# Application of Supercritical Carbon Dioxide for the Preparation of a Piroxicam-β-Cyclodextrin Inclusion Compound

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*Received May 10, 1999; accepted September 7, 1999*

***Purpose.*** Piroxicam is a poorly soluble NSAID, whose solubility is enhanced when included into β-cyclodextrin. The preparation of a piroxicam-β-cyclodextrin inclusion compound using supercritical $CO_2$ was investigated.

***Methods.*** The solubility and the stability of piroxicam in supercritical $CO_2$ were determined. Then, the influence of the temperature, the pressure and the time of exposure on the inclusion rate was studied.

***Results.*** The solubility of piroxicam varied over a wide range depending on the temperature and pressure (from 0.006 to 1.500 mg/g of $CO_2$). The temperature and the time of exposure had a great influence on the inclusion yield, while pressure did not and a complete inclusion was achieved by keeping a physical mixture of piroxicam and β-cyclodextrin (1:2.5 mol/mol) for 6 hours at 150°C and 15 MPa of $CO_2$. This complex was characterized by Differential Scanning Calorimetry, differential solubility and Fourier Transform Infrared Spectrometry.

***Conclusions.*** Supercritical carbon dioxide may prove to be a novel useful complexation method of drugs into β-cyclodextrin.

**KEY WORDS:** piroxicam; β-cyclodextrin; supercritical carbon dioxide; inclusion complex.

## INTRODUCTION

Cyclodextrins (CD) are cyclic oligosaccharides, which are produced by enzymatic degradation of starch by a glucosyltransferase most commonly derived from *Bacillus macerans* (1). Cyclodextrins are made up of six or more glucose rings bound by 1,4-ether linkages. α-, β- and γ-cyclodextrins (the three most common natural cyclodextrins) contain six, seven and eight glucose units, respectively (2).

CD are able to include in their cavity large organic molecules by non-covalent interaction forces (hydrogen bounds, Van der Waals forces). The resulting complex may improve the solubility, stability and bioavailability of the guest.

Piroxicam (P) is a non steroidal anti-inflammatory drug (NSAID) that also possesses analgesic and antipyretic properties. The main mechanism of action of P, like that of other NSAIDs, is by inhibition of the enzyme cyclooxigenase, resulting in reduced prostaglandin synthesis (3–4). Literature already describes an inclusion complex of piroxicam with β-CD in a molar ratio of 1:2.5 which, in comparison with plain piroxicam, shows improved wettability and water solubility, higher plasma concentration ($C_{max}$), takes less time to reach the peak concentration ($T_{max}$), and reduces gastrointestinal side effects (4–5). In this study, piroxicam was chosen as a model drug.

As for many non polar drugs, the poor aqueous solubility of piroxicam restricts the possible preparation methods for the preparation of inclusion complexes with CD. More than ten years ago, some preparation methods using organic solvents as media were described (6–7), but the well-known toxicity of these solvents and the high concentration of residues in the inclusion complexes imposed the withdrawal of these methods.

In this paper, we develop a new alternative method for the preparation of inclusion complexes with non polar drugs using supercritical carbon dioxide (SC $CO_2$). Supercritical fluids (SCF) are fluids used at temperatures and pressures above their critical value. They have been established as good solvents for many non-volatile and thermally labile compounds. SCF have gaslike viscosities and diffusivities, promoting mass transfer, while densities are similar to that of liquid solvents. The solvent power can be manipulated over a wide range by adjusting temperature and pressure. Carbon dioxide is the most widely used supercritical fluid because it has a relatively low critical value and a moderate critical pressure (8). The advantage is obviously the lack of toxicity of SC $CO_2$ compared with conventional organic solvents, since SC $CO_2$ returns to the gaseous state after decompression.

In 1990, Kamihira et al. used CD for the entrapment of volatile aromatic compounds after supercritical extraction (SFE) (9). More recently, in 1996, Giordano and co-workers studied the interaction of supercritical fluid with a drug-cyclodextrin inclusion compound and the possibility of extracting the drug from the inclusion compound by SFE (10).

