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DEVELOPMENT OF METHODS FOR THE PREPARATION AND EVALUATION OF CHLORAMPHENICOL PALMITATE ESTER AND ITS BIOPHARMACEUTICALLY EFFECTIVE METASTABLE POLYMORPH

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ABSTRACT

Polymorphism greatly influences bioavailability because among other variations, the different polymorphs of a particular compound have different solubilities due to different arrangements of molecules in the solid state giving rise to different lattice energies. Chloramphenicol palmitate (CMP) has three polymorphs with different thermodynamic stability. Polymorph A (m.p. 90°C) is the stable variety; Polymorph B (m.p. 89°C) is the metastable variety and Polymorph C is the unstable variety. For the preparation of our desired polymorph B, the available literature is quite insufficient and most of them are well guarded by patent secrets. This work was directed towards the development of modified method of Preparation of CMP and subsequent polymorphic modification to give rise to the desired metastable bioavailable variety. The prepared polymorph when compared with reference standard with respect to M.P., TLC Analysis and I.R. Spectroscopy, shows equivalency in all respect. In-vitro Enzymatic Hydrolysis,

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Dissolution Studies and In-vivo Studies on Intestinal Absorption show superiority of polymorph B over polymorph A.

INTRODUCTION

It has been universally accepted that, in case of polymorphs bioavailability is inversely proportional to the chemical stability of that polymorph. In this investigation efforts were made for the preparation of CMP with the metastable-β - polymorphic crystal lattice structure. Various subsequent experiment have been done for comparing the prepared polymorph with the reference standard. Co-relation of the experimental datas indicates the achievement of the desired polymorph.

EXPERIMENTAL

Materials *: All the materials used were of analytical grade. The metastable CMP R.S. was supplied by Courtesy of C.D.L., Calcutta.*

Methods *:*

Step-I - Preparation of CMP ester : The primary object of using chloramphenicol esters in formulations is to overcome the bitter taste of the drug. Many methods have been suggested by many workers among which a few were found suitable. After an intensive research work a convenient modified method has evolved for the preparation of CMP. The method is as follows :

2.2 ml of palmitoyl chloride was added dropwise with stirring to a solution containing 2.0 g of chloramphenicol in 4.5 ml of acetone containing 0.7 ml of pyridine at 20°C and stirring was continued for 2 hrs ; the mixture was added to 32 ml Water containing 0.1 ml HCl at 20°C. The precipitate was filtered off, washed and dried. The product was dissolved in 20 ml of iso-propanol at 55 - 60°C and filtered with activated charcoal. The filtrate was then cooled to 40°C and methyl amine was added to adjust the pH to 8.5 . The resulting solution was

then poured into 18 ml water at 20°C. After precipitation, the residue was washed, filtered and dried. Washing should be repeated until the pH falls to 7.2 to 7.0. The dried residue was then extracted with 2.8 ml of petroleum ether for 2 to 3 times and then kept overnight in a desiccator maintained at 40°C.

Step II - Preparation of β - polymorph (metastable variety) of CMP : For the preparation of desired polymorph, many trials were carried out. But all of them became unsuccessful. Finally, the preparation of the metastable variety was achieved by removing camphor from eutectic mixtures with CMP[5]. This method as standardised with repeated trials is given below.

5.0 g of camphor and 3.0 g of CMP were taken in a dry mortar - pestle and well mixed until a uniform clumpy mass was formed. It was then removed to a hard glass tube and melted on an oil bath at 160°C. The melted mass was chilled quickly to 0 - 5°C and was kept in a refrigerator for one hour. Then the whole mass was transferred to a flat container and kept under vacuum of 12 cms in a desiccator at 40°C. The lid of the desiccator was opened three times a day to allow fresh air to go inside and by this way the rate of removal of camphor slowed down. This operation was continued for 3 days and then the mass was vacuum dried at 50°C for 8 hrs. The product was kept overnight in the desiccator at low pressure. Then a self-life study of the prepared polymorph was conducted for about 8 months.[4]

