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RESEARCH PAPERS

PHYSICO-CHEMICAL STUDY OF INCLUSION COMPOUND PHENOTHIAZINE - β-CYCLODEXTRIN

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KEYWORDS

β-cyclodextrin, phenothiazine, spray dried, NMR, Differential Thermal Analysis, inclusion compound.

SUMMARY

In this work, the authors have made many assays to prove the inclusion of phenothiazine in β-cyclodextrin.

The inclusion compound was prepared in two various ways and we have checked the inclusion using NMR and Differential Thermal Analysis tests.

This experimentation leads to other assays making inclusion compounds with molecules using the phenothiazine core and used in humans medicines.

INTRODUCTION

The accomplishment of molecular compounds including cyclodextrins can aim at increasing the solubility of lipophiles molecules inside an aqueous medium as well as improving conditions of stability and biodisponibility.

In this context, we've considered studying the inclusion of phenothiazine within cyclodextrin molecules.

Phenothiazine, which is scarcely soluble in water, is used by vets and can be seen as a leader for various molecules used to cure human beings such as chlorpromazine.

The acknowledgement of the compounds obtained through various methods was achieved, among other means through Nuclear Magnetic Resonance and Differential Thermal Analysis.

MATERIALS AND METHODS

1. Materials

β-cyclodextrin (Roquette Frères - Lestrem, France and Rhône Poulenc Chimie - Courbevoie, France); phenothiazine (Merck-Clévenot S.A. - Nogent-sur-Marne, France). Those products have been used without any special purifying method.

The other products used here have analytical grade.

2. Apparatus

Spectrophotometer UV-Vis. Perkin Elmer 550 SE; NMR Brucker AC 200; melting point equipement Gallenkamp MPA (Fisons PLC Equipement Division); differential thermoanalyser DSC/TADS System Perkin Elmer; Rotovapor CR Büchi; spray drier Minor Mobil Niro Atomizer (Denmark); peristaltic pump Masterflex Cole Parmer Instrument.

3. Preparation of solid complexes

Among several methods in use to achieve molecular compounds [1] [2] [3], two methods have been used:

- evaporation under vacuum control;
- spray drying.

In both cases a molar ratio of 1/1 has been used in mixture β-cyclodextrin-phenothiazine [4] [5] [6].

Both processes which give birth to the compound, proceed from the same preparation of β-cyclodextrin - phenothiazine.

Phenothiazine is made soluble in ethanol under light screen then it is stirred and added to an aqueous solution of β-cyclodextrin which is heated up to 55° C. After three hours stirring, cold water is added which accelerates the inclusion mechanism (tab. I).

3.1 Preparation of the compound through evaporation under vacuum control.

The solution M is evaporated under vacuum control at a temperature of 55° C inside an equipement of type Rotovapor until a fine powder is obtained (compound E).

3.2 Preparation of the compound through spray drying process

The solution M is injected by means of a peristaltic pump into the spray drying system inside which the solution is dried by being sprayed in a hot air.

The optimization of the spray drying process through a method using the analytical plan has allowed to set up work conditions (tab. II) [7] [8].

The powder obtained (compound N) is, as it was previously, kept under light screen and in a dry place.

4. Analytical methods

4.1. Melting point

The sample is introduced into a tube which is strongly heated, with constant increment, allowing to acknowledge two parameters:

- the aspect of the sample;
- the temperature of fusion and/or of carbonization of the product.

4.2. Nuclear Magnetic Resonance (NMR)

The analysis of NMR is achieved at 200 MHz. First, the samples are crystallized three

TABLE 1
Solution of β-cyclodextrin – phenothiazine (solution M)

β-cyclodextrin	28.1 g
distilled water	300.0 ml
phenothiazine	4,93 g
EtOH	61.0 ml
distilled water ad to	500.0 ml

TABLE 2
Spray drying process parameters

air pressure	4 kg/cm2
inlet temperature	160°C
flow rate	0.67 ml/s

times in D_2O then placed in a solution into D_2O or into DMSO. The sampling tubes are type RMN 5 UP.
The internal sample is 3 (trimethylsilyl) propionic-2,2,3,3 D4 acid for spectrum realized in D_2O.