## MATERIALS

Piroxicam (99.5%, Ph. Eur. 3rd Edition) was purchased from Certa (Braine-l'Alleud, Belgium) and β-cyclodextrin (Ph. Eur. 3rd Edition, 13.74% of $H_2O$) from CNI (Neuilly-sur-Seine, France). $CO_2$ was of quality N48 from Air Liquide (99.998%, Liege, Belgium). Acetonitrile was of HPLC grade (Acros 28826-0025 far UV). All other reagents and solvents were of analytical grade.

## METHODS

### Solubility of Piroxicam in SC $CO_2$

A 1 ml stainless steel cartridge was filled with about 100mg of pure piroxicam, completed with glass beads. 3g SC $CO_2$ were passed through the cell at a flow rate of 1ml/min. The solubilized P was drawn away by SC $CO_2$ and entrapped in 5ml methanol after the decompression of the gas. Piroxicam solubility is obtained by diluting the samples with 5ml HCl 0.02N and measuring the UV absorbance at 344nm (Hitachi U-3000), if necessary after dilution with methanol-HCl 0.02N-1/1 v/v.

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### Preparation of Inclusion Complexes

Inclusion experiments with SC $CO_2$ were performed in a SUPREX SF Extractor Autoprep 44 (Pittsburgh, PA, USA) (Fig. 1). A 1 ml cell was filled with approximately 0.7g of a physical mixture of P-β-CD 1:2.5 (mol-mol), pressurized at the required pressure (in ±1 min) and left in a static mode. At the end of the process, depressurization occurs within 15 seconds. The contents of the cartridge were ground and homogenized in a mortar.

Freeze-dried and spray-dried products were isolated from an aqueous solution of P-β-CD 1:2.5 (0.05 mol–0.125 mol), adjusted to a pH value of 10.0 with $NH_4OH$, according to a method described by Acerbi *et al.* (11). Freeze-drying was performed in a EDWARDS EIPTC (Milano, Italy) at −50°C and 0.01 MPa during 24 hours and spray-drying was performed in a NIRO Atomizer "mobile minor"(Copenhagen, Denmark) with an inlet temperature of 175°C, a flow rate of 15 ml/min and a spray pressure of 0.2–0.3 MPa.

### Thermal Analysis

The DSC patterns of samples (10–15 mg) were obtained with a Mettler TC11 TA Processor DSC apparatus between 30° and 230°C at a heating rate of 10°C/min under a $N_2$ gas stream. Limits for the integration of the piroxicam melting peak were fixed at 10°C on both sides of the endothermic peak.

### Infrared Absorption Spectroscopy

Studies of the IR Spectra of the products were conducted with a FTIR Perkin-Elmer 1750 spectrophotometer using the KBr disc method (4000 to 450 $cm^{-1}$).

### UV Spectroscopy

Samples were also spectrophotometrically assayed for total and free piroxicam contents with a Hitachi U-3000 U.V. Spectrophotometer. Total piroxicam was measured at $\lambda_{max}$ 334 nm in HCl 0.1N-acetonitrile 1:1 v/v and free piroxicam was measured at $\lambda_{max}$ 334 nm in acetonitrile acidified with HCl (2.08 ml of 12N HCl in 500 ml of acetonitrile).

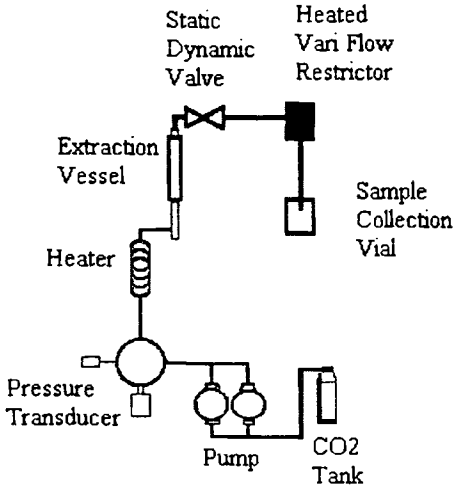


**Fig. 1.** Schematic diagram of the apparatus.

### Water Content Determination

The percentage of water contained in the samples after treatment in SC $CO_2$ was determined on about 50 mg of product with a Karl-Fischer titrator (Mettler DL35).

