Development of a 100 mg theophylline sustained release formulation using POVIAC polymer for infants

Raisa González, Jiovanna Contreras, Mirelly Pérez, Guillermo Jordán,** Mireya López and Alberto Suzarte.

Centro Nacional de Investigaciones Científicas, Avenida 25 y Calle 158, Cubanacán, Playa, Apartado Postal 6414, Ciudad de La Habana, Cuba. **Laboratorios Medsol, Avenida 25 y Calle 212, Playa, Ciudad de La Habana, Cuba.

RESUMEN. El objetivo del presente trabajo consistió en desarrollar una formulación de teofilina de 100 mg, en forma de tabletas, adecuada para ser usada en el tratamiento preventivo del asma en infantes. Se utilizó polímero POVIAC 0, compuesto de polivinilacetato de elevada pureza y adecuado peso molecular, como único constituyente de la matriz reguladora de la liberación del fármaco. Para lograr esto se determinaron las cinéticas de liberación *in vitro* de diferentes formulaciones preparadas en concordancia con un diseño de experimentos 2^3 para tabletas confeccionadas a partir de dos granulados de diferente composición y en el que las variables independientes estudiadas fueron X_1 (contenido de polímero en el granulado de bajo contenido), X_2 (contenido de polímero en el granulado de alto contenido) y X_3 (fracción de la totalidad de la masa de las tabletas que corresponde al granulado de bajo contenido de polímero). También se estudió la cinética de liberación *in vitro* de patrones de reconocido prestigio como Theo-Dur de Key Pharmaceuticals y Slo-Bid de Rhone Poulenc. En base a los resultados se elaboraron formulaciones a partir de un solo granulado que contenía una cantidad de polímero igual que el promedio de aquellas cuya cinética de liberación era más cercana a la del patrón Slo-Bid. Una de las referidas tabletas presentó una muy buena cinética de liberación. Con esta formulación se efectuaron experiencias *in vivo* en perros Beagle (n = 8) y en humanos voluntarios adultos (n = 8) en dosis única y en dosis múltiple. Los resultados aunque preliminares en base al número relativamente reducido de sujetos estudiados fueron muy satisfactorios.

ABSTRACT. The goal of the present work is the development of an oral pharmaceutical formulation of theophylline in tablets and with a 100 mg dosis of active principle, suitable for preventive use in the treatment of infants who suffer from asthma. A polyvinylacetate polymer (POVIAC 0) with a very high purity and an appropriate molecular mass is used as the unique component of the release regulating matrix. The *in-vitro* theophylline release kinetics of different formulations were followed. The tablets were prepared in accordance with a 2^3 experimental design using mixtures of two granulates with different composition of theophylline and in POVIAC 0. The independent variables of the design were X_1 (polymer content of the granulate with lower polymer content), X_2 (polymer content of the granulate with higher polymer content), X_3 (fraction of the total mass of the formulation that corresponds to the polymer lower content granulate. The release kinetics of two prestigious pharmaceutical sustained release commercial formulations (Theo-Dur from Key Pharmaceuticals and Slo-Bid from Rhone Poulenc) were also followed for comparison. Two formulations were also prepared by using only one granulate in their composition. Each of these two granulates presented a polymer content equal to the polymer average content of the two formulations of the mentioned design with the closest similar release kinetics to the Slo-Bid formulation. One of these formulations showed a very good release kinetics. Single and multiple dosis *in vivo* experiments on Beagle dogs (n = 8) and human volunteers (n = 8) were performed. The results, although should be considered as preliminary on account of the relative reduced number of subjects studied, have shown themselves as highly satisfactory.