4.3. Differential Thermal Analysis (DTA)

The sealed sample is placed in the oven of the equipement. The analysis is carried out by calculating differential measures in relation with an inert sample.

5. Control methods

The control methods recommended by European and French Pharmacopoeia, as far as the various substances used are concerned, have been realized.

RESULTS AND DISCUSSION

Through the various analysis methods used, we have tried to make clear the inclusion process between β-cyclodextrin and phenothiazine.

1. Melting point

The different melting points are given in table III.

The assays which are carried out show a quick carbonization of β-cyclodextrin in opposition to the spray dried compound.

TABLE 3
Melting points

Phenothiazine:	185.5° C
β-cyclodextrin (Roquette):	296.6° C
β-cyclodextrin (Rhône Poulenc):	302.0° C
Compound N:	291.0°C

2. NMR

- in D_2O

The spectrum NMR of β-cyclodextrin (fig. 1) as well as of compound N (fig. 2) have been achieved at 200 MHz.

In 3.6–4.O ppm zone (fig. 2), a general modification of the set of peaks can be noticed as well as a group of protons moving left, which tends to prove the inclusion between β-cyclodextrin and phenothiazine.

- In DMSO

The spectrum NMR of phenothiazine (fig. 3), of β-cyclodextrin (fig. 4),as well as of compound N (fig. 5) have been also realized at 200 MHz in DMSO.

In spite of water reaching a peak at d=3.4 ppm, it has been shown that compound N contained phenothiazine (9).

3. Differential Thermal Analysis (DTA)

Phenothiazine (fig. 6) shows an endothermic peak situated between 180 and 197° C which fit with 264 calories per gram.

β-cyclodextrins Roquette and Rhône Poulenc shows an endothermic peak between 70 and 190° C with respectively 546 and 509 calories per gram (fig. 7 and 8), which tends to demonstrate that there wouldn't be any significant difference between those two dextrins [10] [11] [12] [13].

The analysis of compound N shows two peaks, one from 70 to 186° C and the other from 186 to 201° C with respectively 279 and 9.32 calories per gram (fig. 9).

By comparing with spectrum of pure β-cyclodextrin (fig. 7 and 8), a reduction of the endothermic peak can be noticed, which would tend to prove that inclusion has been realized [10]. This reduction can be explained by new links being etablished during inclusion.

The analysis of compound E (fig. 10) shows three endothermic peaks respectively at 70 – 185° C (674 calories per gram), 186 – 207° C (16.19 calories per gram), 218 – 242 calories per gram).

In compound E, the peak of β-cyclodextrin is the same as that of pure β-cyclodextrin. This shows that the compound hasn't been achieved and that it's just a simple mixture (fig. 11).