## RESULTS AND DISCUSSION

### Solubility and Stability of Piroxicam in SC $CO_2$

Table I presents piroxicam solubility data over a wide range of temperature (50–150°C) and pressure (15–50 MPa). The reported values are the average of three consecutive measurements. The relative standard deviation of these measurements was ±10% since these solubilities are low and difficult to obtain, principally because of partial clogging of the restrictor and consequently possible fluctuations of flow rate.

As it can be seen, the solubility of P can vary over a wide range depending on temperature and pressure. The lowest solubility was about 0.006 mg per g of $CO_2$ at 150°C and 15 MPa, while the highest solubility (1.5 mg per g of $CO_2$) was measured at 150°C and 45 MPa.

Examination of the Fig. 2 shows that the influence of pressure is clear. At constant temperature, higher densities lead to greater solubility in SCF (12). An increase of pressure leads to an increase of density and solubility. For example, raising the pressure from 15 to 45 MPa increases the solubility of piroxicam from 0.029 to 0.32 mg/g of $CO_2$ at 50°C and from 0.006 to 1.500 mg/g of $CO_2$ at 150°C.

However, increasing the temperature induces a surprising yet explainable effect. At low pressures (15 and 25MPa), the increase of temperature does not increase the solubility of P, which remains below 0.116 mg/g. At 35 MPa, the increase of temperature induces an increase of the solubility of P (from 0.224 mg/g at 50°C to 0.382 mg/g at 150°C) and this increase of solubility becomes very important at 45 Mpa, though it is combined with a decrease of the density of SC $CO_2$.

Above 35 MPa, the decrease of density is compensated by an increase of the volatility of piroxicam. This effect which has been described previously by several authors (13–15) is in

**Table I.** Solubility of Piroxicam in Supercritical Carbon Dioxide (n = 3)

| Temperature (°C) | Pressure (MPa) | $CO_2$ density ($g/cm^3$) | Solubility of P ± SD (mg/g of $CO_2$) |
|---|---|---|---|
| 50 | 15 | 0.705 | 0.029 ± 0.003 |
| | 25 | 0.838 | 0.115 ± 0.010 |
| | 35 | 0.902 | 0.224 ± 0.002 |
| | 45 | 0.947 | 0.320 ± 0.017 |
| | 50 | 0.966 | 0.404 ± 0.027 |
| 100 | 15 | 0.340 | 0.026 ± 0.002 |
| | 25 | 0.596 | 0.116 ± 0.006 |
| | 35 | 0.720 | 0.324 ± 0.029 |
| | 40 | 0.760 | 0.504 ± 0.091 |
| | 45 | 0.794 | 0.748 ± 0.113 |
| 150 | 15 | 0.238 | 0.006 ± 0.001 |
| | 25 | 0.421 | 0.110 ± 0.006 |
| | 35 | 0.561 | 0.382 ± 0.029 |
| | 40 | 0.612 | 0.699 ± 0.052 |
| | 45 | 0.655 | 1.500 ± 0.194 |