Step III - T.L.C. analysis of prepared polymorph of CMP and its reference standard : T.L.C. analysis was carried out for checking up the identity and purity of the prepared sample. Silica gel G plate was taken which has been activated at 110°C for one hour. The developing solvent was chloroform : methanol : glacial acetic acid in 18:1:1 proportion. After development, the plate was air dried for some time. The stannous chloride solution was then sprayed and dried in hot air oven at 80°C for 15 minutes. The plate was then taken out and the colouring

reagent, p-dimethyl amino benzaldehyde was sprayed. Yellow spots of CMP were developed.

Step - IV - I.R. spectroscopy of prepared polymorph and reference standard of CMP[3] : Two samples were analysed under the identical instrumental and environmental condition (Frequency 4000 to 600 cm^{-1}). The cell path used was Nujol, scan speed ---> slow and slit ---> N.

Step - V - In-vitro studies on enzymatic hydrolysis of Polymorph A and prepared Polymorph B of CMP : When administered orally, the esterified drug first gets hydrolysed by the enzyme present in the G.I. tract, then it becomes available for absorption. Therefore, this hydrolysis step is the rate limiting step in its absorption. Study has been carried out to determine the rates of enzymatic hydrolysis of polymorph A and prepared Polymorph B.

2.0 g of CMP was taken and 100 ml of a uniform suspension was made using polysorbate 80 . 1.0 ml of this suspension was added to 99.0 ml of simulated intestinal juice (U.S.P. XVII [2]), and the whole content with continuous stirring was mounted on a water bath maintained at 37 $\pm$ 0.5°C. The samples drawned at definite intervals, were treated to get the clear solution which was analysed spectrophotometrically at 278 nm[9]. Log of % of ester to be hydrolysed was plotted against time.

Step - VI - Comparison of the dissolution characteristic of Polymorph A and prepared polymorph B of CMP : CMP is insoluble in water. In order to have a comparative idea regarding the relative dissolution characteristics of the above two polymorphs, two discriminating solvent systems were choosen. The first one was absolute alcohol and the second one was 35% (v/v) mixture of tertiary butanol and water[10].

Dissolution tests were carried out with excess ester. The temperature and stirring rate were kept constant throughout the

operation. Sampling were done at suitable intervals. Each sample was filtered, diluted accordingly and the amount of ester dissolved was determined spectrophotometrically[9].

Step VII - In-vivo studies on intestinal absorption of Polymorph A and prepared Polymorph B of CMP : Eight rabbits of same sex (male) and of more or less uniform weight (1.25 to 1.32 kgs) were taken. Prior to the experiment they were aclamatised for ten days with same diet and environment to minimize the environmental variation. On the day of experiment, they were divided into two equal groups. A dose of 30.0 mg was administered orally to each animal of one group with Polymorph A and another group with Polymoprh B. 3.0 ml blood sampling was done by heart puncture after an interval of one hour and was continued for 8 hrs. So we get total 64 samples which were centrifuged separately for 40 minutes at 10,000 r.p.m. 1.0 ml of serum was collected from each tube and treated individually and finally the amount of CMP was determined spectrophotometrically[9]. At a particular time, four different concentrations were got for four animals of the same group. Hence, the mean of four different concentrations was taken and on the basis of the mean data the blood level curve was plotted.

RESULTS AND DISCUSSION

In step I, the yield was 82.0% of the theoritical value and the m.p. of the product was 88 - 89°C. The product if recrystallised from benzene yields stable polymorph with the m.p. 90°C, which is the m.p. prescribed by Merck Index[1]. $[\alpha]_D^{26}$ + 24.6° (C = 5 in ethanol). This method if further be modified to recover the solvent it will become more economic and effective one and can easily be employed in industrial practice.

In step II, the m.p. of the product was 89°C, which indicates the formation of desired Polymorph. The self life study shows a steady m.p. of 89°C for the period tested.