INTRODUCTION

Cuba is one of the countries in the world with the highest indexes of asthma in the population. This is not limited to a close time period and it has behaved as an own and permanent phenomenon of the Cuban population. It is known that the number of people suffering from bronchial asthma in a higher or lower degree is higher than 900 000. All the year, but particularly in winter, many thousands of people visit the Emergency services in hospitals and polyclinics. A great number of the patients are children of all ages. One of the most efficacious and cheapest drugs is theophylline. As this drug shows a narrow therapeutic rank (10-20 μg/mL), the use of sustained release formulations is safest, and allows to reach steady levels of the drug in time, in order to minimize the adverse effects of the drugs containing them. This makes possible their use in a preventive way. These general considerations are more valid when the treatment of infants is considered.

The international market shows many sustained released formulations of theophylline. Generally, they are all relatively expensive. The best formulations are produced as capsules (for example, Slo-Bid) or tablets containing microspheres, where the drug is encapsulated. (for example, Theo-Dur).

This work has had the purpose of developing a non-expensive formulation of sustained release theophylline with a high effectiveness, with

appropriate doses for the preventive treatment of crisis in infants suffering from asthma. This formulation in tablets has as a control matrix for the drug a polymer (POVIAC) developed and produced in Cuba.

The use of this polymer has been authorized because of its innocuousness and the manufacturing of the formulation in conventional tablets makes possible the obtainment of a very low cost drug.[7]

MATERIALS AND METHODS
Materials

The monohydrated theophylline (agrees with the analytical requirements of USP XXIII), magnesium estearate (agrees with the analytical requirements of BP 88), a POVIAC polymer obtained at industrial stage in the National Center for Scientific Research with the required characteristics and specifications described of purity and molecular mass.[1,2]

Obtainment of the tablets

The granules were produced by conventional humid way and they contained monohydrated theophylline, magnesium estearate as lubricant and POVIAC polymer as a controller matrix for the release. The tablets were produced in an eight-station diesinking machine (Ronchi Model MP-8). For each type of tablet 1 kg of formulation was prepared and die-pressed.

Selection of the reference drugs

Drugs from the international market were selected, having the best characteristics to be used as reference drugs. The Physician Desk Reference (PDR-1955) was consulted and two drugs were chosen, considering the reputation of their manufacturers, as well as their dosage, appropriate for therapy in asthmathic children:

The Slo-Bid Gyrocaps capsules of Rhone Poulenc Rorer (100 mg dose).

The Theo-dur tablets formed with microspheres of Key Pharmaceuticals (100 mg dose).

In vitro release assays

The *in vitro* release assays were performed in a Pharma Test II equipment, according to the technique described in the USP XXII, by using the method of blades at a velocity of 100 r/min . Distilled water (900 mL) at 37 ºC was used as dissolution medium. Samples of 1 mL were taken every hour for 12 h . The quantifica-

tion of those samples was performed in a UV-Visible Spectrophotometer, by using quartz trays with 1 cm of light passage at a 273 nm wavelength. For the evaluation and interpretation of the dissolution data, the Peppas proposed equation was used for the analysis of the release mechanisms of the swellable and non-swellable controlled liberation systems.[3,4]

Unique-dosis bioequivalence assays in Beagle dogs

Male Beagle dogs weighing 13-18.2 kg and 8-12 months old were used. A dosis was orally exhibited in a 400 mg experiment (Theo-Dur) and in the others, 200 mg anhydrous theophylline (four or two tablets of 100 mg, respectively) were employed for each formulation, by following a crossed design. Washing time of 1 week. The blood samples (3 mL) were collected at different moments, in heparinized tubes for 32 h after the oral dosis. The samples were immediately centrifugated and the plasma was stored at –20º C until their analysis. The quantification was performed by a HPLC technique.[5]

The BIOPHARM 1.1 pharmacokinetic program processed the data obtained and the 0.8-1.25 interval was used as acceptation criterion for the equivalence, regarding the reference taken as unit, according to what is accepted by the American FDA. The data were analyzed for a reliability interval 90 % and for one probability (p = 0.05).

Unique-dosis bioequivalence assays in humans

The experiment was performed with wealthy non-smoker male voluntary adults. The relative bioequivalence assays were performed according to a cross design. Washing time: 2 weeks. The blood was collected during 32 – 48 h after the oral dosis and it was stored until the moment of the HPLC analysis. The data were processed in the same way used for the samples of the experiments with dogs.