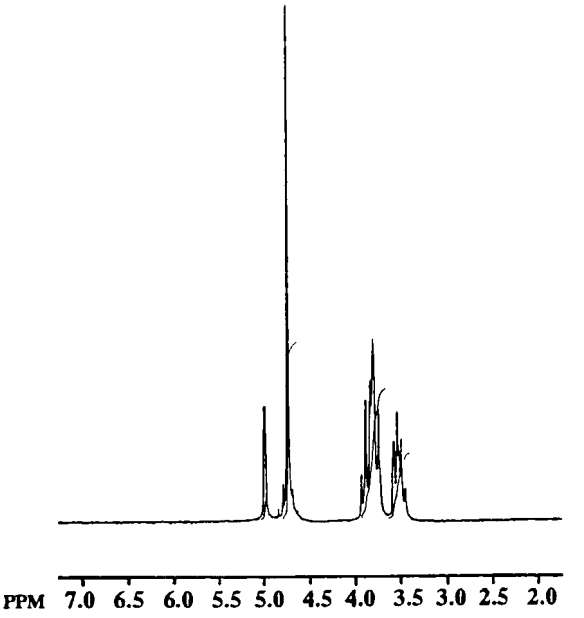

FIGURE 1
Spectrum NMR of β–cyclodextrin (Roquette) Solvent D_2O

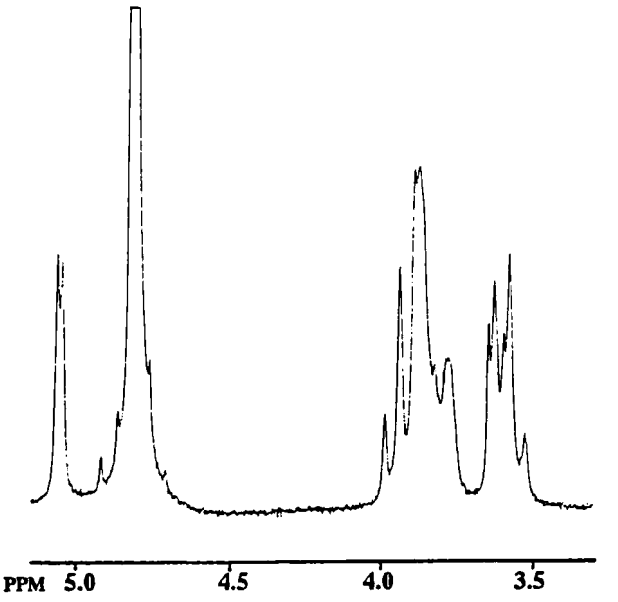

FIGURE 2
Spectrum NMR of compound N – Solvent D_2O

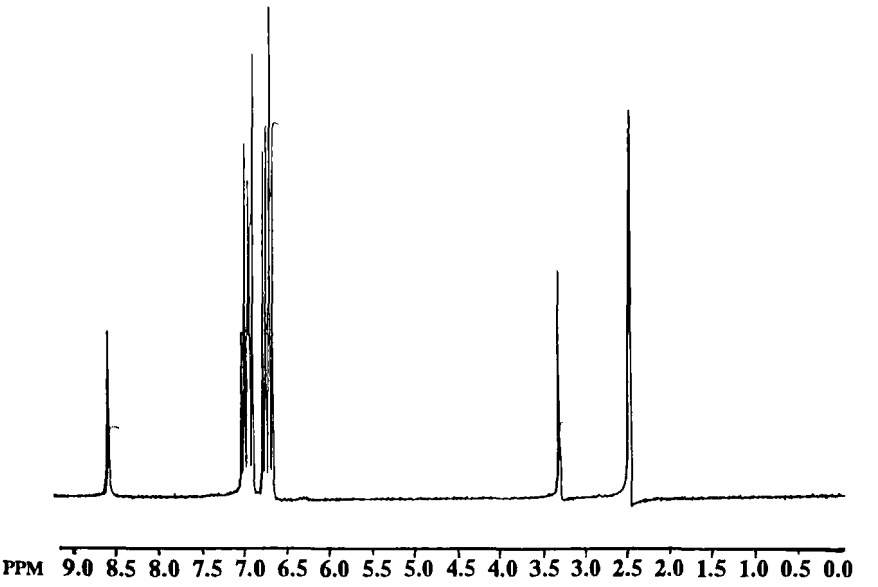

FIGURE 3
Spectrum NMR of phenothiazine – Solvent DMSO

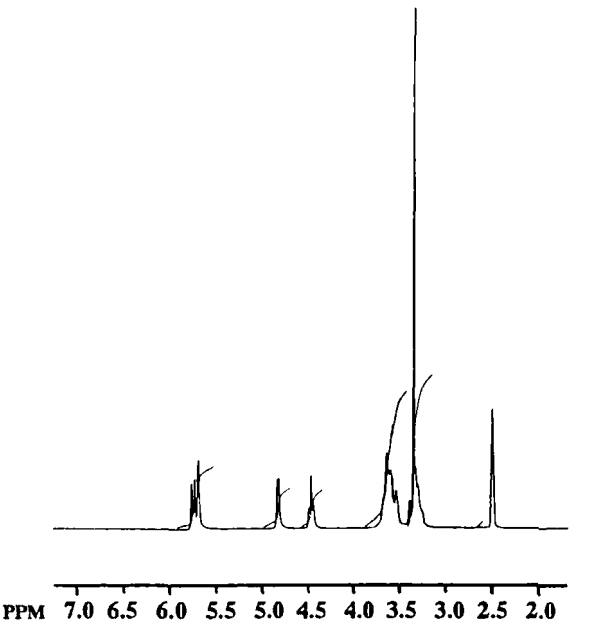