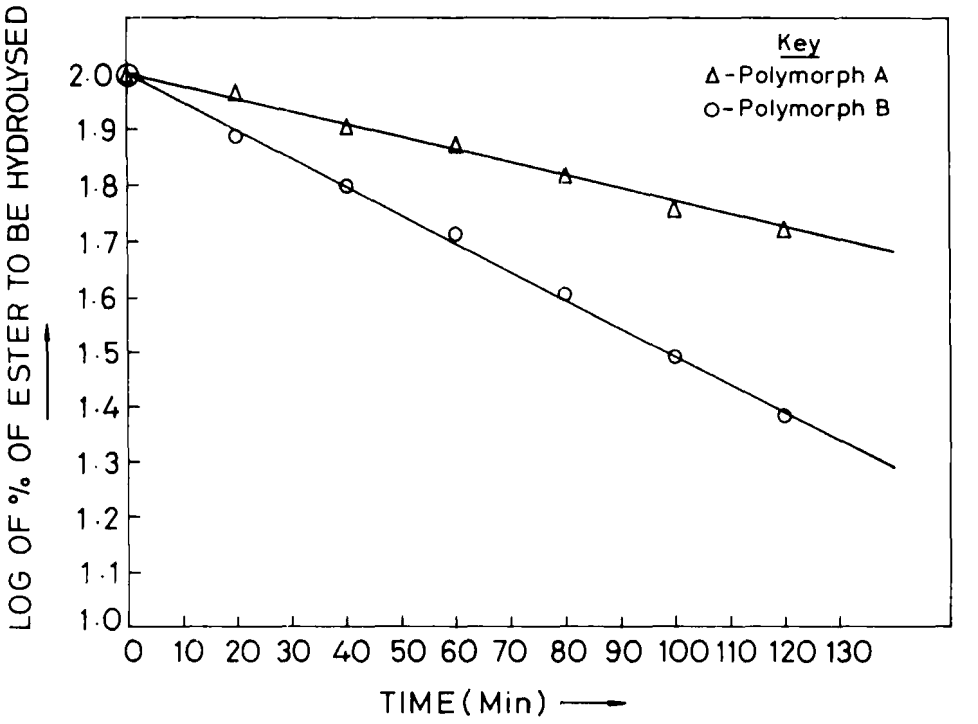

FIGURE 1

Log of % of ester to be hydrolysed versus time plot.

Therefore, if the time consumption could be reduced, the method would become an ideal industrial method for preparation of metastable CMP.

In step III, the R_f values of CMP R.S. and that of our prepared sample were calculated. In both the cases it was same.

From the results of the I.R. spectroscopic analysis of step IV, it was found that all the peaks of the prepared polymorph were almost equal (within the permissible limit) with that of the reference standard. Thus, we can conclude that the polymorphic nature of the prepared sample and that of the reference standard are identical.

In step V, the log % of ester to be hydrolysed vs. time plots give the straight lines (fig. 1) which indicates the first

order reaction. The K value of Polymorph B is about 2.2165 times greater than that of Polymorph A.

It has been observed from fig. 2 and fig. 3 that in both absolute alcohol and 35% (v/v) tert - butanol - water systems the saturation solubility of prepared Polymorph B is about 3.3 times greater than that of Polymorph A. Hence, it has been again established that the rate and extent of dissolution of prepared polymorph B is much greater than that of Polymorph A. Another attempt was made to see whether the change in the type of solvent has any effect on free energy differences between polymorphs or not.

The free energy differences were calculated from the formula

$$\Delta_{GT} = RT \ln \frac{C_s \text{ of Polymorph A}}{C_s \text{ of Polymorph B}}$$

In 35% (v/v) tert - butanol - water system,

$$\Delta_{GT} = 1.9807 \times 301 \times \ln \frac{0.13}{0.43} = -713.19358 \text{ cal./mole.}$$

and in absolute alcohol system,

$$\Delta_{GT} = 1.9807 \times 301 \times \ln \frac{9.5}{31.5}$$

$$= -714.65126 \text{ cal./mole.}$$

Hence, the variation is about 0.204% which can be neglected. Most of data evaluated, were found similar with the standard data.

