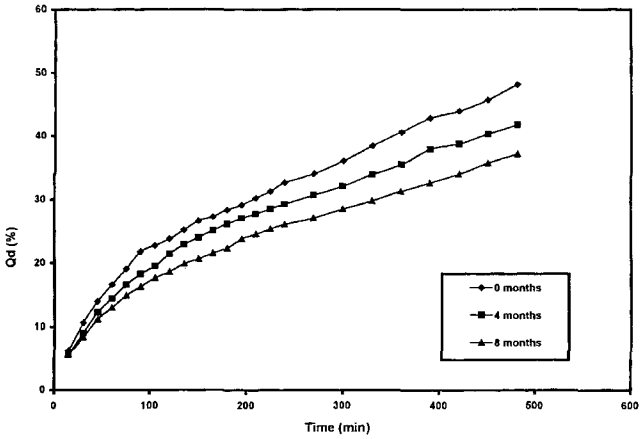


**Fig. 1.** Mean percentages of theophylline dissolved up to 8 h at time zero and after 4 and 8 months at 45°C pH 7.4. Each sample was assayed by triplicate.

1987; Junkman, 1989; Mohs, 1989; Peracchia *et al.*, 1992). In contact with the dissolution medium, the matrix gradually disintegrates and dissolves. Then, the drug diffuses slowly through the membrane of the granules. Finally, the granules dissolve completely. In this matrix system, the drug transport is mainly governed by Fickian diffusion.

After applying the Kruskal-Wallis test to the n values obtained when the formulation was stored at 35°C and 45°C, no significant differences were found within the pH range studied. This fact indicates that the mechanism implied in the diffusion process, which corresponds to Fickian diffusion, is not modified by the storage conditions studied.

The values of the dissolution rate constant obtained (Table I) ranged from 0.036 to 0.043 $min^{-n}$, without significant differences being found among the temperatures and pHs assayed.

In all the cases, the values obtained for lag time were smaller than 15 min.

### Model-independent parameters

Tables II and III summarise the mean values of the percentage of drug dissolved at 8 h ($Qd_{8h}$) and, the dissolution efficiency at 8 h ($DE_{8h}$), as well as their standard deviations obtained under all the experimental conditions assayed in the in vitro theophylline release tests.

After applying a one-way ANOVA to the percentage of drug dissolved at 8 hours (Table II), significant differences ($\alpha$=0.05) were found between pHs 2, 4 and 6, 7.4 as well as among storage times. As storage time increases, the percentage of drug dissolved decreases.

The percentages of drug dissolved show that when the formulation is kept without any controlled storage (time zero), a mean value of 34.44% is obtained for pHs 2 and 4 and, of 47.39% for pHs 6 and 7.4

After storing the formulation at 35°C for 16 months, the mean percentage of drug dissolved was 25.61% (pHs 2, 4) and, 36.12% (pHs 6, 7.4), representing a 26% and 24% diminution, respectively. Similar results were obtained

**Table I.** Mean values of the dissolution rate transport coefficient (n) and of the dissolution rate constant (K) as well as their standard deviations, from three replicates, obtained after storage at 35°C and 45°C, for the pHs studied.

| Temperature (°C) | | | 35 | | | 45 | |
|---|---|---|---|---|---|---|---|
| Time (months) | | 0 | 4 | 8 | 16 | 4 | 8 |
| pH 2 | n | 0.37 (0.02) | 0.34 (0.03) | 0.34 (0.02) | 0.34 (0.04) | 0.38 (0.03) | 0.39 (0.01) |
| | K ($\times 10^2$) ($min^{-n}$) | 3.58 (0.28) | 3.66 (0.81) | 3.06 (0.40) | 3.03 (0.68) | 2.35 (0.63) | 1.97 (0.14) |
| pH 4 | n | 0.43 (0.01) | 0.40 (0.01) | 0.38 (0.01) | 0.34 (0.02) | 0.44 (0.01) | 0.39 (0.06) |
| | K ($\times 10^2$) ($min^{-n}$) | 2.18 (0.13) | 2.75 (0.19) | 2.57 (0.07) | 3.20 (0.35) | 1.61 (0.02) | 2.16 (0.14) |
| pH 6 | n | 0.54 (0.02) | 0.54 (0.06) | 0.47 (0.02) | 0.48 (0.03) | 0.55 (0.02) | 0.45 (0.01) |
| | K ($\times 10^2$) ($min^{-n}$) | 1.43 (0.35) | 1.45 (0.07) | 1.95 (1.19) | 1.78 (0.19) | 1.10 (0.24) | 2.08 (0.08) |
| pH 7.4 | n | 0.46 (0.06) | 0.47 (0.07) | 0.47 (0.02) | 0.40 (0.01) | 0.44 (0.05) | 0.44 (0.05) |
| | K ($\times 10^2$) ($min^{-n}$) | 2.67 (0.09) | 2.32 (0.80) | 2.03 (0.17) | 2.91 (0.26) | 2.75 (0.74) | 2.39 (0.73) |