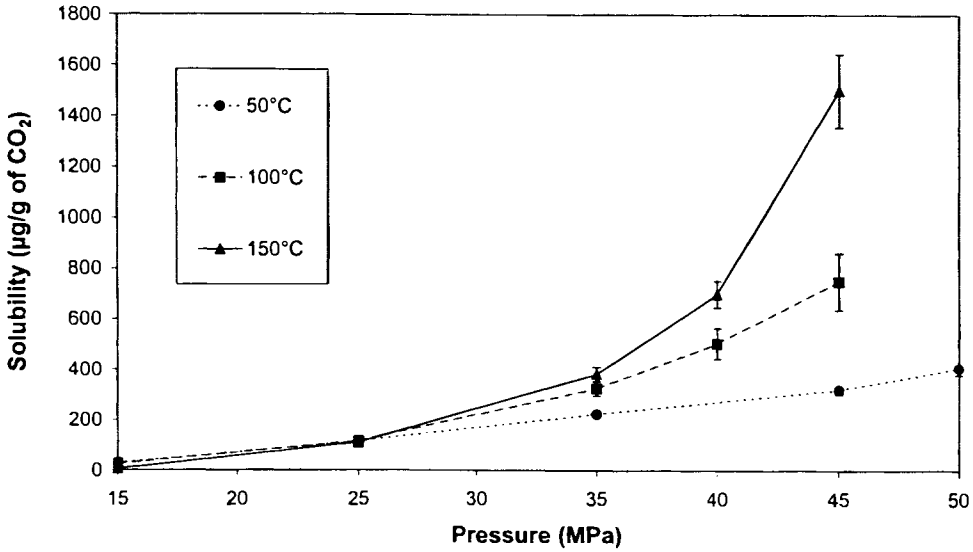


**Fig. 2.** Solubility of piroxicam in supercritical carbon dioxide (n = 3) at 50, 100 and 150°C in function of the pressure.

contrast to the common statement that the density of a supercritical fluid has to be increased in order to increase both solubility and extraction efficiency. While increasing density at constant temperature generally does increase solubility, increasing density at a constant pressure (by lowering temperature) actually does not influence piroxicam solubility at low pressure and lowers it at high pressure.

The crossover pressure (above which the solubility increases with increase in temperature) is around 25 MPa, in agreement with Macnaughton *et al.* who expected this value to be not far above 22 MPa (16). At this pressure, there is no significant difference between the values of solubility obtained at the different temperatures ($p > 0.2$).

It has been reported that at constant temperature, the logarithm of solubility increases linearly with density (17). Figure 3 shows the linear relation ($r^2 > 98.5\%$) between the solubility of P (expressed as the ln values of the solubility in µg/g $CO_2$) and the density of $CO_2$. The solubility value obtained at 100°C and 15 MPa is unexplained and has not been included in the calculation of the linear regression.

The stability of piroxicam in supercritical carbon dioxide was also studied by HPLC. The method is described in a project for a new monograph of P for the testing of related substances (18). Pure piroxicam left for six hours at 150°C and 45 MPa in SC $CO_2$ meets the specifications of the European Pharmacopoeia and shows less than 0.2% of aminopyridin and less than 0.6% of other impurities.

### Effects of Solubility, Temperature, Pressure, and Time on the Complex Formation

After showing that piroxicam was soluble and stable in SC $CO_2$, we studied the influence of temperature, pressure

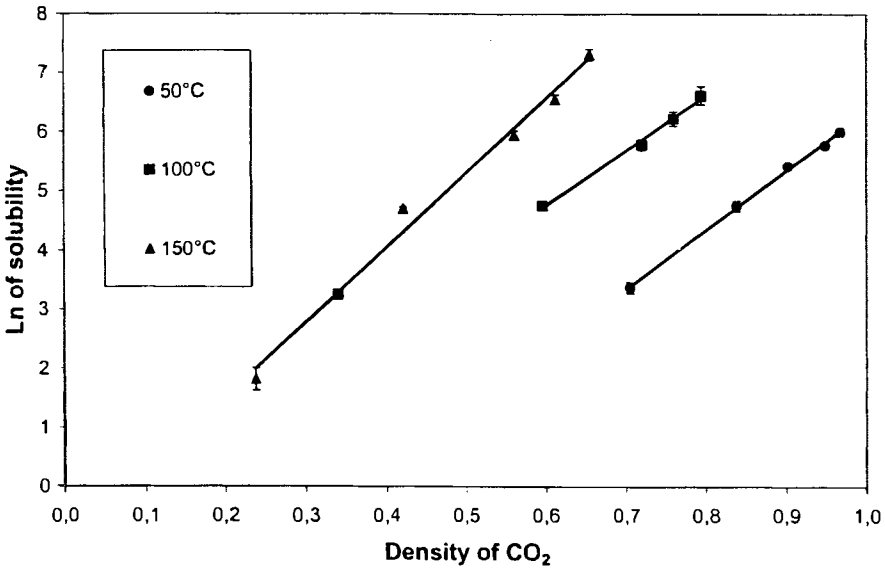


**Fig. 3.** Influence of the density of SC $CO_2$ on the solubility of piroxicam (ln values) at different temperatures.

and time of exposure to SC $CO_2$ on the complex formation. Preliminary studies showed that the time of exposure had a positive influence as increasing the contact time ensures a better inclusion. In some cases, a nearly complete inclusion could already be obtained after 3 hours in SC $CO_2$. Temperature was shown to have an important effect, while the degree of solubility of P and the pressure seem to have a weak influence on the inclusion yields.