Multiple-dosis assay in voluntary humans

The voluntary humans took 800 mg of theophylline a day in two daily 400 mg dosis (four 100 mg tablets) every 12 h for a whole week. It was necessary to decrease the dosis to 600 mg a day for one of the voluntary. At the end of the week, the levels of serum were determined for the

12 h after the last ingestion of tablets. The quantification of the samples was performed in the same way above mentioned.

Selection of the formulations used for the *in vivo* assays

From the *in vitro* released data, the use of the expression:

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

makes possible to obtain, through the value of the exponent (n), criteria on the type of the kinetics of the diffusion of the drug to the liquid medium and with the value of the k constant, the release velocities of different formulations can be compared, even for those cases that the kinetic release models do not coincide. For cylindric geometries (tablets):

a) n < 0.5 show a diffusion mechanism according to the Fick laws.

b) 0.5 < n < 0.89 show a controlled diffusion mechanism by the growth of the difference between the initial content of drug in the matrix and its content in every moment of release, as well as, by the relaxation of the matrix molecules as a consequence of the plastification and the swelling of the polymer caused by the aqueous medium diffusion in the matrix.

c) n > 0.89 it shows a mechanism mainly controlled by the polymer molecules' relaxation. The equation is only applicable for values with a concentration up to 60 % released drug.[3,4]

The release with zero kinetic order, steady and constant in time can be achieved only when the relaxation of the matrix takes place so fast and deep that it is able to compensate the normal progressive decrease of the velocity resulting from the decrease in time of the quantity of drug retained by the matrix. Therefore, this ideal release can only be achieved with relatively high values of n coefficient. From the chosen standards, the Slo-Bid shows the highest value of n (n = 0.66, three releases' average). For this reason, the authors tried to achieve a formulation equivalent or similar to that of the Slo-Bid since the very beginning.

RESULTS

To achieve the purpose with the desired quality, it was used mixed matrixes. In order to obtain those matrixes two granulates were mixed

with a different content of the same POVIAC 0 polymer.

Eight formulations of tablets were prepared with 100 mg doses of anhydrate theophylline, by using mixtures of granulates containing 5, 11, 14 and 20 % POVIAC 0 and similar content of lubricant (magnesium estearate 1 %). The granulates were mixed in binary combinations with 30 parties of one and 70 parties of the other. Those tablets, manufactured at pilot scale (5 kg), were characterized and their release profile was obtained *in vitro* in triplicate. From those profiles, the **n** and **k** values of the expression 1 were calculated.

Table 1 shows the results of the technological characterization with the **n** and **k** values obtained for the Theo-Dur and Slo-Bid standards, as well as for the eight formulations evaluated at first. Those are the results obtained when applying the expressions corresponding to a design of experiments 2^3. To do this, each mixed matrix was considered as the mixture of two granulates, one with a low content of polymer (low level 5 %, high level 11 %) and a granulate with a high content of polymer (low level 14 %, high level 20 %). This way, the three independent variables are:

X_1 content of polymer in the low content granulate.

X_2 content of polymer in the high content granulate.

X_3 fraction of the total mass of the tablets corresponding to the granulate with a low content of polymer, fraction that can correspond to two different levels (0.30 or 0.70).

Considering **n** as a dependent variable, we obtained for it the codified polynomial once the non-significant coefficients, lower than the value of the coefficient of the triple interactions ($B_1, B_2, B_3 = 0.040\,0$) have been removed.

$$n = 0.537\,5 - 0.065\,833X_1 - 0.045\,833X_2 - 0.038\,38X_3$$

This result means that if you want to increase the value of **n** in the rank of values studied for the different variables, the values of the polymer concentrations of both granulates must be decreased, but fundamentally in the granulate with the lowest content. That has as limiting feature the necessity of achieving an appropriate velocity of release.