FIGURE 4
Spectrum NMR of β-cyclodextrin (Roquette) – Solvent DMSO

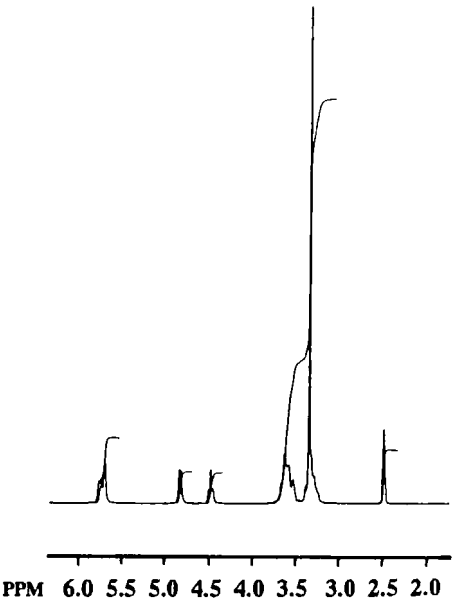

FIGURE 5
Spectrum NMR of compound N – Solvent DMSO

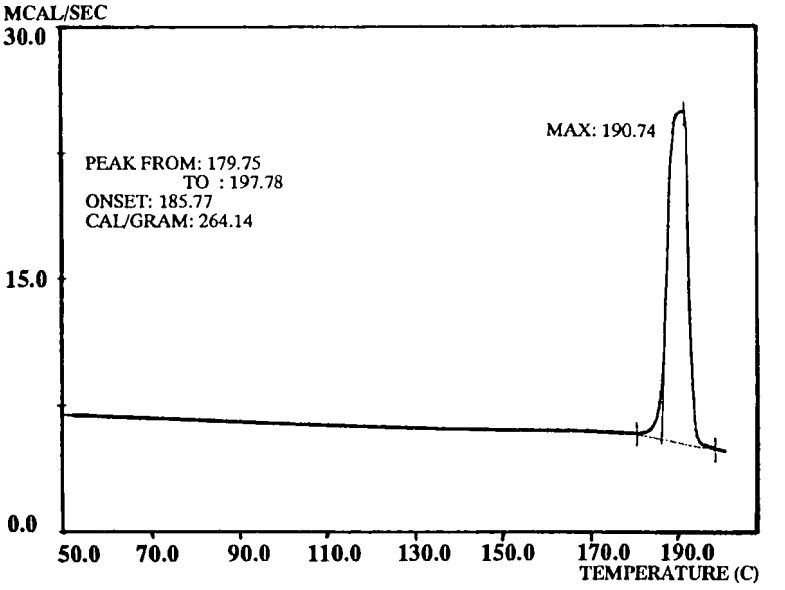

FIGURE 6
Phenothiazine (DTA)

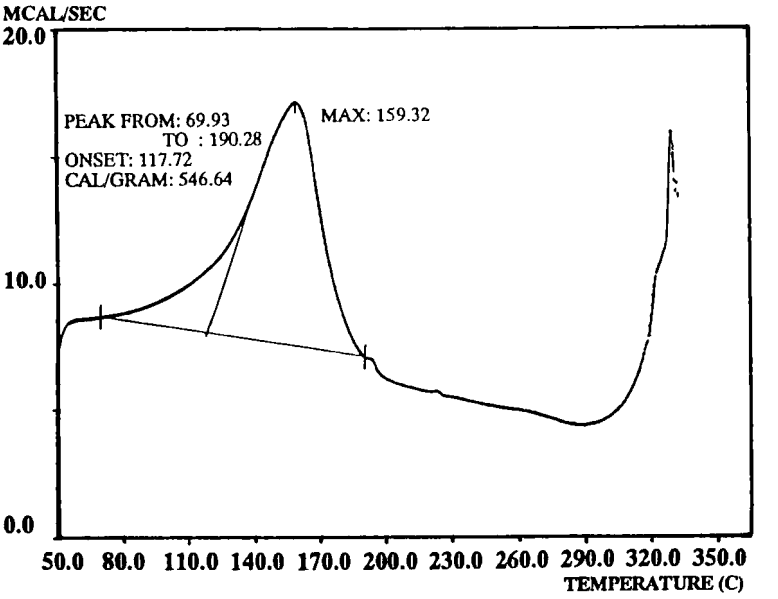

FIGURE 7
β-cyclodextrin (Roquette) (DTA)

