DRUG LOADING INTO SOLID CARRIERS USING A CONTROLLED PARTICLE DEPOSITION (CPD) METHOD FOR IMPROVED DRUG DISSOLUTION

DIE BELADUNG VON FESTEN TRÄGERN MIT ARZNEISTOFFEN MITTELS CONTROLLED PARTICLE DEPOSITION (CPD) ZUR VERBESSERUNG DER AUFLÖSUNGSGESCHWINDIGKEIT DER ARZNEISTOFFE

DISSERTATION

der Fakultät für Chemie und Pharmazie
der Eberhard-Karls-Universität Tübingen
zur Erlangung des Grades eines Doktors
der Naturwissenschaften

2008

vorgelegt von

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Tag der mündlichen Prüfung	31. Januar 2008
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am Lehrstuhl für Pharmazeutische Technologie der Eberhard-Karls-Universität Tübingen.

Meinem geehrten Doktorvater, Herrn Prof. Martin A. Wahl, danke ich für die Möglichkeit, diese Arbeit in seinem Arbeitskreis anfertigen zu können, die Überlassung des interessanten Themas sowie für die Schaffung optimaler Arbeitsbedingungen. Darüber hinaus danke ich ihn für seine jederzeit gewährte Unterstützung, seine Bereitschaft zur Hilfe und Diskussion sowie für den persönlichen und menschlichen Einsatz. Für die Möglichkeit der Teilnahme an Weiterbildungsveranstaltungen zur Fachapotheker für Pharmazeutische Technologie sowie für den Besuch von Kongressen und Seminaren, die meinen technologischen Horizont erweitert haben, bedanke ich mich herzlich!

Besonders danke ich auch Herrn Prof. Dr. Michael Türk vom Institut für Technische Thermodynamik und Kältetechnik der Universität Karlsruhe für die Anfertigung des zweiten Gutachtens, die hervorragende Betreuung während des gesamten Promotionsvorhabens sowie für seine freundliche Hilfs- und Gesprächsbereitschaft.

Herrn Prof. Dr. Rolf Daniels danke ich für die Teilnahme an der Prüfungskommission, die inhaltlich reichen Fachgespräche sowie für die Möglichkeit der Teilnahme an der Weiterbildung zum Fachapotheker für Pharmazeutische Technologie. Ebenso dankbar bin ich für seinen freundlichen menschlichen Einsatz während meiner Promotionszeit.

Beim Prof. Dr. Peter C. Schmidt bedanke ich mich herzlich für die Annahme als Doktorand am Lehrstuhl für Pharmazeutische Technologie der Universität Tübingen, die zahlreichen und wertvollen Hinweise sowie für die hilfreichen wissenschaftlich-fachlichen Diskussionen.

Herrn Prof. Dr. Stefan Laufer danke ich für die Leitung des Promotionsausschusses und für die Möglichkeit, die FTIR-Spektroskopie an seinem Lehrstuhl anfertigen zu dürfen .

Frau Marlene Crone und Herrn Dip.-Ing. Mathias Löwengberg vom Institut für Technische Thermodynamik und Kältetechnik der Universität Karlsruhe bedanke ich mich für die Herstellung der Cyclodextrin-Komplexe sowie für die gute Zusammenarbeit und die intensiven und inhaltsreichen Gesprächen.

Meinen Kollegen, Frau Martina Brenn, Frau Renate Beer, Herrn Roland Walker und Herrn Klaus Weyhing, danke ich für ihre Hilfsbereitschaft und Freundschaft. Sie haben mir bei der Bewältigung von kleinen und großen Problemen viel geholfen.

Ebenso danke ich Herrn Dr. Hans-Jürgen Martin für die Unterstützung meiner anfänglichen Tätigkeit am Lehrstuhl für Pharmazeutische Technologie der Universität Tübingen.

Herrn Dr. Martin Maus danke ich ganz herzlich für seine Freundschaft, stetige Hilfe und Offenheit sowie für die zahlreichen Sprachkorrekturen und dem Korrekturlesen.

Allen meinen Kollegen – ehemalige und derzeitige – danke ich für die gute Zusammenarbeit und die wertvollen Vorschläge und Anmerkungen.

Ich danke meinen Freunden, insbesondere Herrn Lucian-B. Brujan, für ihre persönliche Unterstutzung. Ohne sie wäre mein Leben im Ausland sicherlich schwieriger gewesen.

Für die Korrektur meiner Dissertation bedanke ich mich bei Herrn Dr. Abebe Endale, Frau Laure Almairac und Herrn Patrick Rother.

Ein ganz besonderer Dank gilt meiner Familie, die meine Ausbildung und Promotion möglich gemacht und mich dabei so liebevoll begleitet und unterstützt hat.

ZUSAMMENFASSUNG

Fast 60 % der Arzneistoffe bei den Neusynthesen und 40 % der Arzneistoffe bei den Neuentwicklungen besitzen eine geringe Wasserlöslichkeit. Der Anteil an Substanzen, die eine Löslichkeit unter 0,1 mg/l aufweisen, nimmt dabei ständig zu (Merisko-Liversidge, 2002) und bereitet daher Schwierigkeiten bei der Aufbereitung der Wirkstoffe zu Arzneimitteln.

Bei der Applikation von Arzneistoffen sollen die meisten von ihnen möglichst schnell ihre Wirkung entfalten. Um eine ausreichend hohe Plasmakonzentration am Wirkort durch die Resorption erreichen zu können, müssen diese Arzneistoffe in gelöstem Zustand im Magen-Darm-Trakt vorliegen. Darüber hinaus erreichen die schwerlöslichen Arzneistoffe nach peroraler Gabe sehr oft keine ausreichende Bioverfügbarkeit. Die Gründe sind in der zu geringen Sättigungslöslichkeit und Lösungsgeschwindigkeit der Substanzen zu suchen (Buttle, 2004; Müller, 1998). Von daher ist das Ziel der Arzneiformenentwicklung, den Wirkstoff innerhalb kürzester Zeit in die gelöste Form zu bringen, um eine bessere Absorption und damit eine höhere Bioverfügbarkeit erzielen zu können.

Um dieses Problem zu bewältigen, wurden verschiedene Methoden entwickelt, wie z.B. die Reduktion der Partikelgröße bzw. die Vergrößerung der Oberfläche, wobei jedoch Probleme durch Agglomeration der Wirkstoffpartikel auftreten können (Martin, 2003). Dies deutet darauf hin, dass die Benetzung der Partikel einen entscheidenden Faktor in der Löslichkeitsverbesserung der Arzneistoffe darstellt.

Mit dem Ziel einer produktschonenden Herstellung von submikronen Arzneistoffen mit guten Benetzungs- und Auflösungseigenschaften wurde das Controlled Particle Desposition Verfahren (CPD-Verfahren) entwickelt. Das CPD-Verfahren ist eine vielversprechende Methode zur direkten Abscheidung feinster Partikel auf Trägermaterialien durch die Verwendung überkritischer Fluide als Alternative zu organischen und toxischen Lösungsmitteln. Bei diesem Prozess handelt es sich im

Prinzip um ein statisches Gleichgewicht, bei dem die verwendeten Versuchsstoffe (Arzneistoff, Trägermaterialien) getrennt vorgelegt werden. Dann wird zunächst CO_2 eingeleitet, und zum Erreichen des überkritischen Zustandes werden Druck und Temperatur erhöht. Dabei entsteht eine überkritische Lösung des Wirkstoffes in CO_2. Diese Lösung dringt dann, bedingt durch die gasähnlichen Transporteigenschaften überkritischer Fluide, in das Trägermaterial ein. Anschließend wird der Wirkstoff aus der überkritischen Lösung durch eine schnelle Druckabsenkung und somit Verringerung der Löslichkeit direkt in bzw. auf dem Träger abgeschieden.

Zu Beginn dieser Arbeit wurde ein Optimierungsprozess für die CPD-Anlage sowie die CPD-Versuchsparameter durchgeführt. Als Trägermaterial wurde β-CD aufgrund seiner Fähigkeit, andere Moleküle, beispielsweise pharmazeutisch aktive Substanzen, als „Gast“ in seine Kavität aufzunehmen, ausgewählt. Bei dieser Art der Komplexierung wird das eingeschlossene Molekül nur durch Van-der-Waals-Kräfte oder Wasserstoffbrückenbindungen fixiert.
Ibuprofen wurde als Modell für Wirkstoff mit schwerlöslichen Eigenschaften eingesetzt. Ibuprofen als Arzneistoffmodell zeigt wesentlich eine gute Löslichkeit in $scCO_2$. Außerdem sind Ibuprofen/β-CD–Komplexe bereits mit verschiedenen klassischen Verfahren hergestellt worden (Kurozumi *et al*., 1975; Nozawa *et al.,* 1994; Mura *et al.,* 1998; Khan *et al.,* 2001).
Zur Bestimmung der bestmöglichen Komplexierungsbedingungen in dem CPD-Verfahren wurden die Parameter: Haltezeit, Druck sowie Temperatur variiert. Die Auswertung der Versuche erfolgte schließlich durch die Bestimmung der eingeschlossenen und nicht-eingeschlossenen Anteile an Wirkstoff in den Trägermaterialien.

Mit den durchgeführten Experimenten konnte gezeigt werden, dass die Herstellung von Ibuprofen/β-CD-Komplexen mit dem CPD-Verfahren möglich ist. Die Untersuchungen zum Einfluss der Versuchsbedingungen in dem begrenzten experimentellen Bereich zeigen, dass Temperatur und Druck positiven Einfluss auf die Komplexbildung haben, solange sich durch sie die Wirkstofflöslichkeit erhöht. Der dritte wichtige untersuchte Prozessparameter ist die Zeit, in der die gesättigte

überkritische Wirkstofflösung in direktem Kontakt mit dem Trägermaterial ist. Im Falle Ibuprofen hat diese Zeit keinen erheblichen Einfluss auf die Herstellung von Ibuprofen/β-CD-Komplexen aufgrund der hohen Löslichkeit von Ibuprofen in $scCO_2$. Jedoch konnte die Kontaktzeit bei der Anwendung von Wirkstoffen mit geringer Löslichkeit in $scCO_2$ einen positiven Effekt zeigen (Moribe *et al.,* 2007).

Nach der Optimierung der CPD-Anlage und der Prozessparameter konnte ein gleichförmiges reproduzierbares Produkt mittels CPD-Verfahren hergestellt werden.
Das erhaltene CPD-Produkt mit einem Gesamtgehalt von Ibuprofen 2,8 ± 0.22 % wt. wurde mit 5,5 % wt. Ibuprofen/β-CD-Komplexen, die durch andere klassische Verfahren (Gefriertrocknung, Kopräzipitation bzw. physikalische Mischung) hergestellt wurden, verglichen.
Die CPD-Komplexe weisen eine fließende Pulverform auf, während die Gefriertrocknungs- und die Kopräzipitationsprodukte Klumpen bilden oder zumindest Agglomerate enthalten.
Mit Hilfe der HPLC-Analytik konnten ca. 97 % wt. Inklusionsausbeute im Gefriertrocknungsprodukt und ca. 50 % wt. bei den CPD- und Kopräzipitationsmaterialien nachgewiesen werden, wobei in der physikalischen Mischung nur 3 % wt. eingeschlossene Ibuprofenanteile ermittelt wurden.
Die physikochemische Charakterisierung (FTIR, XRD und DSC) erlaubte den Nachweis der Interaktion zwischen dem Gastmolekül und β-CD. Diese Interaktion war deutlich in dem Gefriertrocknungsprodukt zu sehen, in den CPD- und Kopräzipitationsmaterialien verlief die Komplexbildung vollständig.
In der morphologischen Untersuchung (REM) konnte die entstehende Komplexbildung bei allen Ibuprofen/β-CD-Komplexen unabhängig vom Herstellungsverfahren beobachtet werden.
Aufgrund der Komplexbildung war die Verbesserung der Auflösungsgeschwindigkeit im Vergleich zu reinem Ibuprofen in allen Ibuprofen/β-CD-Produkten zu sehen: Diese Verbesserung war am deutlichsten in den CPD- und Gefriertrocknungsprodukten.