To confirm these preliminary results, a $3^k$ experimental design has been used. Temperature and pressure (k = 2) were studied at three levels (50–100–150°C; 15–30–45 MPa). The time of contact with SC $CO_2$ was fixed at three hours. The results of the influence of the pressure, the temperature and the density of SC $CO_2$ are given in Table II. Data on piroxicam content (free and total), inclusion yield, water content and enthalpy are mentioned in comparison with data obtained with a physical mixture, a physical mixture kept in an oven for 6 hours at 150°C, a spray-dried complex and a freeze-dried complex, each of them containing the same ratio piroxicam/β-cyclodextrin.

The percentage of inclusion was evaluated by UV spectrometry, according to the following equation

$$\% \text{ of inclusion} = \frac{(\text{total piroxicam content} - \text{free piroxicam content})}{\text{total piroxicam content}} * 100$$

UV assay for total piroxicam content proved that there was no excessive loss of piroxicam during $CO_2$ treatment. The apparent increase in total piroxicam content for the runs at 150°C may be explained by the loss of water during the experiment. Taking into account the loss of water, there is no significant difference in the piroxicam content before and after treatment at 50°C and 30 MPa and at 150°C for each pressure ($p > 0.05$). All the other conditions induced a slight decrease of piroxicam content.

The decrease of the endothermic melting peak of free piroxicam in the samples compared with a physical mixture of P-β-CD in the same ratio gave also an appreciation of the inclusion. Furthermore, there was a good correlation between the inclusion percentage and the enthalpy ($r^2 > 99.5\%$).

**Table III.** Comparison Between the Experimental Inclusion Yields and Those Calculated with the Theoretical Model

| Temperature (°C) | Pressure (MPa) | Inclusion yields (%) [experimental] | Inclusion yields (%) [theoretical] |
|---|---|---|---|
| 50 | 15 | 4.17 | 4.37 |
| | 30 | 3.43 | 3.48 |
| | 45 | 1.39 | 1.13 |
| 100 | 15 | 3.97 | 2.39 |
| | 30 | 2.44 | 4.55 |
| | 45 | 5.88 | 5.24 |
| 150 | 15 | 84.6 | 85.87 |
| | 30 | 93.4 | 91.07 |
| | 45 | 94.0 | 94.80 |

By multiple regression on the 27 experiments, the effects of the temperature and the pressure were studied. The relation ($r^2 = 99.7\%$) can be expressed as follows:

$$\text{Inclusion (\%)} = 94.29 - 2.66 * T - 0.12 * P + 0041 * T * P + 0.017 * T^2 - 0.0032 * P^2$$

Table III compares the experimental data and those calculated from the theoretical model.

As expected from the data, the temperature was shown to have a significant effect on inclusion of piroxicam into β-CD ($p < 0.0001$). Temperature at the 2nd power has a positive influence while the term at the 1st degree has a negative influence. The pressure does not have a significant effect on the inclusion ($p > 0.66$) even at the 2nd power ($p > 0.46$), but the interaction between the temperature and the pressure is significant and positive.

This model can explain why inclusion was only effective at 150°C (at 50° and 100°C, the inclusion yield was below 6% after 3 hours), and why an increase of pressure induced a small decrease of the inclusion percentage at 50°C, but increased it slightly at 150°C.

**Table II.** Data of the Inclusion Experiments with SC $CO_2$ (n = 3) in Comparison with Data Obtained from Physical Mixtures and Complexes Prepared by Other Methods