**Table II.** Mean values of the percentage of theophylline dissolved at 8 h ($Qd_{8h}$) and their standard deviations, from three replicates, obtained after storage at 35ºC and 45ºC for the pHs studied

| | $Qd_{8h}$ (%) | | | | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | | 35 | | | 45 | |
| Time (months) | 0 | 4 | 8 | 16 | 4 | 8 |
| pH 2 | 34.63 (2.45) | 28.57 (1.67) | 26.81 (0.10) | 24.95 (0.26) | 24.62 (2.22) | 22.83 (0.69) |
| pH 4 | 34.26 (0.19) | 32.65 (0.94) | 27.84 (0.79) | 26.28 (2.27) | 25.32 (1.30) | 23.10 (0.74) |
| pH 6 | 46.70 (1.73) | 43.26 (0.13) | 38.21 (2.32) | 35.63 (2.64) | 36.75 (3.06) | 36.36 (1.35) |
| pH 7.4 | 48.09 (0.82) | 44.54 (2.59) | 41.06 (2.16) | 36.61 (0.63) | 41.73 (0.86) | 37.04 (0.45) |

**Table III.** Mean values of the dissolution efficiency of theophylline at 8 h ($DE_{8h}$) and their standard deviations, from three replicates, obtained after storage at 35°C and 45°C for the pHs studied

| | $DE_{8h}$ (%) | | | | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | | 35 | | | 45 | |
| Time (months) | 0 | 4 | 8 | 16 | 4 | 8 |
| pH 2 | 24.72 (1.33) | 20.83 (0.60) | 19.15 (0.26) | 18.10 (0.13) | 17.20 (1.83) | 15.92 (0.63) |
| pH 4 | 22.50 (0.41) | 22.48 (0.40) | 19.42 (0.40) | 18.98 (1.42) | 16.79 (0.64) | 16.28 (0.56) |
| pH 6 | 27.65 (0.80) | 26.29 (0.11) | 24.54 (1.18) | 22.75 (1.39) | 21.75 (1.99) | 23.83 (0.87) |
| pH 7.4 | 31.02 (0.31) | 28.48 (0.33) | 26.47 (1.01) | 24.34 (0.62) | 27.52 (1.45) | 24.26 (0.69) |

**Table IV.** Values of the similarity factor, $f_2$

| | $f_2$ | | | | |
|---|---|---|---|---|---|
| Temperature (°C) | | 35 | | 45 | |
| Time (months) | 4 | 8 | 16 | 4 | 8 |
| pHs 2 and 4 | 49.30 | 39.75 | 36.06 | 32.15 | 29.28 |
| pHs 6 and 7.4 | 48.36 | 39.26 | 33.89 | 35.84 | 34.73 |

after storing the formulation at 45°C for 8 months, corresponding to 33.3% (pHs 2, 4) and 22.5% (pHs 6, 7.4) reduction, respectively.

The dissolution efficiency at 8 h (Table III), also shows a similar behaviour. Significant differences ($\alpha$=0.05) were found between pHs 2, 4 and 6, 7.4 at both temperatures assayed. Moreover, the said parameter diminishes as storage time increases, resulting in 21.47% (pHs 2, 4) and 19.47% (pHs 6, 7.4) reduction when the formulation is maintained for 16 months at 35°C and, 31.8% (pHs 2, 4) and 24.04% (pHs 6, 7.4) diminution when kept 8 months at 45°C, respectively.

Table IV summaries the values of the similarity factor, $f_2$. Since significant differences were obtained between pHs 2, 4 and 6, 7.4, these two groups of pH were used to estimate the similarity factor. Moreover, the comparison of dissolution profiles was established by using the mean average profiles at time zero and the ones corresponding to the different storage times.

From the $f_2$ values obtained it can be observed that, in all the cases, the factor is lower than 50, which indicates the lack of similarity among curves. Also, it can be seen that the factor decreases as the storage time increases, at both temperatures assayed.

In conclusion, storage of the formulation at 35°C and 45°C, with a 60% of R.H., produced a modification in the dissolution profile of the drug, becoming the drug release rate slower after storage. Major modifications occurred after 16 and 8 months, when the formulation was stored at 35°C and 45°C, respectively.

The modifications observed in the percentage of drug dissolved, ranging between 22.57% at pHs 6 and 7.4 after 8 months at 45°C and, 33.37% at pHs 2 and 4 under the same conditions, could have an influence in the pharmacological effect of theophylline, taking into account its narrow therapeutic index and moreover, that they are produced at temperatures and storage times feasibly achieved during its shelf-life.

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