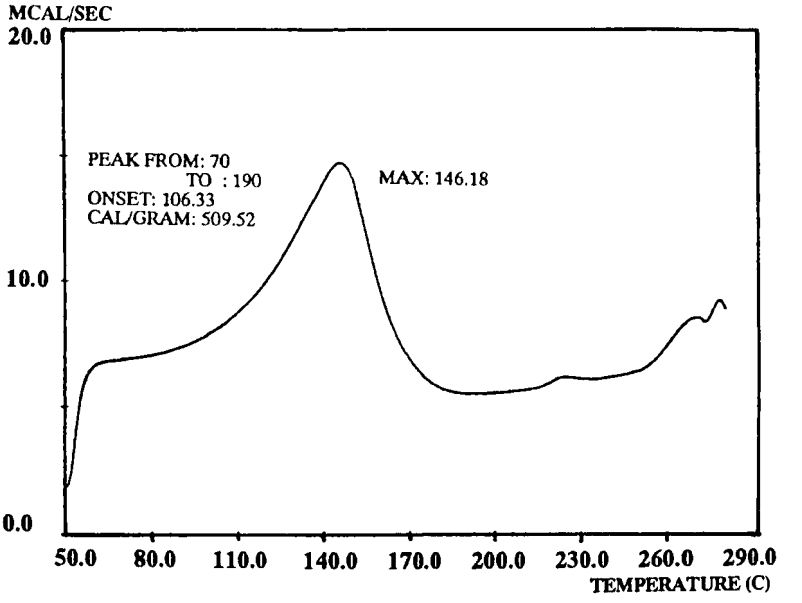

FIGURE 8
β-cyclodextrin (Rhône Poulenc) (DTA)

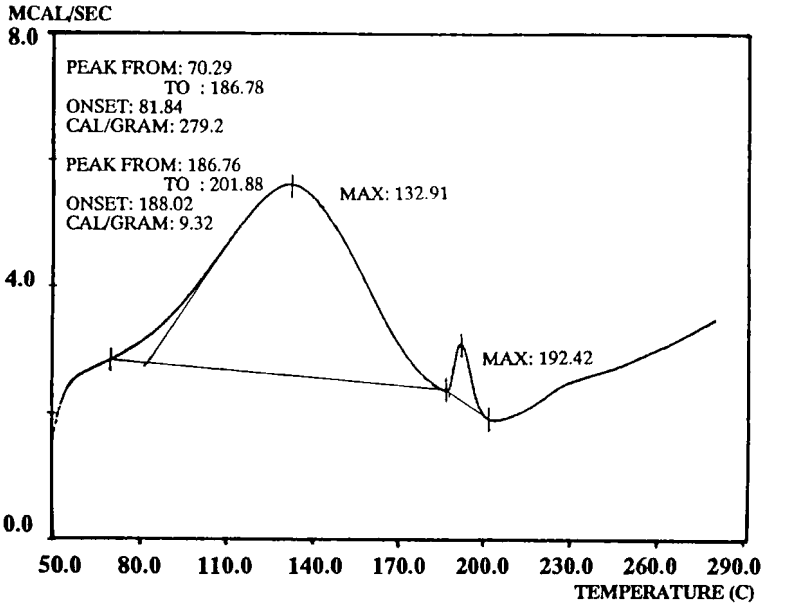

FIGURE 9
Compound N (DTA)