Eine Erhöhung der β-CD-Komplexierung von Ibuprofen durch Zusatz von wasserlöslichem Polymer konnte gezeigt werden. Die Anwesenheit geringer Mengen

an PVP (1 % wt.) erhöht das Ausmaß der Komplexierung auf bis zu 9 % wt. des Ibuprofens, mit dem ein ternärer Komplex aus Ibuprofen, β-CD und PVP beladen werden konnte. In diesem Produkt konnte eine Verbesserung der Auflösungsgeschwindigkeit im Vergleich zu reinem Ibuprofen bzw. der physikalischen Mischung von Ibuprofen/β-CD/PVP erzeugt werden.

Als weitere Anwendung des CPD-Prozesses, die Bildung von Ibuprofen/Methyl-β-CD-Komplexen wurde erfolgreich bewerkstelligt. Methyl-β-CD schmilzt unter den Versuchsbedingungen (39,5 °C, 24,6 MPa und 15 h). Dabei lassen sich höhere Wirkstoffanteile in die Matrix einarbeiten. Die entstehende feste Dispersion war unregelmäßig in Form und Größe, was eine unregelmäßige Freisetzung des Ibuprofens verursacht. Die physikochemische Charakterisierung weist auf Einschlussverbindungen aus Ibuprofen und Methyl-β-CD hin.

Schließlich wurden die β-CD-haltigen Granulate noch durch einen Feuchtgranulierungsprozess hergestellt und mittels CPD- bzw. „Solution-Immersing“ Methoden mit Ibuprofen beladen. Beladung mittels CPD ließ aber einen deutlich größeren Gehalt an Ibuprofen erkennen. Nur ein Teil des Wirkstoffanteils, mit dem die Matrix beladen wurde, weist eine kristalline Form auf (DSC und XRD). Diese Ergebnisse konnten mit der signifikanten Verringerung der Oberfläche der mit Hilfe der CPD beladenen Granulate verglichen mit den unverarbeiteten β-CD-Granulaten bzw. den mittels „Solution Immersing“ beladenen Granulaten bestätigt werden. Die Verbesserung die Löslichkeit war allerdings in beiden beladenen Produkten zu sehen.

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ABBREVIATIONS

α-CD	α-Cyclodextrin
β-CD	β-Cyclodextrin
BCS	Biopharmaceutics classification system
° C	Celsius degree
CAN-BD	Carbon dioxide-assisted nebulization with a bubble dryer
CD	Cyclodextrin
CI	Confidence interval
Conc.	Concentration
CPD	Controlled particle deposition
DMSO	Dimethylsulfoxide
DSC	Differential scanning calorimetry
DTA	Differential thermal analysis
DTG	Differential thermogravimetric analysis
e.g.	exempli gratia
et al.	et alii
Exp.	Experiment
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
i.e.	id est
GAS	Gas antisolvent precipitation
GC	Gas chromatography
γ-CD	γ-Cyclodextrin
GIT	Gastro intestinal tract
GLC	Gas liquid chromatography
h	Hour
HP-β-CD	Hydroxypropyl-β-cyclodextrin
HP-γ-CD	Hydroxypropyl-γ-cyclodextrin
HPLC	High performance liquid chromatography
K	Kelvin
Kv	Kilovolt

mA	Milliamper
mbar	Millibar
M-β-CD	Methyl-β-cyclodextrin
MCC	Microcrystalline cellulose
mg	Milligram
min	Minute
µl	Microlitter
ml	Milliliter
mM	Millimol
MPa	Megapascal
nm	Nanometer
NMR	Nuclear magnetic resonance spectrophotometry
No.	Number
NSAID	Non-steroidal anti-inflammatory drug
P_{eff}	Effective jejunal permeability
Ph. Eur.	Pharmacopoeia Europea
PVT phase	Pressure volume temperature phase
PVP	Polyvinyl pyrrolidone
RESS	Rapid expansion of supercritical solutions
rpm	Revolutions per minute
SAA	Supercritical assisted atomization
$scCO_2$	Supercritical carbon dioxide
SCF	Supercritical fluid
SD	Standard deviation
sec	Second
SEDS	Solution enhanced dispersion by supercritical fluids
SEM	Scanning electron microscopy
SLG	Solid-liquid-gas
TG	Thermogravimetry
USP	The United State pharmacopoeia
UV-VIS	Ultraviolet-visible spectrophotometry
% wt.	Weight in percent
XRD	X-ray diffractometry

Parts of this thesis have been published:

As research papers:

Hussein, K., Türk, M. and Wahl, M. A.
"Comparative evaluation of ibuprofen/β-cyclodextin complexes obtained by supercritical carbon dioxide and other conventional methods"
Pharm. Res. **24**(3), 585-592 (2007)

Türk, M., Upper, G., Steuernhlater. M., Hussein, Kh. and Wahl, M. A.
"Complex formation of ibuprofen and β-cyclodextrin by controlled particle deposition (CPD) using SC-CO_2"
J. Supercrit. Fluids **39** (3), 435-443 (2007)

Hussein, K., Türk, M. and Wahl, M. A.
"Drug loading into β-cyclodextrin granules using a supercritical fluid process for improved drug dissolution"
Eur. J. Pharm. Sci. (in press)

As oral communications:

Hussein, K., Türk, M. and Wahl, M. A.
"Herstellung und Charakterisierung von Ibuprofen/β-Cyclodextrin Komplexen mittels überkritischer Fluide Technologie"
DPhG Doktorandentagung, Freudenstadt-Lauterbad, Germany (2004)

Hussein, K., Türk, M. and Wahl, M. A.
"Preparation and evaluation of drug/β-cyclodextrin solid inclusion complexes by supercritical fluid technology"
9th Meeting on Supercritical Fluids, Trieste, Italy (2004), ISBN 2-905267-9

Türk, M., Hils, P., Hussein, K. and Wahl, M. A.
"Herstellung von mit submikronen Wirkstoffen beladenen Arzneistoffträgern mit überkritischen Fluiden"
GVC-Fachausschusssitzun, Hochdruckverfahrenstechnik, Karlsruhe, Germany (2004)

Türk, M., Crone, M., Hussein, K., Wischumerski, R. and Wahl, M. A.
"Complex formation of ibuprofen and β-cyclodextrin by controlled particle deposition (CPD)"
3th International Meeting on High Pressure Chemical Engineering, Erlangen, Germany (2006)

Türk, M., Crone, M., Hussein, K., Wischumerski, R. and Wahl, M. A.
"Controlled particle deposition (CPD): A novel process for the enhancement of the dissolution rate of poorly water-soluble drugs"
ACHEMA, Frankfurt am Main, Germany (2006)

As poster presentations:

Hussein, K., Türk, M. and Wahl, M. A.
"Loading of powder carriers by CPD (controlled particle deposition) method using sc-CO_2"
DPhG Jahrestagung, Mainz, Germany (2005), ISBN 3-00-016844-3

Hussein, K., Türk, M. and Wahl, M. A.
"Controlled particle deposition (CPD): A new technique to load drugs in submicron porous carrier using supercritical fluids"
AAPS Annual Meeting and Exposition, Nashville, USA (2005), ISBN 0-9711767-7-9

Hussein, K., Türk, M. and Wahl, M. A.

"Influence of preparation method on dissolution behaviour of drug/β-cylodextrin complex prepared using SC-CO_2 and other conventional methods"

10th European Meeting on Supercritical Fluids, Colmar, France (2005), ISBN 2-905267-47-X

Hussein, K., Türk, M. and Wahl, M. A.

"Controlled particle deposition (CPD): A new technique to load powder carriers using scCO_2"

5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Geneva, Switzerland (2006)

Hussein, K., Türk, M. and Wahl, M. A.

"Drug loading by controlled particle deposition (CPD) into β-cyclodextrin granulates for improved drug dissolution"

AAPS Annual Meeting and Exposition, San Antonio, USA (2006), ISBN 0-9787711-0-9

CHAPTER 1

INTRODUCTION

1.1 Bioavailability and dissolution of poorly water-soluble drugs

1.1.1 Bioavailability of drugs

Bioavailability is defined by the food and drug administration (FDA) in § 320.1 as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action". This definition focuses on the processes by which the active ingredients or moieties are released from an oral dosage form and move to the site of action (FDA guidance for industry, 2003).

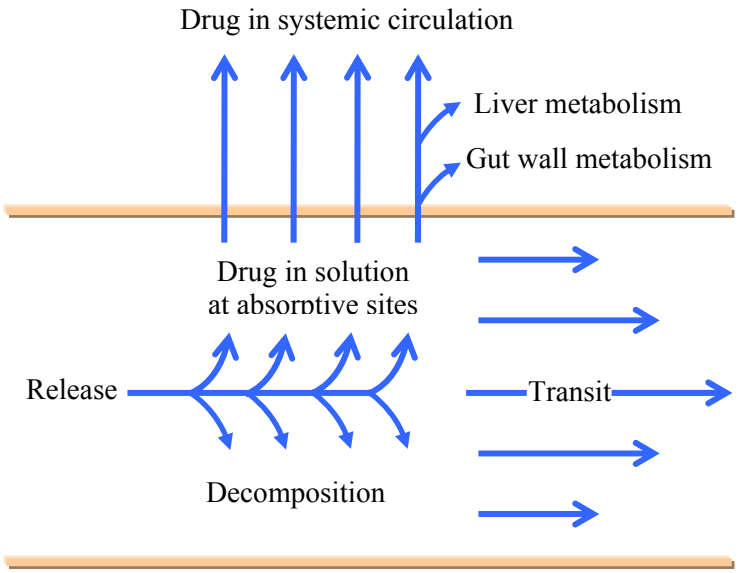

Figure 1.1 *Events in the GIT following administration of an oral dosage form according to* Dresseman and Reppas (2000).

In other words, for oral administration (gastrointestinal route), the drug must be dissolved in the physiological fluid and thereafter absorbed through entrance ports to act on target structures. Due to cost, convenience and compliance, the oral application of solid forms is the preferential way of application. The bioavailability of orally applied drugs depends on the velocity of dissolution rate and absorption, since only dissolved drug can pass the gastrointestinal membrane. Drug metabolism in the intestinal lumen, the intestinal wall and the liver may reduce its bioavailability (Chan and Stewart, 1996). Figure 1.1 depicts events in the gastrointestinal tract (GIT) following administration of an oral dosage form.

The major biopharmaceutical factors that affect the rate and extent of the absorption are solubility, dissolution rate and intestinal permeability of drugs. According to these three parameters, Amidon *et al*. (1995) proposed the biopharmaceutics classification system (BCS) which is incorporated later in the guidelines of the FDA (FDA guidance for industry, 2000).

1.1.2 Biopharmaceutics classification system

The biopharmaceutics classification system (BCS) is based on determining the underlying process that controls the drug absorption rate and extent, namely drug solubility and intestinal membrane permeability. The intention of this system was set up to a theoretical background for the *in vitro* dissolution with the *in vivo* bioavailability of drugs correlation (*in vitro – in vivo* correlation). According to the BCS, drugs can be categorized into four basic groups (Figure 1.2).