| Temperature (°C) | Pressure (MPa) | Density ($g/cm^3$) | Total P content (%) | Free P content (%) | Inclusion yield (%) | % water | Enthalpy (J/g) |
|---|---|---|---|---|---|---|---|
| 50 | 15 | 0.71 | 7.73 ± 0.14 | 7.41 ± 0.08 | 4.17 | 12.6 ± 0.4 | 8.70 ± 0.38 |
| | 30 | 0.87 | 7.85 ± 0.18 | 7.59 ± 0.09 | 3.43 | 13.0 ± 0.4 | 8.85 ± 0.27 |
| | 45 | 0.95 | 7.75 ± 0.11 | 7.65 ± 0.03 | 1.39 | 12.6 ± 0.2 | 9.10 ± 0.12 |
| 100 | 15 | 0.34 | 7.66 ± 0.06 | 7.36 ± 0.11 | 3.97 | 12.4 ± 0.7 | 8.65 ± 0.22 |
| | 30 | 0.66 | 7.81 ± 0.15 | 7.62 ± 0.10 | 2.44 | 12.7 ± 0.6 | 8.83 ± 0.18 |
| | 45 | 0.79 | 7.48 ± 0.07 | 7.04 ± 0.15 | 5.88 | 12.7 ± 0.1 | 8.03 ± 0.28 |
| 150 | 15 | 0.24 | 8.18 ± 0.08 | 1.26 ± 0.22 | 84.6 | 10.4 ± 0.3 | 0.59 ± 0.13 |
| | 30 | 0.49 | 8.44 ± 0.13 | 0.56 ± 0.10 | 93.4 | 9.4 ± 0.2 | 0.18 ± 0.04 |
| | 45 | 0.66 | 8.24 ± 0.05 | 0.49 ± 0.08 | 94.0 | 9.0 ± 0.2 | 0.17 ± 0.06 |
| Physical mixture | | | 8.09 ± 0.14 | 7.89 ± 0.23 | | 12.7 ± 0.1 | 10.23 ± 0.56 |
| Physical mixture kept in an oven for 6 hours at 150°C | | | 9.85 | 9.38 | 4.77 | 0.9 | 9.7 |
| Spray-dried complex | | | 10.51 | 0.03 | 99.7 | 4.4 | 0.19 |
| Freeze-dried complex | | | 9.43 | 0.05 | 99.5 | 10.3 | 0.31 |

The degree of solubility of piroxicam in SC $CO_2$ did not influence the inclusion of piroxicam into β-CD. At 30 MPa and for the three temperatures, solubility of P in SC $CO_2$ was intermediate and similar for each temperature, but inclusion is only significant at 150°C. On the other hand, at 150°C, the solubility of P was strongly influenced by the pressure (see Table II), but not the inclusion yield.

At this point, parameters have been optimized and a nearly complete inclusion (inclusion yield > 98.5%) could be achieved after 6 hours of contact with supercritical $CO_2$ at 15 MPa and 150°C. This complex has been characterized and compared with complexes prepared by freeze-drying and spray-drying.

The usefulness of SC $CO_2$ was also demonstrated by maintaining a physical mixture of piroxicam and β-CD for 6 hours in an oven at 150°C. In these conditions, inclusion was not possible, proving that the inclusion resulted from a combined effect of both temperature and supercritical $CO_2$.

## Characterization of the Complex

### *DSC.*

During all the preliminary study, the disappearance of the endothermic peak was considered as a clear evidence of the inclusion phenomenon. In Fig. 4, typical curves of both physical mixture and inclusion compound are depicted. The DSC curve of the physical mixture shows two peaks: a broad endotherm between 50° and 150°C corresponding to the water loss of the β-cyclodextrin, followed by the endothermal melting peak at 195°–205°C characteristic of crystalline piroxicam. However, when working with supercritical fluids, the disappearance of endothermic peaks of the drugs may be attributed to several events: the loss of product (piroxicam could be extracted by $CO_2$ during the treatment), or the transformation of the drug into an amorphous state, or the formation of an inclusion complex or a combination of these three events.

Though amorphous piroxicam has a high tendency to crystallize, Redenti *et al.* (19) showed that it was possible to obtain amorphous piroxicam by rapidly cooling molten crystalline piroxicam (at an ultra-fast cooling rate of 3–4 million degrees/min) and by storing the glassy substance at very low temperature (−80°C).

Rapid decompression of a solution of piroxicam in SC $CO_2$ (resulting in supersaturated solution and rapid cooling of the product due to the endothermic expansion of $CO_2$) could lead to the formation of amorphous piroxicam stabilized by the presence of β-CD.