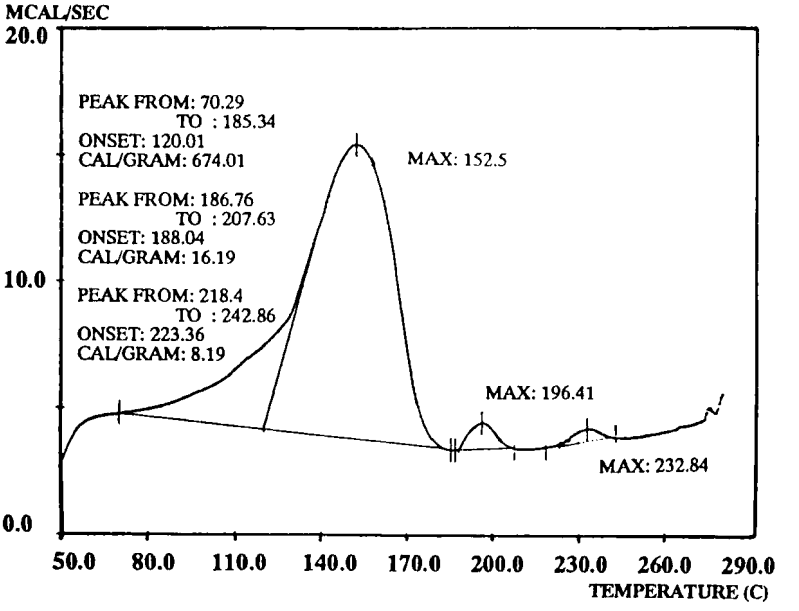

FIGURE 10
Compound E (DTA)

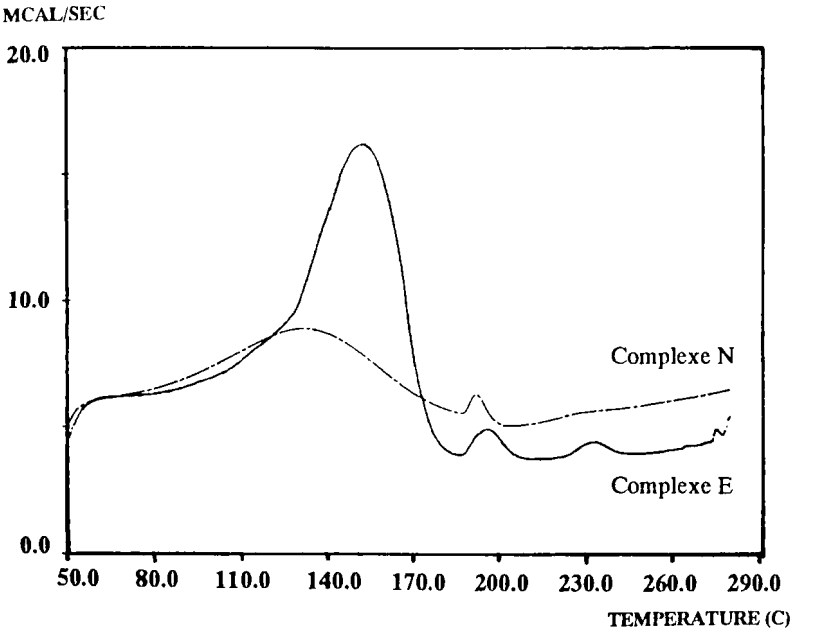

FIGURE 11
Comparing compounds N (spray dried) and E (evaporated under vacuum control) (DTA)

The yellowish appearance of compound E, coming from the colour of phenothiazine, contrasts with the white colour of compound N which corroborates the previous results.

DISCUSSION

The reducing of the melting point when developping compound N and the results obtained by analysis (NMR and DTA) leads us to acknowledge a change as far as the physico-chemical properties of compound N are concerned.

By those means of two analysis methods which are easier and easier to be carried out nowadays, we've been able to demonstrate this inclusion process [14] [15].

The molecular fitting and the links which have been established between β-cyclodextrin and phenothiazine couldn't be detailed because of the resolution (200 MHz) of the equipement to NMR, but the purpose which was to make clear the development of a cyclodextrin - phenothiazine compound has been achieved.

CONCLUSIONS

Those analysis processes are preliminaries to the optimization of the development into inclusion compound of phenothiazine.

They are a proof that inclusion is possible with the aim of making further investigations on phenothiazine and their derived products taking into account the galenic interest that their development into an inclusion compound would represent [16] [17] [18].

Other assays with a formulation at ratio 2:1 (β-cyclodextrin : phenothiazine) [19] with derived products to be used in therapy and not so easy to handle, could be considered with that purpose.

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