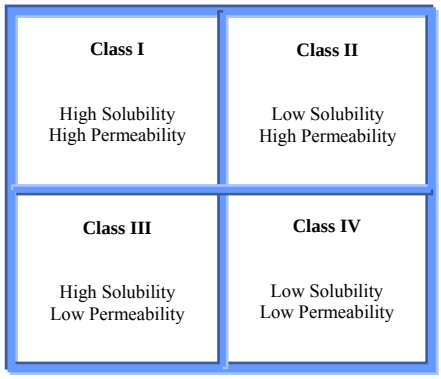

Figure 1.2 Biopharmaceutics classification system scheme according to Amidon *et al.* (1995).

BCS class I: Materials consist of water-soluble drugs that are well absorbed from GIT. For compounds of this class, formulated as immediate release products, dissolution rate generally exceed gastric emptying rates. Therefore, nearly 100 % absorption can be expected if at least 85 % of a product dissolved within 30 min. Drugs in this class are frequently lipophilic with a molecular weight less than 500 Da and aqueous solubility ≥ 1 mg/ml (Loftsson *et al*., 2004).

BCS class II: Compounds relatively lipophilic and water-insoluble drugs, that when dissolved, are absorbed from GIT. For this drug class the dissolution is limiting factor for their absorption.

BCS class III: Drugs are water-soluble but not ready to permeate biomembranes. A membrane permeability of these drugs is limiting factor for their absorption.

BCS class IV: Compounds consist of water-insoluble drugs which, when dissolved do not ready by permeate biomembranes. Oral drug delivery formulations of these drugs tend to be very difficult. (Dressman *et al*., 1998; Van de Waterbeemd *et al*., 2003)

Based on this classification system, a drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous medium over the pH range from 1.0 to 7.5. The estimated volume of 250 ml is derived from the standard glass of water, which should be consumed during the oral administration of the dosage form.

The effective permeability (P_{eff}) is generally described in terms of units of distance of molecular movement per unite time (10^{-4} cm/s). A drug is defined as high permeable if the extent of oral absorption is determined to be ≥ 90 % of an administrated oral dose (Loftsson *et al*., 2004). The permeability may be determined by *in vitro* or *in vivo* methods that can predict the extent of drug absorption in humans. The rapid dissolution class boundary is defined in terms of the *in vitro* dissolution being grater than 85 % during 30 min in 900 ml aqueous media at pH 4.5 and 6.8 using USP Apparatus I (100 rpm) or Apparatus II (50 rpm) (Avdeef, 2001; Yazdanian *et al*., 2004).

The key parameters controlling the drug absorption have been suggested by Amidon *et al*. (1995) and can be expressed by three dimensionless numbers; a dose number (Do), dissolution number (Dn) and an absorption number (An); representing

the fundamental processes of drug dose, dissolution and membrane permeation, respectively.

$$Dose\ Number = Do = \frac{M_0}{C_s V_0} \qquad \text{Eq.1.1}$$

M_0: Dose of drug administrated

C_s: Saturation solubility

V_0: Initial gastric volume

$$Dissolution\ Number = Dn = t_{res} / t_{Diss} \qquad \text{Eq.1.2}$$

t_{res}: Mean residence time

t_{Diss}: Time required for a drug particle to dissolve

$$Absorption\ Number = An = \frac{P_{eff}}{R} t_{res} \qquad \text{Eq.1.3}$$

P_{eff}: Effective permeability

R: Radius of the intestinal segment

For Class II drugs, limits are imposed on the absorption by the solubility Do or dissolution rate Dn either in general or on regional basis within the GIT leading incomplete absorption and bioavailability, despite the high membrane permeability (An). Generally, for these drugs increasing the dissolution rate increases their bioavailability (Persson, 2006).

1.1.3 Drug solubility

The solubility of a substance is the amount of substance that has passed into solution when equilibrium attained between the solution and excess, i.e. undissolved substance at given temperature and pressure. Values for solubility of drugs (mg/ml) were obtained from standard references (Merck Index, 2001; USP DI, 2004) defined the solubility of drugs as listed in table 1.1 (Kasim *et al*., 2003; Takagi *et al*., 2006).

The water-solubility of a drug can be determined using several methods such as the flask method and column elution method. In addition, it can be estimated using automated and miniaturized methods (Loftsson and Hreinsdottir, 2006).

Table 1.1 *Solubility definitions according to* Kasim *et al*. (2003) *and* Takagi *et al*. (2006).

Solubility definitions	Parts of solvent required for one part of solute	Solubility range (mg/ml)	Solubility assigned (mg/ml)
Very soluble	< 1	≥ 1000	1000
Freely soluble	1 - 10	100 - 1000	100
Soluble	10 - 30	33 - 100	33
Sparingly soluble	30 - 100	10 - 33	10
Slightly soluble	100 - 1000	1 - 10	1
Very slightly soluble	1000 - 10000	0.1 - 1	0.1
Partially insoluble	≥ 10000	< 0.1	0.01

1.1.4 Drug dissolution

In vitro dissolution testing provides useful information at several stages in the drug development process, formulation of dosage forms, quality control and *in vivo* - *in vitro* correlation.

Mechanisms of dissolution kinetics of crystals have been intensively studied in the pharmaceutical science. However, the dissolution of a solid substance can be described in two steps. At first molecules are released from the surface to surrounding dissolution media which create a saturated layer (stagnant layer), adjacent to the solid surface. Then, the drug diffuses into bulk of the solvent regions of high drug concentration to regions of low drug concentration (Figure 1.3).

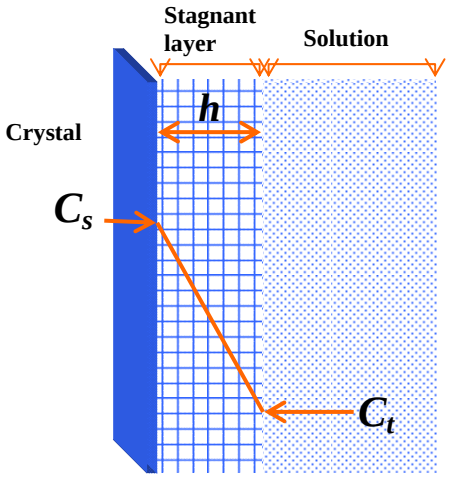

Figure 1.3 Concentration gradient during the dissolution process.

To understand the dissolution of a solid material a layer model has been employed (Bruner and Tolloczko, 1900; Nernest, 1904; Brunner, 1904). Based on this model, the mass rate of the dissolution (dM/dt) can be described as given in the Noyes - Whitney (1897) equation:

$$\frac{dM}{dt} = \frac{D \cdot S}{h} (C_s - C_t) \qquad \text{Eq.1.4}$$

D: Diffusion rate coefficient

S: Total surface area

h: Thickness of the dissolution layer

C_s: Saturation solubility

C_t: Drug concentration in the bulk solution at time t

The dissolution rate is proportional to both S and C_s. In other side, C_s is influenced by the composition of the dissolution medium and its pH. Therefore, the drug solubility and dissolution rate are not constant throughout the GIT. In the case of BCS class II drugs, the dissolution rate is influenced both by the physicochemical properties of the substance and by the prevailing physiological condition in the GIT. These parameters were discussed in details by Dressman and Reppas (2000) and are given in table 1.2. According to Noyes and Whitney (1897), the dissolution rate of a drug can be increased either by increasing the surface area (S) available for the solvent e.g.

reducing particle size or by increasing the saturation solubility C_s e.g. solubilisation of drugs into micelles, liposomes or into cyclodextrins (Pouton, 2006; Stegemann et al., 2007).

Table 1.2 Physical and physiological factors that can influence drug dissolution in GIT (Dressman and Reppas, 2000).

Parameter	Physical factor	Physiological factor
Surface area	Particle size	Surfactants in gastric juice and bile
Diffusion coefficient	Molecular size	Viscosity of luminal contents
Stagnant layer thickness		Motility patterns and flow rate
Solubility	Hydrophilicity and crystal structure	pH, buffer capacity, bile and food components
Concentration of drug in solution		Permeability
Volume of GI contents		Secretion and administration fluids

For the determination of *in vitro*-dissolution, numerous tests for solid dosage forms are reported in the literature and pharmacopoeias. The two general methods included in the Ph. Eur. 5.8 are the paddle and basket apparatus. These methods belong to the closed system and prescribe to measure dissolution from immediate release oral tablets and capsules or modified release oral dosage forms and other non-oral types of dosage form (Dyas and Shah, 2006). These methods, however, do not simulate the absorption in the GIT and are not suitable to measure the dissolution of poorly soluble drugs, for which sink condition is rather difficult if not impossible to reach.

In vitro systems for poorly water-soluble drugs should ideally maintain sink conditions and the dissolving solid should be investigated in fresh solvent. Such a situation is actually achieved in the open system e.g. flow through the cell (Ph. Eur. 5.8) or Stricker apparatus (Stricker, 1969). The apparatus as a model for drug dissolution test

can be employed as a useful tool to simulate an *in vivo* environment and used for low-solubility powders or immediate-release dosage forms.

1.2 Ibuprofen as model drug

Ibuprofen (Figure 1.4) a non-steroidal anti-inflammatory drug (NSAID) was invented in 1969. As NSAID, ibuprofen act through inhibition of cyclooxygenase enzyme (COX), thus inhibiting prostaglandin synthesis. There are at least two variants of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) and ibuprofen inhibits both. It appears that analgesic, antipyretic and clinical efficacy is achieved principally through COX-2 inhibition; whereas COX-1 inhibition is responsible for several "housekeeping" physiologic functions. However, the inhibition of COX-1 by conventional NSAIDs produces much of the toxicity and adverse drug events, particularly gastrointestinal toxicity, associated with these agents (Cannon and Breedveld, 2001).

Ibuprofen is used for the treatment of acute and chronic pain e.g. dysmenorrhoea and rheumatic diseases, as single doses of 200 - 800 mg three times to four times a day (maximum dose per day is 3.2 g) (Martindale, 1993).

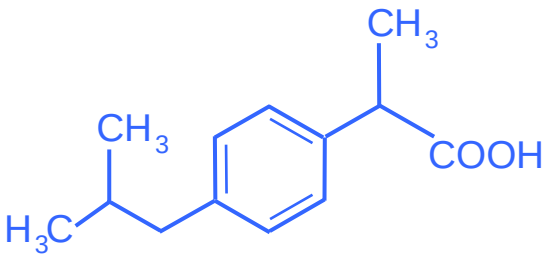

Figure 1.4 Chemical structure of ibuprofen.

Ibuprofen has been chosen in this study as a model of poorly water-soluble drug, since it is particularly insoluble in acidic aqueous solution and therefore it was regarded as representative of drugs that are only sparingly soluble in water (Herzfeldt and Kümmel, 1983). The bioavailability of ibuprofen is limited by its dissolution; once dissolved it readily gets absorbed throughout the gastrointestinal tract, therefore its fall in BSC class II (Wilson *et al.*, 1989).

Table 1.3 lists the important chemical, physical and pharmacokinetic data of ibuprofen.

Table 1.3 Chemical, physical and pharmacokinetic characteristics of ibuprofen.

	Ibuprofen
Systemic name	2-[4-(2-methylproply)phenyl]propanoic acid
CAS number	15687 - 27 - 1
Formula	$C_{13}H_{18}O_2$
Mol. mass	206.3 g/mol
Appearance	White, crystalline powder or colourless crystals
pK_a	4.4 - 5.2
Solubility	Practically insoluble in water, freely soluble in acetone, in methanol and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides and carbonates
Melting point	75 - 77 °C
Bioavailability	49 - 73 %
Protein binding	99 %
Metabolism	Hepatic
Half life	1.8 - 2 h
Extraction	Renal

1.3 Cyclodextrins as strategy to enhance water-solubility of drugs

In the fact, that about 40 % of the drugs being in the development pipelines and up to 60 % of the compounds coming directly from synthesis are categorized as poorly water-soluble (Merisko-Liversidge, 2002) and more than one third of the drugs listed in the US pharmacopoeia are poor water-soluble or water insoluble (Pace *et al*., 1999). The increasing number of poorly water-soluble drug candidates in the pharmaceutical sciences provides challenges for the oral formulation since their water solubility and rate of dissolution are a limiting step for their absorption and biological availability (Hörter and Dressman, 2001).