In the case of the formulation 3, the increase of the content of polymer of the granulate with the highest content does not produce a decrease of **n** because the content of the other granulate is low and especially, because the rate of the granulate with the high content is the highest. This determines a very low release velocity K = 0.14). For low velocities, the percentage of drug released in the period of study is low and the release tends to an apparent and false linearity, what is shown by the high value of **n**. The discussed results indicate that the best formulations are the formulations 2 and 5. The design polynomial suggests that the formulations can be still optimized; for instance, by reducing the polymer content of the low-content granulate of the formulation 2 and slightly increasing the rate of the other granulate to obtain an appropriate release velocity with a high value of **n**.

Nevertheless, the formulation 5 was chosen (Table 1) to be evaluated *in vivo* because of its relative nearness of the **n** value to the corresponding value of the Slo-Bid capsules. To do that, a bioequivalence study was first performed in eight Beagle dogs at a unique dosis of 200 mg theophylline (two tablets 100 mg oral dosis) (Fig. 1).
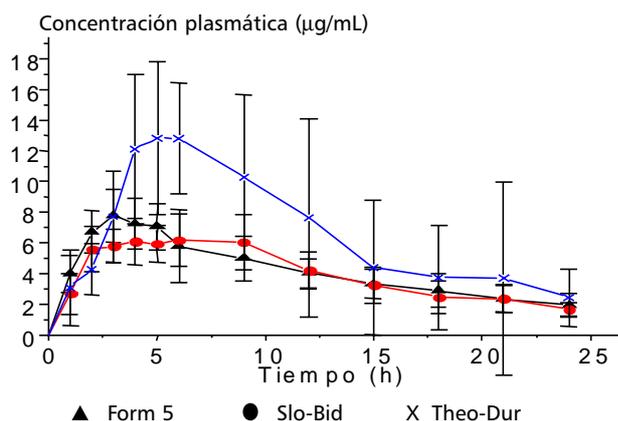

Fig. 1. Plasmatic concentration versus time in experiences with unique dosis in Beagle dogs (n = 8). Formulation 5: Two 100 mg tablets of anhydra theophylline. Slo-Bid: Two 100 mg capsules of anhydra theophylline.

Table 1. Composition of the controller matrix. The values in percentage correspond to the rate of POVIAC 0 in each granulate used, the given values with the p letter (parts) correspond to the rate of mixture of the granulate with different content of polymer.

Formulation	Technological characterization				Release		Composition of the polymeric controller matrix.
	Weight (mg)	Height (mm)	Hardness (kgf)	Friability (%)	n	k	
1	125	3.61	12	0.11	0.58	0.25	5 % (30p) & 14 % (70p)
2	128	3.68	12	0.40	0.63	0.21	11 % (30p) & 14 % (70p)
3	132	3.75	12	0.37	0.71	0.14	5 % (30p) & 20 % (70p)
4	135	3.78	12	0.03	0.40	0.16	11 % (39p) & 20 % (30p)
5	120	3.60	11	0.03	0.63	0.22	5 % (70p) & 14 % (30p)
6	126	3.62	11	0.03	0.52	0.22	11 % (70p) & 14 % (30p)
7	123	3.61	10	0.03	0.49	0.24	5 % (70p) & 20 % (30p)
8	129	3.73	12	0.10	0.37	0.28	11 % (70p) & 20 % (30p)
Slo-Bid Gyrocaps	–	–	–		0.66	0.19	
Theo-Dur	–	–	–		0.45	0.27	

Table 2 shows an equivalence only for the area under the curve from 0 to infinite (AUC) and for Tmax. The area under the curve for 0 to 32 h (AUC lqc) and the Cmax./AUC parameter show value only slightly apart from the interval accepted as a bioequivalence criterium. The Cmax value is significantly different from the mentioned criterium. However, for some pharmaceutical products, the 0.70 – 1.43 rank has been considered as an acceptation criterium of bioequivalence for Cmax.[8]

Theo-Dur was also evaluated, and it was observed that it had a biological disponibility a little higher in relation to the other two formulations.