In the last step, emphasis has been drawn to establish a relationship between polymorphism and its influence on bioavailability. If we refer the fig. 4, we find that peak plasma concentration of the prepared Polymorph B is achieved in or about 2 hrs. But in case of Polymorph A, it takes 1 - 2 hrs. The peak plasma concentration of Polymorph B is 3.75 μg/ml and that of Polymorph A is 0.525 μg/ml.

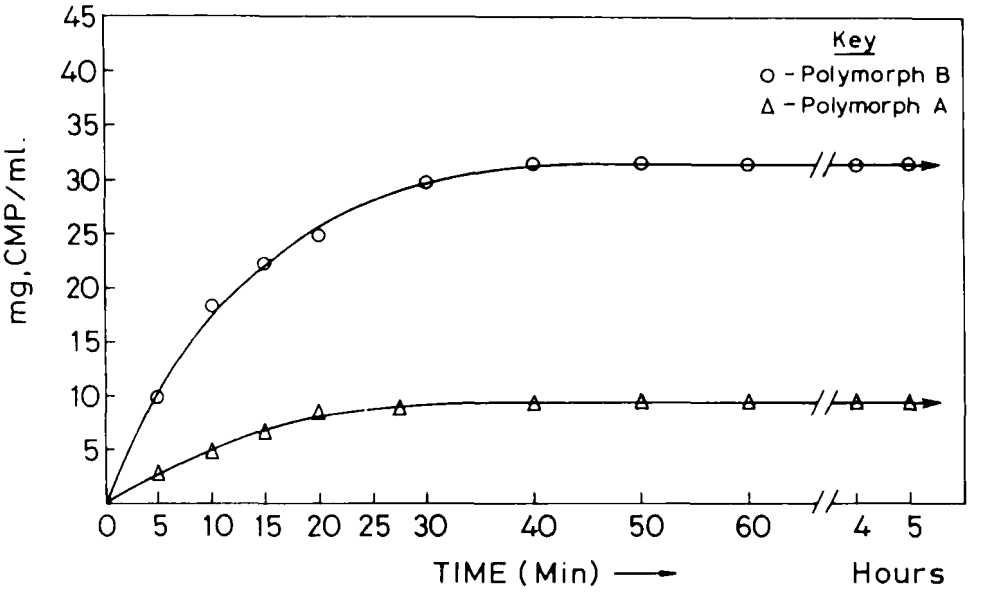

FIGURE 2

Dissolution behavior of CMP polymorphs in absolute ethanol at 28°C.