Several strategies for enhancing water-solubility and dissolution rate of these drugs have been attempted including particle size reduction to the nanoscale (Loth and Hemgesberg, 1986; Türk *et al*., 2002; Martin, 2003), preparation of solid dispersions (Serajuddin, 1999), drug loading in solid porous carriers (Vallet-Regi et *al*., 2001; Charnay *et al*., 2004; Andersson *et al*., 2004; Salonen *et al*., 2005) and liposome formulation (Gulati *et al.,* 1998). Cyclodextrin and their derivatives, however, have been successfully applied in the pharmaceutical industry as solubility enhancer because of the ability to form inclusion complexes with poorly water-soluble drugs (Frömming and Szejtli, 1994; Loftsson *et al*., 2004).

1.3.1 Fundamental

Cyclodextrins (CDs) are cyclic oligosaccharides, consisting of (α-1, 4)-linked α-D-glucopyranose unites, produced by degradation of starch using the glucosyltransferase enzyme (GCT). The first substance isolated and proved to be a cyclodextrin was published by Villiers in 1891 and characterised in 1903 by Schardinger (Szejtli, 1998). However, it took more than 50 years to establish and confirm the structure of CDs (Dodziuk *et al*., 2006). The molecular structure (Figure 1.5) of these glucose derivatives, which approximates a truncated cone or tours, generates a hydrophilic exterior surface and a non-polar cavity interior, due to the fact that hydroxyl functions are oriented on the cone to the exterior with the primary hydroxyl groups of the glucose residues at the narrow edge of the cone and the

secondary hydroxyl groups at the wider edge. The central cavity is lined by skeletal carbon and ethereal oxygen atom of the glucose, which give it a lipophilic character (Loftsson *et al*., 2004).

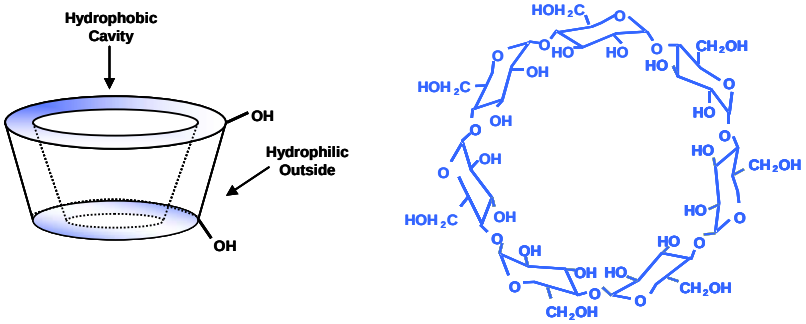

Figure 1.5 Chemical structure and toroidal shape of β-cyclodextrin molecule

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The natural α-, β- and γ-cyclodextrin consist of six; seven and eight glucopyranose unites. Cyclodextrins with more than eight glucose unites have been described, but they are instable (Duchene and Wouessidjewe, 1992).

Natural CDs, in particular β-cyclodextrin, have limited water solubility. The chemical and physical properties of natural CDs are listed in table 1.4.

Table 1.4 Characteristics of α-, β- and γ-cyclodextrin (Frömming and Szejtli, 1994; Saenger, 1980).

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
No. of Glucose unites	6	7	8
Formula	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Molecular weight [g/mol]	972.86	1135.01	1297.15
Inner diameter [nm]	50	60	80
Outer diameter [nm]	146	154	175
Height [nm]	79	79	79
Cavity volume 1 g CD [ml]	0.10	0.14	0.20
Water solubility [g/100ml]	14.5	1.85	23
Water molecule in cavity	6	11	17
pKa	12.332	12.202	12.081

A lot numbers of modified CDs have been prepared and show to have research applications. However, only the derivates containing the hydroxypropyl, methyl and sulfobutyl ether substitutes are used as pharmaceutical excipients. Derivatives improve dramatically the water solubility of CDs.

Due to CDs application in the food and the pharmaceutical industries, it's necessary to investigate the metabolism and toxicity of CDs. After the oral administration, the natural CDs are hydrolysed only in the colon. This hydrolysis resembles that of starch, but with a lower initial rate due to the fact that CDs are resistant to β-amylases active on the end of groups and sensitive only to α- amylases active in the middle of the chains (Duchene and Wouessidjewe, 1992).

The oral administration of CDs does not result in an acute toxicity; moreover, no significant change in the organs and the biological values was observed after the long-term administration of CDs.

Intramuscular administration of β-CD results in ulceration and its intravenous administration has nephrotoxic and haemolytic effects. These effects seem to be less in the γ-CD probably due to their better water solubility.

Lipophilic cyclodextrin derivatives, such as the methylated cyclodextrin, are to some extent absorbed from GIT into systemic circulation and have been shown to be toxic after the parenteral administration (Loftsson *et al*., 2004).

1.3.2 Pharmaceutical applications of CD

CDs are widely applied in the pharmaceutical industries. About 30 pharmaceutical products containing CDs are now on the market worldwide (Loftsson *et al*., 2004). However, the most common pharmaceutical application of CDs is to enhance the solubility, bioavailability and the stability of drugs. In addition, CDs can be used to decrease the side effect of drugs (gastrointestinal or ocular irritation), masking unpleasant odour or taste, transform gases or liquids into solids and for prolonged release drug formulations (Loftsson and Brewster, 1996).

1.3.3 Cyclodextrin complexes

CDs by their hydrophobic cavity are able to form inclusion complexes (Figure 1.6) with compounds having a size compatible with dimensions of the cavity. However, geometrical and chemical factors are determining the kind of guest molecules, which can penetrate into the CD cavity (Frömming and Szejtli, 1994).

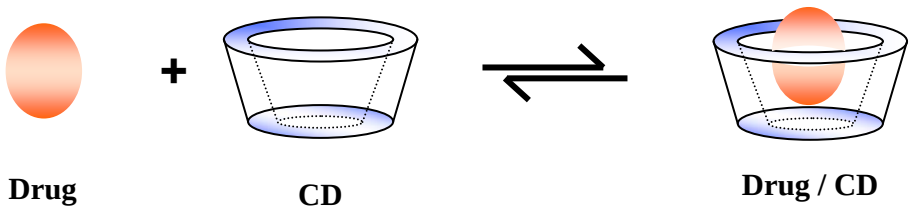


Figure 1.6 *Schematic illustration represent equilibrium binding of drug and cyclodextrin from a 1:1 complex according to* Rajewski and Stella (1996).

This knowledge was first suggested by Cramer in the 1940s. The term "inclusion compound" i.e. "Einschlussverbindung" was introduced by Schlenk (1950). The most important pharmaceutical application of CD complexes was patented in 1953 by Freudenberg, Cramer and Plieninger (Szejtli, 1998).

During the drug/CDs complex formation, no covalent bonds were formed or broken. The driving force leading to the inclusion complex formation includes release of enthalpy from substitution of the water molecule from the cavity, electrostatic interaction, Van der Waal's interaction, hydrogen bonding, release of conformational strain and charge-transfer interaction (Loftsson *et al*., 2004; Frömming and Szejtli, 1994). All these forces are relatively week, allowing free drug molecules in solution to be in rapid equilibrium with drug bound within the CD cavity (Rajewski and Stella, 1996). However, the complex formation of hydrophobic drugs with CDs through an equilibrium process can be specified quantitatively by an association or stability constant ($K_{a:b}$).

$$Drug_a + CD_b \rightleftharpoons Drug_a / CD_b \text{ complex}$$

$$k_{a:b} = \frac{[Drug_a / CD_b]}{[Drug]^a\ [CD]^b} \qquad \text{Eq.1.5}$$

a and b: Molar ratio of the sequestered drug molecule to the CD

The knowledge of this stability constant can be used to compare the binding effectiveness of different CDs, or prediction of the chemical stability of the inclusion compound or the possible release of the included molecule. Various complexes with different ratio of drugs/CDs can be formed depending of the CD type and physicochemical properties of drugs.

1.3.4 Preparation of drug/CD binary system

Various techniques to prepare drug/CD complexes in solution, suspension, paste or by dry mixing have been reported in the literature (Frömming and Szejtli, 1994; Cabral-Marques, 1994; Hedges, 1998). However, these methods are similar to each other, using successively less water.

1.3.4.1 Preparation of drug/CD in a solution

The presence of water is necessary to prepare a drug/CD complex in this method. Addition of organic solvent may be necessary when the guest molecule is hydrophobic. Only limited organic solvent e.g. ethanol, methanol, propanol and diethyl ether can be used.

The common procedure is to stir or shake the aqueous/organic solution for a certain time (hours - weeks) at a certain temperature (20 - 80 °C). After stirring, the mixture is stored at a temperature of 3 - 5 °C, then filtered or centrifuged. The crystalline product is dried to constant weight.

In case of highly soluble CDs the solution can be dried using spry-drying or freeze-drying.

The use of organic solvent and high temperature limit the applications of these methods in the industry; hence, it is described in the literature as a laboratory method.

1.3.4.2 Slurry method

In the so-called slurry method, the CD and the guest are not dissolved, but at least suspended in the solution. The complex is collected in this method after stirring, filtering and drying processes. This method can be varied by the use of even less water to produce the complex "pasta-like"; it is then called "knead" method. The slurry method, however, is the most feasible method for industrial purpose (Frömming and Szejtli, 1994).

1.3.4.3 Preparation of drug/CD in a solid

The complex can be prepared by a simple mechanical mixing of CD and the guest substance using a vibration mill or a ball mill, since the mixing times can range from hours to days. However, no significant extent of complexation was observed in this case for β-CD.

The disadvantages of some of these conventional complex formation techniques are limiting the use of the complexation in a large scale in the industrial applications. Including the requirement of organic or toxic solvents, the latter may result in residues in the products, environmental pollution may occur and a multi-stage processing including long drying steps may affect drug stability. To overcome these disadvantages, alternative methods for preparing drug/CD complexes using supercritical fluid (SCF) technology have been developed, since its non-toxic, environment-friendly behaviour, which dose not leave traces in the product, and no drying steps are required. SCF techniques used to prepare the CDs complexes are delineated in detail in section 1.5.

1.3.5 Preparation of drug/CD/polymer ternary system

For reasons of costs, production capabilities and toxicology, only limited amount of CDs must be used in drug formulation. In fact, the isolated crystalline

product obtained by each complexation procedure can contain an uncomplexed part of a drug or empty CD. Methods to improve the inclusion efficiency and optimise the CD complexation are reported in the literature (Mosher and Thompson, 2006). However, the addition of a small amount of water-soluble polymers to the aqueous complexation media increases the complexation efficiency of CDs towards the drug by increasing the stability of the complexes, resulting in enhanced drug solubility. This knowledge was confirmed using spectrophotometry, NMR and thermodynamic methods. Furthermore, the increase of the drug bioavailability through this addition was reported (Loftsson, 1998; Mura *et al*., 2001; Faucci *et al*., 2001; Pose-Vilarnovo *et al*., 2002; Valero *et al.,* 2003).

1.3.6 CDs in granules formulations

Although the advantages of granules and tablets as solid dosage forms compared to powders; only a few numbers of papers and patents formulate the CD complexes for a solid dosage form.