According to Redenti (19), DSC allows an evaluation of the inclusion complex purity with regard to the free piroxicam content despite its amorphous or crystalline state. But as thermal analysis provides only negative evidence, additional techniques were also used to characterize the complex and to show that the disappearance of the melting peak of piroxicam was the result of the formation of an inclusion complex.

### *Differential Solubility.*

A second evidence of the complexation is the differential solubility of the physical mixture and of the inclusion compound in acetonitrile. Once included into β-CD, piroxicam is no longer soluble in acetonitrile unlike free piroxicam. The separate assay for free P (in acidified acetonitrile) and total P (in a mixture HCl 0.1N-acetonitrile 1:1 v/v) in addition with DSC allows to determine the part of amorphous, crystalline and included piroxicam.

### *Spectroscopic Methods.*

Figure 5 shows the IR spectra of piroxicam, β-cyclodextrin, physical mixture and product treated by supercritical $CO_2$. Spectrum of the physical mixture can be considered as the result of the addition of piroxicam and β-CD's spectra. On the contrary, in the spectrum of the inclusion compound, shifts, disappearances or attenuation of the characteristic piroxicam bands reveal a modification of the environment of piroxicam. For example, the symmetric $SO_2$ stretching band at 1354 $cm^{-1}$

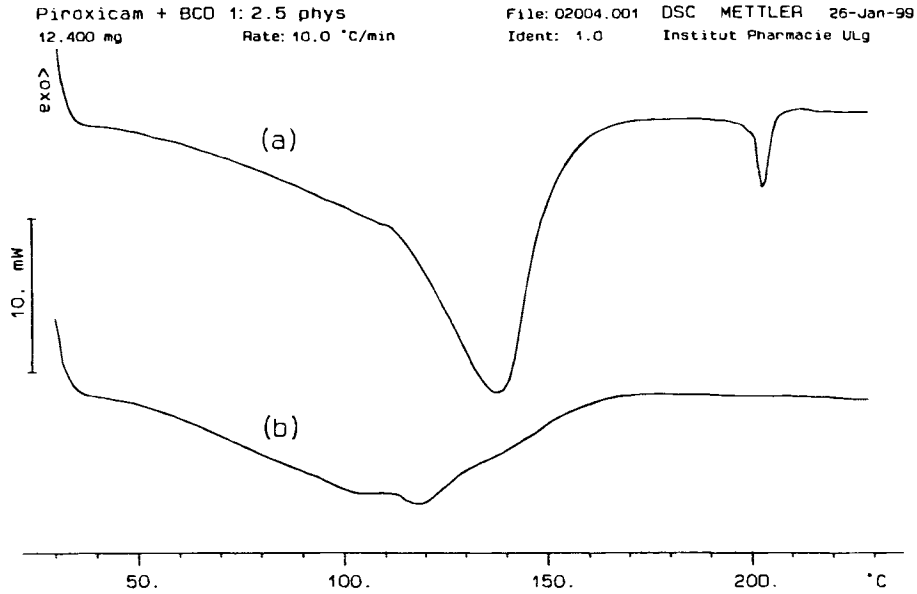


**Fig. 4.** Typical DSC curves: (a) physical mixture; (b) product obtained after 6 hours at 150°C and 15 MPa.

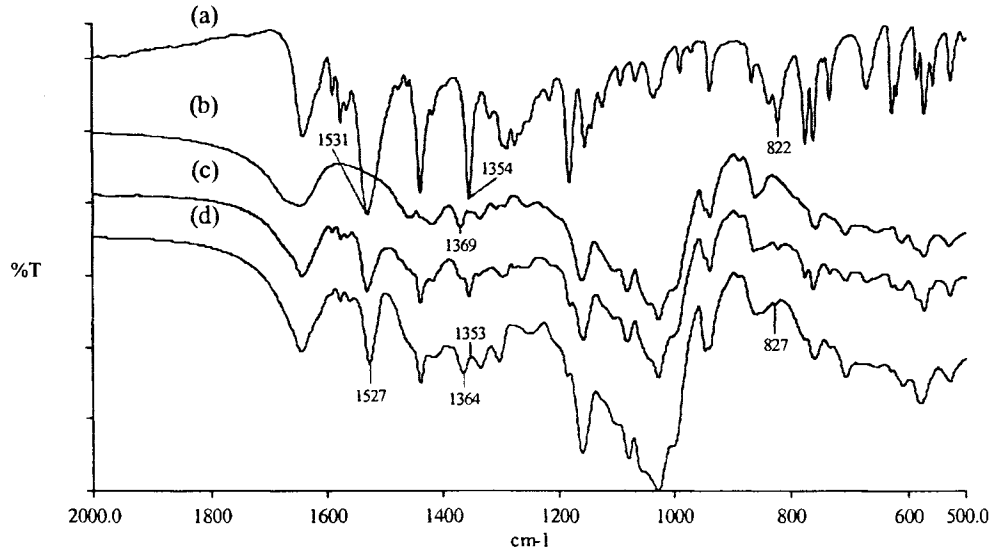


**Fig. 5.** IR spectra of pure piroxicam (a); β-cyclodextrin (b); physical mixture (c) and; product treated by SC $CO_2$ (d).

is no more detectable at this wavelength in the complex. The band at 1531 $cm^{-1}$ belongs to the amido NH of piroxicam and shifts to 1527 $cm^{-1}$. The CH aromatic deformation band at 822 $cm^{-1}$ for piroxicam and the CH aliphatic deformation band at 1369 $cm^{-1}$ for β-CD were also changed to 827 $cm^{-1}$ and to 1364 $cm^{-1}$ respectively. These changes are probably related to the interaction and the formation of intermolecular bounds between the guest (P) and the host (β-CD).

### Hypotheses About the Mechanism of Inclusion

At this stage of our research, we have tried to propose hypotheses about the complexation mechanism in supercritical fluids.

Contrary to spray-drying and freeze-drying wherein both the drug and the cyclodextrin are dissolved in a solvent prior to the crystallisation or precipitation of the inclusion compound by temperature variation, in SCF, the CD is practically insoluble and it has been shown that the solubility level of P has a minor effect on the inclusion.