Afterwards, a bioequivalence study was performed in eight healthy voluntary subjects at a unique dosis of 200 mg theophylline (two tablets 100 mg) for each formulation (5 formulation and Slo-Bid).

The results (Fig. 2 and Table 3) show that both formulations has an *in vivo* behavior very similar, even though they are not strictly bioequivalent.

Both formulations are particularly attractive for the treatment of infants by virtue of a delivery of the considerably steady drug. That decreases the risks of adverse effect during the treatment because it is easier to keep the drug's plasma levels into the therapeutic rank. Figure 2 shows that the average concentration of drug in plasma, although it moves in relatively low levels that can be attributed to the low dosis administered, it is kept from 0.6 and 0.9 μg/mL from the first hour to the following twenty four hours.

Even though the results shown let us to consider the formulation 5 as a good solution, the authors tried to compare the results obtained from tablets produced from two granulates to those obtained from new tablets produced with only one granulate and containing a global quantity of POVIAC 0 similar to the first tablets. This was performed in order to simplify, if possible, the technology for the production of the formulation finally chosen. Table 4 shows the results of the *in vitro* dissolution study

for two formulations (formulations 9 and 10) with a total content of POVIAC 0 equal to that of the 1 and 5 formulations of Table 2 (11.3 and 7.7 %, respectively), but manufactured from only one granulate.

Tables 1 and 4 show that the release profile of formulation 1 is different from the profile of the tablets of formulation 9, while in the case of the formulation 5, the *in vitro* release profile is very similar to that of the formulation 10. Considering

this result, a semi-industrial batch of tablets of the formulation 10 was manufactured and employed in a study at multiple dosis in voluntary healthy adult humans (Fig. 3 and Table 5).

Figure 3 shows the constancy of the serum levels of theophylline for the 12 h elapsing between one dosis of the drug and the following one is highly satisfactory. The drug level reached corresponds to the lower sector of the therapeutic rank, what is appropriate for the given doses.

Table 2. Results of the study of bioequivalence of the formulation 5 regarding Slo-Bid in Beagle dogs (n = 10).

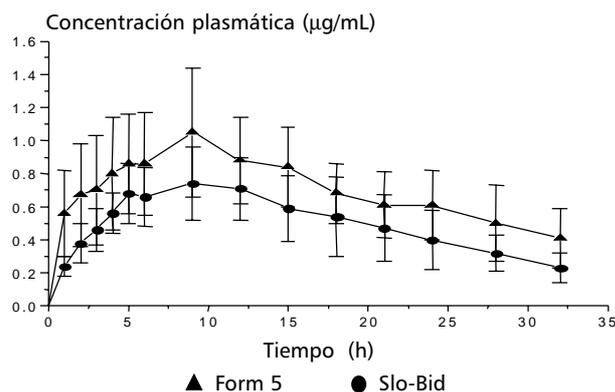| Parameters	IC 90 % (Criterium 0.80 – 1.25)	Bioequiv.- punctual (%)
AUC	0.90 – 1.21	104
AUC lqc	1.00 – 1.26	112
Cmax./AUC	0.98 – 1.27	112
Cmax.	1.03 – 1.32	106
Tmax.	–	100

Concentración plasmática (µg/mL)

Fig. 2. Plasmatic concentrations versus time of the formulations 5 and Slo-Bid in study with unique dosis in adult humans n = 8. Dosis for each formulation: 200 mg in two tablets or two capsules.

Table 3. Results of the study of bioequivalence of the formulation 5 regarding Slo-Bid in voluntary adult humans (n = 8).

Parameters	IC 90 % (Criterium 0.80 – 1.25)	Bioequiv.- punctual (%)
AUC	1.12 – 1.48	129
AUC lqc	1.29 – 1.60	143
Cmax./AUC	0.96 – 1.32	113
Cmax.	1.27 – 1.66	145
Tmax.	–	100

Table 4. Technological characterization, release constants and composition of the polymeric matrix of the formulations 9 and 10.