Frömming and Szejtli (1994) in their detailed review reported some examples for preparation of granules containing CDs. A preparation of a CD complex as granules containing drug was reported as well by Ghorab and Adeyeye (2001 and 2003). The authors in this study prepared ibuprofen-β-CD granules by mixing the drug and β-CD in a conventional wet granulation process. The complex formation was confirmed using different characterisation methods; in addition, the enhanced bioavailability of process-induced fast-dissolving ibuprofen co-granulated with β-CD was investigated.

In 1998 Gazzaniga *et al*. investigated the use of β-CD as a pelletization agent with microcrystalline cellulose (MCC) in an extrusion/spherounization process. The addition of MCC conferred mechanical strength to the obtained pellets and reduced the amount of β-CD in the final formulation. In related work, Gainotti *et al*. (2004) produced drug-free pellets of β-CD and MCC using a high-shear mixer and loaded the obtained pellets with ibuprofen using powder and solution layering processes.

1.3.7 Characterisation of CD systems

There is no guarantee that the material obtained by any complexation method is a true homogeneous inclusion complex. In many cases, the isolated solid product is a mixture of complex, uncomplexed guest and empty CD. Therefore, the formation of an inclusion complex has to confirm using quantitative analysis methods, thermoanalytical methods, solid-state spectroscopic methods and morphological mechanical methods. However, the selection of a specialised technique frequently depends on equipments available and properties of the guest.

1.3.7.1 Determination of guest content

The quantitative determination of the guest content can be performed by analytical methods such as UV, GLC, GC and HPLC.

1.3.7.2 Thermoanalytical methods

The complex formation can be confirmed using different thermoanalytical methods (TG, DTG, DSC and DTA). These methods can only be used when the guest has a melting/boiling temperature below the thermal degradation range of the cyclodextrin at about 250 °C. Using the DSC, no energy absorption is observed at the melting range of the guest when the guest is completed. In fact the guest molecule is surrounded by the CD. Therefore, an endothermic melting peak can be observed for the drug and its physical mixture with CD but will be absent for the complex in case of complete complex formation of drug in CD (Frömming and Szejtli, 1994).
In addition, the uncompleted fraction of the guest can be estimated and quantified from the melting peak data of the guest using DSC measurement especially if this amount is large (Salonen *et al*., 2005; Mura *et al*., 2003).

1.3.7.3 Solid-state spectroscopic methods

Several methods such as NMR, FTIR and X-ray are used to prove that the complex is formed.
Among solid-state analytical techniques, ^{13}C-NMR is a powerful one for the identification of CD inclusion complexes and to obtain information on corresponding

binding modes. The inclusion formation can be confirmed from the unique splitting pattern of the host lattice spectrum. This method also provides information about the guest molecule orientation and location of cavity water molecules before and after the inclusion (Schneider *et al*., 1998; Injoon *et al*., 1998; Frömming and Szejtli, 1994; Cabral-Marques, 1994).

Fourier transform infrared spectroscopy (FTIR) has also been used for analyses of CD complexes in some case. Upon the host/guest interaction, bands due to the included part of the guest are generally shifted or their intensities altered. In the literature most often, FTIR studies of such CD complexes are reported which have a carbonyl group-bearing guest. This is due to the adequate and well separated bands of the carbonyls stretching bands that appear between 1680 and 1700 cm^{-1} which is significantly covered and shifted by CD complexation (Frömming and Szejtli, 1994).

In the recent years, X-ray crystallography is reported as the only exact method for elucidation of the molecular structure of CD inclusion complex (Saenger, 1980). Powder X-ray diffractometry is a simple and exact method for the detection of CD inclusion compounds in powder or microcrystalline state. In case of crystalline guest molecules, the complex formation can be suggested by missing or appearance of a reflection band in the CD diffractogram, this method has been exploited to confirm the complex formation of β-CD with NSAID (Cabral-Marques, 1994; Frömming and Szejtli, 1994).

1.3.7.4 Morphological mechanical properties

The morphological investigation using scanning electron microscopy (SEM) of the obtained microcrystalline powder can be utilised to confirm the complex formation by the appearance of a new crystal population compared to the CD and the guest crystals and their physical mixture. This method has been used widely in the recent years.

1.4 Utilisation of SCF for formation of CD complexes

1.4.1 Fundamental

Supercritical fluids are described as fluids and gases in the temperature and pressure state of above critical point (Fukushima, 2000). The critical point represents the highest temperature and pressure at which the substance can exist as a gas and liquid in equilibrium. The range of pressures and temperatures that define the supercritical fluid region of the diagram are shown in the PVT phase diagram for the pure compound (Figure 1.7). The first description of a supercritical phase was in 1822 by Baron Cagniard de la Tour. Despite this early discovery, applications development reached the initial peak during the period from the second half of the 1960s to the 1970s followed by a secondary peak about 15 years later (Fukushima, 2000)

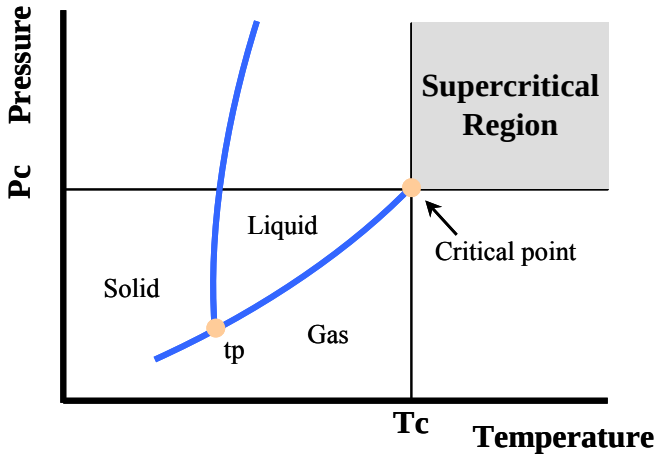


Figure 1.7 Phase diagram for a pure substance.

The physicochemical properties of a supercritical fluid vary over a wide range depending on pressure and temperature but are generally intermediate between those of gases and liquids. A comparison of some physicochemical properties of gases, liquids and supercritical fluids are given in table 1.5 according to McHugh and Krukonis (1986).

Table 1.5 Physiochemical properties of gases, liquids and supercritical fluids.

	Density [g/cm^3]	Diffusivity [cm²/s]	Viscosity [g/(cm.s)]
Gas	10^{-3}	10^{-1}	10^{-4}
Supercritical fluid	10^{-1}	10^{-3}	10^{-3}
Liquid	1	10^{-6}	10^{-2}

Supercritical fluids have liquid–like density values enabling appreciable salvation power. Therefore, the SCFs are capable of extracting like a liquid, but without the difficulties of removing liquids. In addition, the viscosity of solutes in supercritical fluids is lower than in liquids and the diffusivity of solutes is higher; hence, the SCFs facilitate mass transfer and have penetrating properties.

The dissolving power of SCFs offers a safe solvent for the extraction processes in comparison with the conventional extraction. Products dissolved in a SCF may be separated out by pressure reduction and/or by changing the temperature avoiding the use of additional drying steps.

The above-discussed particular properties of SCFs resulted in several applications in food and pharmaceutical industries. SCF-extraction is the most commonly used technical process employing SCFs (McHugh and Krukonis, 1986; King and Bott, 1993; Kaiser *et al*., 2001). Moreover, SCFs has been utilised for purification and separation purposes in food processing and distillation industries as well as in analytical chemistry applications for many years (Arai *et al.,* 2002; Valcarcel and Tena, 1997). Decaffeination of tea and coffee beans and purification and fractionation of polymers from residual solvent are examples of its use (McHugh and Krukonis, 1986).

Furthermore, SCFs offer exciting opportunities to the pharmaceutical scientific to cater to various processing needs. They can be used in a recent operation as reaction media during drug discovery process and have been successfully used in the enantioselective synthesis of drugs. SCF acts as ideal medium for enzyme mediated reactions as enzymes retain their activity when exposed to SCFs. Additionally SCFs are commonly used in the polymer engineering technology, particle coating and for generating a

particle in micro/nano-scale using different techniques such as RESS, precipitation using a SCF as anti-solvent (GAS and SEDS) and supercritical carbon dioxide assisted spray-drying (CAN-BD and SAA) (Arai *et al.,* 2002; Pasquali *et al.,* 2006; York *et al.,* 2004).

1.4.2 Supercritical carbon dioxide

Supercritical carbon dioxide ($scCO_2$) is the most widely used supercritical fluid, due to low cost, non-toxic, non-flammable and environment-friendly behaviour. In addition, it exhibits a low critical temperature and pressure (Tc = 30.98 °C, Pc = 73.773 bar) As a consequence, $scCO_2$ is suitable for use as a solvent for thermally labile natural substances or pharmaceutical agents. Moreover, $scCO_2$ is a good solvent for many non-polar (and some polar) molecules with low molecular weight (Devine *et al*., 2002).

1.4.3 Formation of CD complexes using SCFs

In the last ten years, SCF processes were developed and optimised in order to achieve broad applications in drug delivery systems. One of the most frequent technique applications of the SCF process is to enhance the dissolution of poorly water-soluble drug using two main strategies, either by micronizing these compounds into nano/micro particles or to formulate them by encapsulation, CD inclusion and impregnation (Perrut *et al.*, 2004). However, the complex formation of drugs with CDs looks as a very promising solution to enhance the dissolution of poorly soluble agents.

Several SCF processes have been attempted to prepare drugs/CDs complex (Table 1.6).

In 1999, Van Hees *et al.* prepared a piroxicam/β-CD (1:2.5 molar ratio) inclusion complex using a static system with a multistage process by keeping a physical mixture of the drug with a native cyclodextrin three hours in the $scCO_2$ at 150 °C and 45 MPa. The authors assumed the high inclusion yield was due to the high temperature and pressure of CO_2, which could promote exchange of the water molecules with piroxicam inside the cyclodextrin. In the same technique, the formation of miconazole/miconazole nitrate complexes with several CDs (β-CD, HP-β-CD, γ-CD

and HP-γ-CD) complexes was investigated. Additionally, the influence of different molar ratios from 1:0.5 to 1:2.5 and the effect of the addition of ternary alkaline compounds was also studied in temperature of 125-137 and 150 °C, three levels of pressure (15 - 30 and 45 MPa) and two different contact times (60 and 180 min). The author established that the complex formation of miconazole and *γ*-CD or HP-*γ*-CD by treating physical mixtures in $scCO_2$ is a good method to improve the water solubility of miconazole. In both studies, the authors use high temperature processing; however, this is not attractive process for heat-sensitive pharmaceutical substances.

Charoenchaitrakool *et al.* (2002) prepared the complex of ibuprofen with methyl-β-cyclodextrin (M-β-CD) as a solid dispersion using a multi-stage dynamic system by passing a supercritical CO_2/ibuprofen mixture through an M-β-CD packed bed. The authors showed that the maximum of drug loading was 10.8 % wt. at 19 MPa, 35 °C and 24 h static contacting time in CO_2. In this procedure, it should be considered that under these conditions M-β-CD was melted since M-β-CD starts to transform into a liquid at 14.7 MPa and 35°C when contacted with supercritical CO_2. Using other technique the same group prepared a naproxen/CD by supercritical anti-solvent process, using ethanol or DMSO-ethanol or DMSO-acetone mixtures as solvent and carbon dioxide as anti-solvent. The resulting particles were mainly constituted by naproxen/CD complex (as shown by DSC curves) exhibiting a very fast dissolution profile in water at 37 °C, in comparison with a physical mixture or a coevaporated mixture of both compounds. The use of organic solvent in the last study did not provide any advantage compared to the conventional methods for complex formation. However, the use of supercritical anti-solvent as method for the complex formation was reported later in other works (Freiss *et al*., 2003; Jun e*t al*., 2007; Moribe *et al*., 2007).