It is now commonly accepted that the mechanism of inclusion of a guest in a CD cavity is essentially a substitution of the included water molecules by the less polar guest. During the first step of the complex formation reported by Frömming and Szejtli (2), the water molecules escape from the CD cavity and move to an energy level corresponding to that of the gaseous state.

The high temperature and pressure of $CO_2$ could promote the exchange of the water molecules with piroxicam inside the CD. At a high temperature (150°C are needed to achieve inclusion), water is still in the liquid state because of the high pressure (the vapor pressure of water at 150°C = 0.48 MPa) (20) but its solubility in $SCCO_2$ is about 12 mole% at 150°C and only about 4 to 7 mole% at 110°C depending of the pressure (21). This greater solubility of water at 150°C could also explain the occurence of compact cylinders of powder collected after the runs at 150°C, indicating the presence of a liquid phase. This liquid phase could be due to the condensation of this water during the rapid depressurization and also to the melting of P (m.p. 198°C under atmospheric pressure) which is depressed with an increase of pressure.

The facilitated escaping of the water molecules from the cavity of the CD is not sufficient to explain the inclusion. The physical mixture kept in the oven lost more water and showed less inclusion.

In addition, as this method is time dependent, we can imagine a reaction in different steps among which one has a limiting rate. Piroxicam needs to be soluble in supercritical carbon dioxide before its inclusion into cyclodextrin but once included, it would no longer be soluble in SC $CO_2$ (like in acetonitrile) and the equilibrium would continually be displaced.

## CONCLUSIONS

In this study, a novel complexation method of drugs into CD has been investigated. A successful inclusion has been achieved with a model drug, without using any additional substances (ammoniac or organic solvents) thus without any residues. This new method will be tested with other substances but might be attractive for non-polar drugs for which no alternative can be applied or for unstable substances, for which the inert character of supercritical carbon dioxide would limit degradation. It could also be interesting to combine this process with techniques of micronization by means of supercritical fluids (Rapid Expansion of Supercritical Solutions or Aerosol Solvent Extraction System for example). (13,22)

## ACKNOWLEDGMENTS

The authors are grateful to the "Fonds Special de la Recherche de l'ULg" for providing the SF Extractor.

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