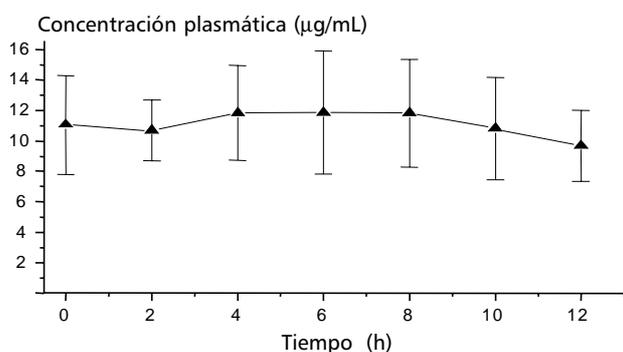| Formulation	Technological characterization (10 tablets average)				Release constants		Composition of the polymeric matrix
	Weight (mg)	Height (mm)	Hardness (kgf)	Friability (%)	n	k	(%)
9	125	3.55	12	0.11	0.52	0.277 6	11.3
10	120.8	3.44	>13	0.2	0.630 17	0.237 4	7.7

Concentración plasmática (µg/mL)

Fig. 3. *Experience with multiple dosis in voluntary healthy humans (n = 8).*

Table 5. Study with multiple dosis in voluntary healthy adults (n = 8). Formulation 10.

$C_{máx.}$ (µg/mL)	$T_{máx.}$ (h)	Vd (L)	V_{dss} (L)	C_{av} (µg/mL)	C_{min} (µg/mL)
12.96	5	0.026	0.028	11.12	9.17

CONCLUSIONS

A 100 mg formulation was developed with the use of POVIAC 0 in the regulating matrix that showed an *in vivo* behavior very similar to Slio-Bid, although it is not bioequivalent. This formulation has a biological disponibility slightly higher than that of Slo-Bid and slightly lower than that of Theo-Dur.

The results of the design of the experiments allowed us to set the premises to obtain optimized formulations formed by a mixture of granulates with different content of POVIAC 0.

A 100 mg formulation was developed with the use of POVIAC 0 in the regulating matrix that showed an *in vivo* behavior very similar to Slo-Bid. This formulation, obtained from only one granulate showed an appropriate release profile for keeping the plasma concentration of theophylline over and close to the lower limit of the therapeutic rank in an steady way for 12 h when it is given in multiple dosis (a way corresponding to the preventive treatment of asthma with sustained release theophylline formulations).

BIBLIOGRAPHY

1. Autorización de uso de Acetato de Polivinilo como Auxiliar Farmacéutico. Centro de Control de Medicamentos (CECMED), Ciudad de La Habana, Cuba, ERM 685/97.
2. Suzarte A. y col. Polímeros derivados del acetato de vinilo como aglutinantes y matrices en formulaciones farmacéuticas de acción sostenida. Patente cubana No. 22199, 1993.
3. Ritger P.L, Peppas N.A. **J. Contrl. Release, 5**, 23-36, 1987.
4. Ritger P.L, Peppas N.A. **J. Contrl. Release, 6**, 37-42, 1987.
5. Contreras J., Ontivero E. , González R., López M., J. **High Resolution Chromatography, 22**, 131, 1999.
6. Contreras J., Pérez N., González R., Ontivero E., López M., Arzneimittel Forschung/**Drug Research, 48**, 3, 259-262, 1998.
7. Arias A. y col. Estudio de toxicidad a dosis única y repetida del polivinilacetato, Informe Final, Centro Nacional de Toxicología, Hospital Militar "Dr. Carlos J. Finlay", Ave. 31 y 114, Marianao , Ciudad de La Habana , 1996.
8. Steinijans V.W., Hauschke D. **Clin. Research & Reg. Affairs, 10**, 203-220, 1993.

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