The preparation of naproxen/β-CD complex using $scCO_2$ and ethanol as cosolvent was reported by Junco *et al*. (2002), this study, however, did not contain any data on dissolution. Bandi *et al*. (2004) prepared the complex of budesonide and indomethacin with amorphous cyclodextrin (HP-β-CD) by exposing physical mixtures of budesonide-HP-β-CD (0.31:1 molar ratio) and indomethacin/HP-β-CD (0.85:1 and 0.35:1 molar ratio) 20 hours to supercritical CO_2 at 40 °C and 21.1 MPa. The authors

explain the improved drug dissolution by a change in the physical form of the drug (from crystalline to amorphous state) and/or HP-β-CD to a more soluble form since supercritical CO_2 treatment of the pure drugs did not enhance the dissolution rate.

Table 1.6 Drug/CDs binary complexes processed by SCFs.

Drug	CDs	SCF	Publication
Budesonide and indomethacin	HP-β-CD	CO_2	Bandi *et al*., 2004
Eflucimibe	β-CD	DMSO and CO_2	Freiss *et al*., 2003
Ibuprofen	M-β-CD	CO_2	Charoenchaitrakool *et al.*, 2002
Ibuprofen	β-CD	CO_2	Hussein *et al*., 2007 and Türk *et al*., 2007
Ibuprofen, flurbiprofen and naproxen	Trimethyl-β-CD	CO_2/ethanol	Moribe *et al.,* 2007
Itraconazole	β-CD	CO_2	Hassan *et al.*, 2004 and Al-Marzouqi *et al*., 2006
Miconazole and miconazole nitrate	β-CD/HP-β-CD/ γ-CD/HP-γ-CD	CO_2	Van Hess *et al*., 2002
Naproxen		Ethanol/DMSO-ethanol or DMSO-acetone and CO_2	Foster *et al*., 2002
Naproxen	β-CD	CO_2 and ethanol	Junco *et al*., 2002
Piroxicam	β-CD	CO_2	Van Hees *et al*., 1999
Simvastatin	HP-β-CD	CO_2 and ethanol	Jun *et al*., 2007

CHAPTER 2

AIM OF THE STUDY

Therapeutic effectiveness of a drug depends on the bioavailability and ultimately on the solubility of drug molecules. For most orally administered, poorly water-soluble drugs (BCS class II), the absorption is rate limited by dissolution. The dissolution of poorly water-soluble drugs depends on the effective surface area, crystal habit, and the energy state within the drug crystals. Although dissolution is directly proportional to the specific surface area of the hydrophobic drugs, other factors such as wettability, air adsorption, and agglomeration also play an important role in drug dissolution (Sharma *et al*., 2005).

To improve the solubility of drugs, Martin (2003) showed in a previous study, that the mircronisation to nano-scale of hydrophobic compound (griseovulvin) using rapid expansion of supercritical solutions (RESS) technology is an important factor to improve their water-solubility, absorption and bioavailability. Otherwise, the high surface energy of the obtained nanosized objects gives rise to unique surface properties and reactions or lead to the agglomeration of particles

With this respect, our aim is to enhance the water solubility of poorly water-soluble drugs by developing a toxic-free, single-step, SCF process for loading a submicron drug into solid carriers under the perpetuation of the positive properties of the carriers. To achieve this goal, the controlled particle deposition (CPD) method is developed.

With the introduction, drug/CD complex formation is a promising strategy to increasing the water solubility of drugs. A native crystalline β-CD is chosen as carrier to be loaded by a model drug (ibuprofen) in the CPD method.

For optimisation of the ibuprofen/β-CD complex formation in the CPD process, the stability and/or the solubility of both drug and carriers in $scCO_2$, the influence of the

CPD experimental parameters on the loading process and the process reproducibility will be investigated.

In order to evaluate this novel technique, the ibuprofen/β-CD complex obtained by the CPD will be compared with ibuprofen/β-CD complexes obtained using conventional methods for the complex formation (freeze-drying, co-precipitation and physical mixture). The obtained solid complexes will be characterised for quantitative determining of the un- and complexed drug content (HPLC), the crystallinity using X-ray diffraction (XRD), differential scanning calorimetry (DSC) and morphologically using scanning electron microscopy (SEM). Furthermore, the interactions between the drug and β-CD will be elucidated using fourier transform infrared spectroscopy (FTIR). Ultimately, the ability of the obtained materials to improve the water solubility compared to the pure drug will be investigated using *in vitro* dissolution test.

The effect of the addition of a water-soluble polymer on the drug/CD binary system was investigated in the aqueous solution or/and in the solid, using classical methods for the complex formation e.g. freeze-drying or spray-drying (Loftsson 1998; Mura *et al*., 2001; Faucci *et al*., 2001; Pose-Vilarnovo *et al*., 2002; Valero *et al*., 2003). To date, the effect of the presence of water-soluble polymers in $scCO_2$ as drug/CD complexation media is not reported. Therefore, the preparation of ibuprofen/β-CD/ PVP ternary system in the CPD and characterization of its physicochemical and dissolution properties are the second objective of the study.

The third objective of the study is to expand the application of the CPD by loading drug in various carriers. Charoenchaitrakool *et al*. (2002) prepared ibuprofen/M-β-CD using a multi-step supercritical process; however, the formation of this complex using a single-step process (CPD) will be investigated in our work.

Finally, granules depending on β-CD prepared by a wet granulation method will be utilise as carrier to be loaded with ibuprofen either by the CPD process or by the solution immersing (SI) as a conventional method for comparison. The drug-loaded granules will be characterised for solid state and dissolution properties.

CHAPTER 3

MATERIALS

3.1 Materials

The materials used in this work are divided in three groups: model drug, excipients and chemicals. All the materials and solvents were of the purest grade available and used as received without further purification.

3.1.1 Drug

Table 3.1 List of drug.

Name	Labelling	Manufacturer/supplier
Ibuprofen	Batch No. 460490	Knoll Pharmaceuticals

3.1.2 Excipients

Table 3.2 List of excipients.

Name	Labelling	Manufacturer/supplier
β-Cylodextrin	Cavamax® W7 Pharma	Wacker-Chemie AG
Methyl-β-cylodextrin	Cavasol® W7 M	Wacker-Chemie AG
Microcrystalline cellulose	Avicel® PH 102	FMC Biopolymer
Polyvinyl pyrrolidon	Kollidon® 25	BASF AG

3.1.3 Chemicals

Table 3.3 List of chemicals.

Name	Manufacturer/supplier
Gases	
Carbon dioxide	Air Liquide GmbH
Helium (Type 4.6)	Messer Griesheim GmbH
Nitrogen (Type 5.0)	Messer Griesheim GmbH
Chemicals	
Acetonitril (gradient grade)	Fischer Scientifics GmbH and J.T.Baker
Aqua ad injectabilia	Own production
Ammoniac solution 25 % wt.	Merck KGaA
Buffer solution pH 4.0	Merck KGaA
Buffer solution pH 7.0	Merck KGaA
Buffer solution pH 9.0	Merck KGaA
Calcium chloride	Merck KGaA
Diethyl ether	BASF AG
D (+) Glucose monohydrate	Merck KGaA
Disodium hydrogen phosphate	Merck KGaA
HEPES 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid	Fluka
n-Hexane	Fischer Scientifics GmbH and Carl Roth GmbH
Magnesium chloride hexahydrate	Merck KGaA
Magnesium sulfate heptahedrat	Merck KGaA
MES [2-(4-Morpholino) ethanesulfonic acid	Fluka

Name	Manufacturer/supplier
Phosphoric acid 85 % wt.	Merck KGaA
Potassium dihydrogen phosphate	Merck KGaA
Potassium chloride	Merck KGaA
Sodium chloride	Merck KGaA
Sodium dihydrogen phosphate	Merck KGaA
Sodium hydroxide 0.1 N	Merck KGaA
Sodium hydroxide 1 N	Merck KGaA

3.2 General equipments

Table 3.4 List of general equipments.

Equipment	Manufacturer
Balance Mettler AT 261 Delta range	Mettler Toledo GmbH
Balance Mettler PC 1616	Mettler Toledo GmbH
Balance Mettler PC 4600 Delta range	Mettler Toledo GmbH
Balance Mettler PM 6100	Mettler Toledo GmbH
Balance Sartorius CP224s	Sartorius AG
Centrifuge Megafuge 1.0R	Heraeus Instruments
Drying oven T5050	Haraeus Holding GmbH
PH Meter 761 Calimatic	Knick GmbH
PH Meter Seven Easy	Mettler Toledo GmbH
Single channel pipette (5 - 50) µl	Eppendorf AG
Single channel pipette (20 - 200) µl	Eppendorf AG
Single channel pipette (100 - 10000) µl	Eppendorf AG
Turbula mixer T2C	Willy A. Bachofen AG
Ultra sonic bad Sonorex Super RK510H	Bandelin electronic GmbH

Equipment	Manufacturer
UV-VIS spectrometer 550 S	Perkin Elmer GmbH
UV-VIS spectrometer Lamda 16	Perkin Elmer GmbH
UV-VIS spectrometer Plattenreader. Synergy HT	Bio-Tek Instruments
Water distillation apparatus	Wagner & Munz GmbH
Water purification system (revers osmosis) Hemo-Ro	Millipore GmbH

3.3 Other consumable materials

Table 3.5 List of other consumable materials.

Name	Labelling	Manufacturer
Crimp caps (HPLC)	Product No.7056	Macherey-Nagel GmbH
Membrane filter 0.45 µm	Regenerated cellulose Ø 25 mm	Sartorius AG
Membrane filter 0.45 µm	Cellulose nitrate Ø 47 mm	Sartorius AG
Vials (HPLC)	Product No.70201	Macherey-Nagel GmbH

3.4 Data processing

Notebook: Acer aspire 1314 LC Athlon XP-M 2400, 512 MB RAM, 40 GB hard disk.

Table 3.6 List of softwares.

Software	Manufacturer
ISIS Draw 2.1.4	MDL®
GraphPad Prism 4.0	GraphPad
Microsoft Windows XP Professional	Microsoft
Microsoft Excel 2003	Microsoft

Software	Manufacturer
Microsoft Photo Editor 2003	Microsoft
Microsoft Power Point 2003	Microsoft
Microsoft Word 2003	Microsoft
Origin 6.0	OriginLab

CHAPTER 4

METHODS

4.1 Controlled particle deposition process

4.1.1 General description

The key idea behind the controlled particle deposition (CPD) process is to dissolve the solute in the SCF followed by permeation of this fluid into the solid carrier, this achieves by in a single-step process without using any organic or toxic solvent or co-solvent.

Drug loading experiments were carried out at the Institut für Technische Thermodynamik und Kältetechnik, Universität Karlsruhe (TH), using a static mode SCF set-up. The static mode, however, uses a fixed amount of CO_2. In the CPD, a known amount of the drug and the carrier are placed in separate cartridges, which have a sieve form inside a high pressure cell. Then the high pressure cell was closed and immersed in a water bath and heated to the desired temperature. The required amount of the liquid CO_2 was condensed in the high pressure cell. As soon as the desired temperature was reached, the drug/scCO_2 precipitated in the solid carrier. During the treatment time, pressure and temperature are controlled. At the end of the experiment, the SCF was removed from the high pressure cell by decreasing the pressure, thereby allowing the fluid to evaporate in gas form. The final product was taken from the carrier chamber. The drug loading process by the CPD is depicted schematically in figure 4.1.