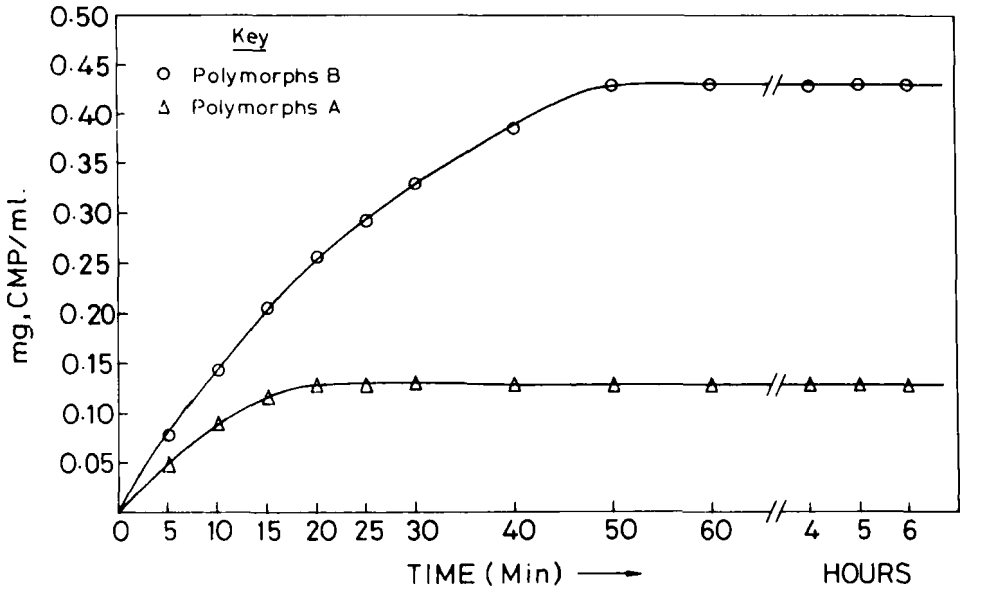

FIGURE 3

Dissolution behavior of CMP polymorphs in 35% v/v tert.-butanol at 28°C.

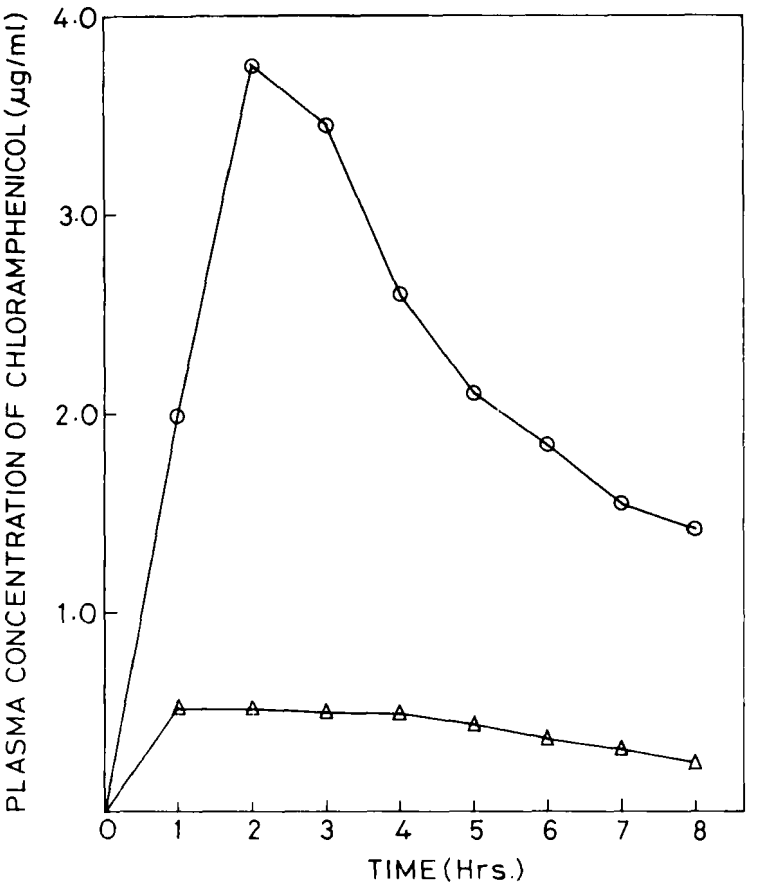

FIGURE 4

Plasma-level curve of Chloramphenicol.

If the data have been compared with the enzymatic hydrolysis data of step V, we can directly correlate the in-vivo and in-vitro hydrolysis and absorption pattern. More or less the bio-pharmaceutically effective polymorph in a single dose administration took about 2 hrs. to be hydrolysed and its absorption was more or less instantaneous. If the other controlling factors of the biological absorption study could be standardised, the in-vitro data then would directly be implemented in the dose calculation and dose gapping.

SUMMARY

1. Methods for the preparation of CMP ester and its metastable polymorph was developed.

2. Prepared polymorph when compared with R.S. with respect to m.p., R_f value and I.R. spectra, shows equivalency in all respect.

3. In-vitro study on enzymatic hydrolysis, dissolution characteristic and in-vivo studies on bioavailability show superiority of Prepared Polymorph B over Polymorph A.

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