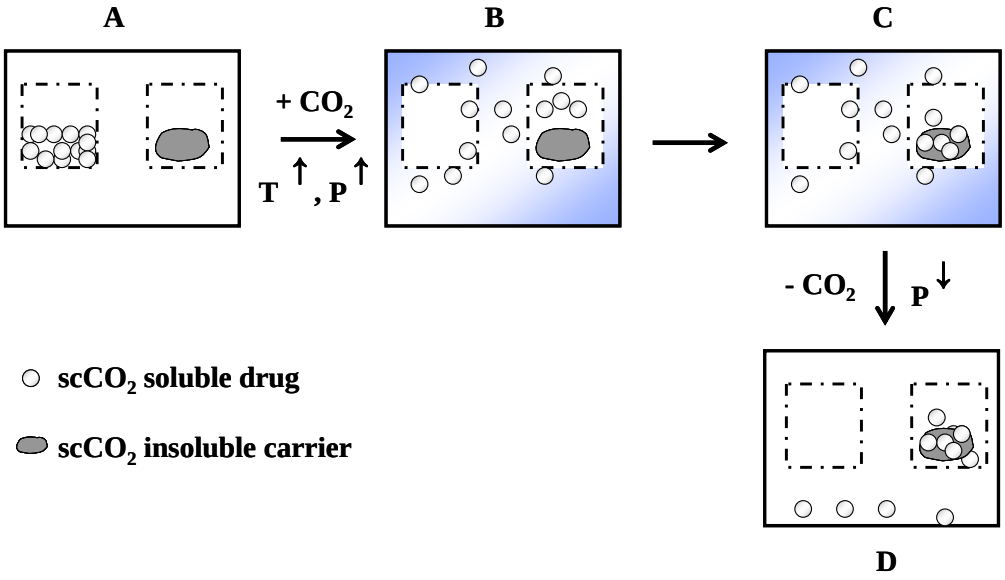


Figure 4.1 Schematic illustration of drug loading by the CPD process:

(A) The drug and the carrier packed in separate vessels.

(B) CO_2 was condensed in the reactor and the system brought to the desired temperature and pressure.

(C) The $scCO_2$ dissolved drug particle permeates in the solid carrier.

(D) After the treatment time depressurisation was occurred and the drug participate in the carrier.

4.1.2 Phase behaviour study

The knowledge of the equilibrium solubility of the drugs in the SCF is an essential factor in designing a drug-loading process in the CPD. The investigations of the phase behaviour were performed at the Institut für Technische Thermodynamik und Kältetechnik, Universität Karlsruhe (TH). The pressure and the temperature of the three phases SLG coexistence curve of the drug/carrier under CO_2 pressure were measured according to the first melting point method (Lemert and Johnston, 1989; Diefenbacher and Türk, 2002). This method depends on the observation of the melting point onset using a high pressure view cell by controlled increase of the temperature. The experiments were carried out using a static mode, described in detail by Türk *et al.* (2007). For a typical experiment, about 1 g of the solid material was placed in the equilibrium cell. Thereafter, the system is evacuated at 0.4 Pa for 5 min and the view cell is heated to the desired temperature. Afterwards, CO_2 is gradually charged from

the gas cylinder into the view cell and the pressure adjusted at the desired value using a manual pump.

4.1.3 Primary CPD experimental apparatus (CPD I)

At the beginning of the work, the loading experiments were performed in a static mode (Figure 4.2 and Figure 4.3). This apparatus enables experiments in the temperature range from 5 to 80 °C and pressures up to 50 MPa (Türk *et al.*, 2007). The temperature inside the high pressure cell is measured with a Pt-100 thermometer (Merz Messfühlertechnik GmbH, Deisenhofen, Germany). The accuracy of the temperature measurement is within the total limit of ±0.06 °C. The system pressure is measured with a piezoresistive pressure gauge (WIKA Alexander Wiegand GmbH & Co. KG, Klingenberg, Germany). The total uncertainty of the pressure measurement is within ±0.06 MPa.

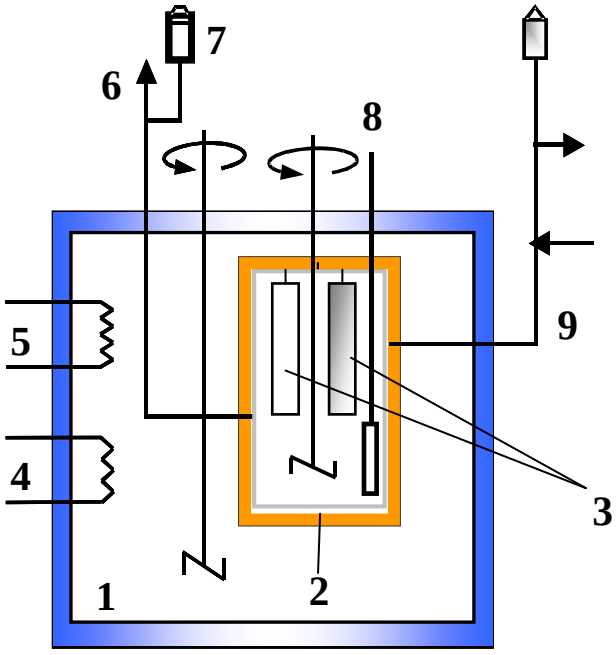
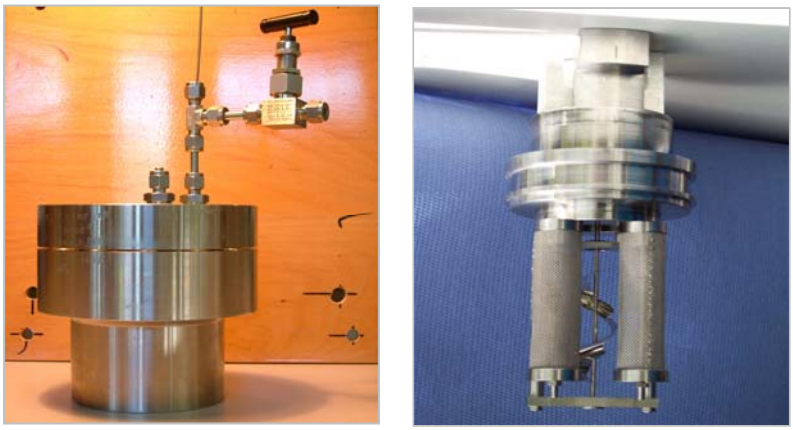


Figure 4.2 Schematic diagram of the CPD I apparatus for preparation of inclusion complexes in SCF (Türk et al., 2007): Liquid thermostat (1), high-pressure cell (2), drug and carrier cartridges (3), heating (4); cooling (5), pressure sensor (6), pressure-relief valve (7), Pt-100 thermometer (8) and inlet/outlet (9).



(A) (B)

Figure 4.3 Pictures of the experimental set-up CPD I

(A) High pressure cell.

(B) Drug and carrier cartridges.

4.1.4 Modified CPD experimental apparatus (CPD II)

A few technical modifications in the primary CPD I apparatus have been applied to receive reproducible and predictable results. The first modification was in the localisation of the drug/carrier cartridges (Figure 4.2, 3), which were placed in horizontal attitude in the upper part of the high pressure cell instead of vertical in the primary set-up. This has been done in order to be sure that the loading experiments occur in the supercritical phase and not in the liquid CO_2 that may be found at the bottom of the high-pressure cell during the depressurisation. The second modification was a change in the position of Pt-100 thermometer (Figure 4.2, 8) which was placed in the water bath in the modified apparatus CPD II. The last stand of the CPD apparatus is shown in the schematically representation (Figure 4.4) and the photograph (Figure 4.5).

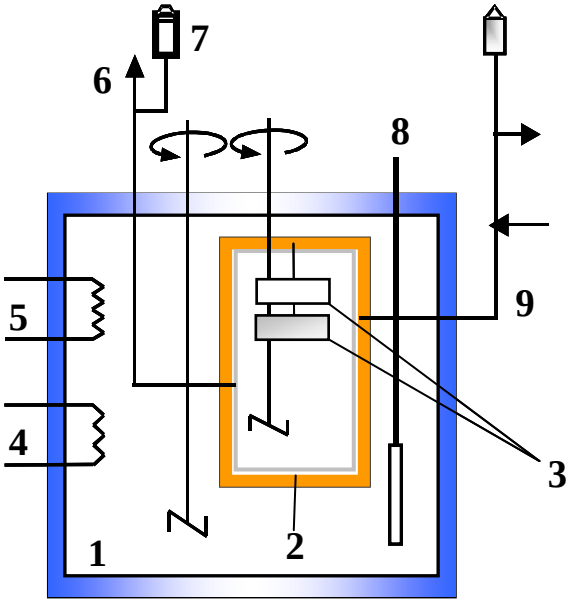


Figure 4.4 *Schematic diagram of the CPD II apparatus for preparation of inclusion complexes in SCF (Hussein et al., 2007): Liquid thermostat (1), high-pressure cell (2), drug and carrier cartridges (3), heating (4); cooling (5), pressure sensor (6) pressure-relief valve (7), Pt-100 thermometer (8) and inlet/outlet (9).*

Figure 4.5 *Picture of modified drug or carrier cartridge in the experimental set-up CPD II.*

4.2 Analytical methods

4.2.1 Determination of ibuprofen content in the binary system using high performance liquid chromatography (HPLC)

The quantitative determination of the guest content in the CD complexes was reported by Frömming and Szejtli (1994). In this, the complex has to be dissolved in 50 % ethanol, and then diluted with pure ethanol since β-CD is insoluble in pure ethanol. The authors in this work recommended other methods for high stable or poorly water-soluble complexes by dissolving the substance in 0.5 - 1 ml DMSO and then diluted with 50 % ethanol. However, the determined amount of guest in these methods does not give information about the un- and complexed part of the guest with β-CD.

An alternative method for determination of the un- and complexed amount of drug was reported by Waleczek *et al.* (2002) and Kaiser (2003), this method is based on the fact that the β-CD and its complexes are insoluble but the free drug is soluble in n-hexane, so the uncomplexed part of the guest can be detected by washing the obtained complexes with n-Hexane. The washed complexes were being dissolved in DMSO to determine the complexed amount of drug. In this study, a similar method using different solvents has been employed to detect the un- and complexed ibuprofen content in the obtained ibuprofen/CD binary system.

4.2.1.1 Preparation of the samples

To determine the free drug content in the complex, a sample (10 mg) of each product was shaken with n-hexane (1 ml). The n-hexane supernatant was separated and dried. The residue from the n-hexane layer was dissolved in acetonitrile (10 ml) and analysed by HPLC. The drug content in the complex was determined by dissolving the previously washed complex with a small amount of DMSO (400 µl) and diluted with acetonitrile. After 12 hours, the β-CD had sedimented, and the supernatant was centrifuged (Megafuge 1.0 R, Heraeus, Hanau, Germany) for 15 min at 1300 rpm,

removed, filtered and was analysed by HPLC.

4.2.1.2 HPLC method for determination of ibuprofen content in the complex

The HPLC system consisted of a Shimadzu LC-6A pump (Shimadzu Europe, Duisburg, Germany), an AS-200A injector (Merck/Hitachi, Darmstadt, Germany), a Nucleosil 100-5 C18 125x4 mm column (Macherey-Nagel, Düren, Germany), a Shimadzu SPD-6A UV-detector (Shimadzu Europe, Duisburg, Germany) at 230 nm, and a Shimadzu C-R6A integrator (Shimadzu Europe, Duisburg, Germany). The mobile phase was 50:50 (v/v) acetonitrile/20 mM K_2HPO_4 pH 2.5 at a flow rate of 1.5 ml/min. The injection volume was adjusted to 20 µl.

The inclusion yield (% wt.) of the ibuprofen/β-CD binary system prepared with various methods was evaluated according to the following equation:

$$Percentage\ of\ included\ ibuprofen = [\frac{complexed\ amount\ of\ ibuprofen}{free\ amount + complexed\ amount}]*100$$

Eq.4.1

4.2.1.3 Calibration and validation of the quantitative determination of ibuprofen by HPLC

For the calibration curve, an amount of 6.66 mg ibuprofen was accurately weighed and dissolved in 50 ml of acetonitrile (stock solution [13.32 mg/100 ml]). Six additional calibration levels (3.33; 2.664; 1.998; 1.332; 0.666 and 0.333 mg/100 ml) were prepared by dilution of the stock solution with acetonitrile. Each dilution was analysed at least six times by HPLC.

The validation of ibuprofen content has been carried out according to ICH guidelines of analytical procedures (ICH Q2(R1), 2005). In this, the linearity of calibration curve was analysed using a linear regression model and correlation coefficients. The calibration curves, residuals and standardized residuals were inspected to assess linearity according to Gottwald (2000). The limits of detection (LOD) and limits of quantification (LOQ) were determined from serial dilution and

were calculated according to the following equations:

$$LOD = \frac{3.3\,\sigma}{S} \qquad \text{Eq.4.2}$$

$$LOQ = \frac{10\,\sigma}{S} \qquad \text{Eq.4.3}$$

σ: The standard deviation of the responses

S: The slope of the calibration curve

The system precision and the recovery was determined by analysing four different concentrations (0.5, 1.0, 2 and 3 mg/100 ml) of ibuprofen. Each determination was repeated at least six times, mean value; standard division and relative standard division were calculated.

4.2.2 Determination of ibuprofen content using UV spectrometer

The determination of the total amount of ibuprofen in the ternary system (ibuprofen, β-CD and PVP), β-CD-granules and the dissolved amount of the drug in all dissolution measurements were performed using UV photometry at wavelengths of 264 or 221 nm (Figure 4.6).

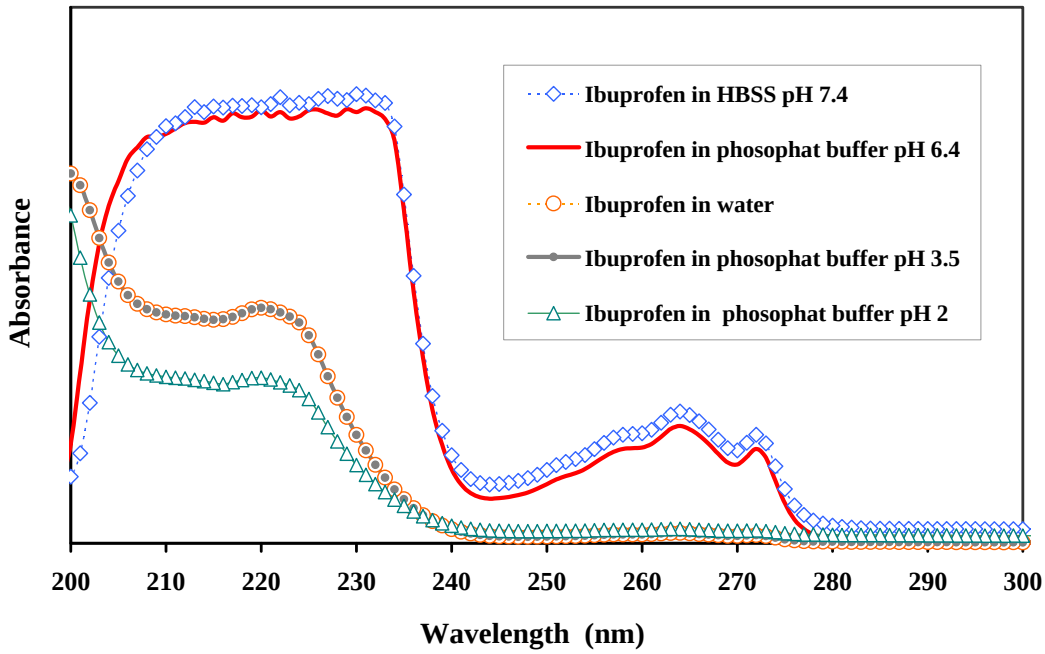


Figure 4.6 UV spectrum of ibuprofen in different pH values.

4.2.2.1 Preparation of hanks balanced salt solution

In this work hanks balanced salt solution (HBSS) has been used as medium for all dissolution experiments, determination of ibuprofen content in the ternary system and β-CD-granules. HBSS (Table 4.1) was supplemented with glucose (1 g/l), buffered with 5.07 g/l HEPES (pH 7.4) or 2.13 g/l MES (pH 6.0) and adjusted to the desired pH using sodium hydroxide (0.1 N or 1 N).

Table 4.1 Composition of hanks balanced salt solution (HBBS).

Name	g/l
$CaCl_2$	0.14
KCl	0.40
KH_2PO_4	0.06
$MgCl_2 \times 6\ H_2O$	0.10
$MgSO_4 \times 7\ H_2O$	0.10
NaCl	8.00
$Na_2HPO_4 \times 2\ H_2O$	0.06

4.2.2.2 Sample preparation of β-CD ternary system and β-CD granules

The drug content loaded in the ternary system and β-CD-granules was determined by UV measurement. Sample amount of 100 mg were dispersed in 10 ml HBSS buffer pH 7.4 and placed in an ultrasonic bath for one hour, the resulted suspension was filtered through a 45 μm, cellulose nitrate membrane filter (Starourious, Göttingen, Germany) and measured by a UV-VIS spectrophotometer (550 S, Perkin Elmer) at 264 nm.

4.2.2.3 Calibration and quantitative determination of ibuprofen by UV spectrometry

For the calibration of ibuprofen at pH 5, six solutions of ibuprofen in HBSS within a range of (0.5240 - 1.8 mg/100 ml) were made by dilution stock from solution. Each dilution was analysed at least six times by UV-VIS spectrophotometer (550 S, Perkin Elmer) at 221 nm. For the calibration of ibuprofen at pH 6, eight solutions of ibuprofen in HBSS within a range of (0.50 - 4.01 mg/100 ml) were made by dilution from stock solution. Each dilution was analysed at least six times by UV-VIS spectrophotometer (550 S, Perkin Elmer) at 264 nm. For the calibration of ibuprofen at pH 7.4, nine solutions of ibuprofen in HBSS within a range of (0.727 - 36.36 mg/100 ml) were made by dilution from stock solution. Each dilution was analysed at least six times by UV-VIS spectrophotometer (550 S, Perkin Elmer) at 264 nm.

4.3 Solid state characterisation

4.3.1 Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy in ATR mode (Attenuated Total Reflection) was performed using a Spectrum One FTIR-spectrometer (Perkin-Elmer Co, USA). A small amount of the powder was placed on top of the crystal and pressed down to achieve good contact between the crystal and the sample. Each spectrum was collected in the range of 650 - 4000 cm^{-1} and a scan speed of 0.5 cm/s. The baseline was corrected for all spectra using the Perkin-Elmer Spectrum software (Perkin-Elmer Co, USA). The FTIR measurements were conducted at the Pharmazeutische Chemie, Eberhard Karls Universität Tübingen.

4.3.2 Thermal behaviour

Differential scanning calorimetry (DSC) measurements were performed using a Mettler DSC system (TA 8000, DSC 820, Mettler Toledo, Giessen Germany). The samples were placed in perforated 40 µl aluminium standard pans and crimped with punched lids. An empty aluminium sample pan was used as a reference. The DSC methods used for different materials are listed in the table 4.2.

Table 4.2 DSC method.

Calibration	Tau-Lag (Gallium, Indium, Tin)
	Temperatures (Gallium 29.8 °C, Indium 156.6 °C, Tin 231.9 °C, Zink 419.6 °C) Heat of fusion (Indium ΔHf = 28.45 J/g)
Flushing gas	Nitrogen gas (10 ml/min)
Cooling	Nitrogen liquid
Temperature range	25 - 200 °C for ibuprofen and their products

Heating rate	10 °C/min for determination of the melting point and 5 °C/min for quantitative determination of the drug loaded in crystalline form
Sample weight	2 - 10 mg per run

The determination of the crystallinity from the melting peak data using DSC measurement was performed according to Mura *et al.* (2003) and Salonen *et al.* (2005). The crystalline drug content was expressed as percent of drug mass fraction in the starting sample; this fraction was estimated by the ratio between the heat of fusion of ibuprofen detected in the amount of drug-loaded sample and that of the amount of pure drug, according to the following equation:

$$Crystalline\ drug\ content = \frac{\Delta Hf_S / m_S}{\Delta Hf_{ib} / m_{ib}} \times 100^{*} \qquad \text{Eq. 4.4}$$

ΔHf_s: Heat of fusion of crystalline ibuprofen in drug-loaded sample (J)

M_s: Sample weight of drug-loaded sample (g)

ΔHf_{ib}: Heat of fusion of pure ibuprofen (J)

m_{ib}: Sample weight of pure ibuprofen (g)

* The heat of fusion (ΔHf) was determined by integrating the melting peak of ibuprofen using STAR^e software, version 8.10 (Mettler Toledo, Giessen, Germany).

4.3.3 X-ray diffraction

Powder X-ray diffraction (PXRD) patterns were carried out using a Guinier step scan diffractometer (G600, Huber Diffraktionstechnik, Rimsting, Germany) with monochromatic CuKα_1 radiation (λ = 1.54056 Å). The voltage and current were at 40 kV and 30 mA. The diffraction patterns were recorded in the rang of $5° \leq 2\theta \leq 60°$,

with a step size of 0.025°, and a 5 sec time per step. The XRD measurements were conducted at the Instiut für Kristallographie, Universität Karlsruhe.

4.3.4 Scanning electron microscopy

The surface morphology of the raw material and the obtained products were examined by the use of a scanning electron microscope (SEM) (DSM 940 A, Carl Zeiss, Oberkochen, Germany). The pictures were taken with a Contax M 167 Mt camera (Yashica-Kyocera, Japan) and digitized using Orion system (Orion 5, E.L.T. sprl). Each sample was fixed on an aluminium pin using double adhesive tape (Tempfix) and then coated with gold, by employing a Sputter Coater (E 5100, Bio-Rad, München, Germany). The samples were sputtered four times for 60 sec and exposed to 20 mA current and 2.1 kV acceleration voltages at a vacuum of 0.02 - 0.03 mbar. The micrographs were taken at 5 kV and 10 kV and at a magnification between 5000 and 20000.

4.3.5 Surface area measurement

The specific surface area was determined using nitrogen gas adsorption at a temperature of -196 °C based on the Brunauer, Emmett and Teller (BET) method according to the Ph. Eur. 5.8. A quantity of test granules, providing a surface area of at least 1 m^2, was accurately weighed and analysed using a SA 3100 Beckman Coulter system (Beckman Coulter, Krefeld, Germany), the outgas temperature was 30 °C and the outgas time 360 min. The surface area was calculated using a SA-VIEW™ software, version 2.12 (Beckman Coulter, Krefeld, Germany). The measurements were repeated three times with different sample quantities.

4.3.6 Determination of the water content

The water content in the selected fraction of β-CD-granules was determined by infrared drying method. A sample (1 g) of granules was placed on an IR-balance (Mettler P160 and Mettler LP12, Mettler Toledo, Giessen, Germany) and dried to a constant weight. The water content % wt. was calculated from the mass loss.

4.3.7 Friability

The friability was measured according to Gainotti *et al*. (2004) using an Erweka friability tester (type PTF1, Pharmatest Apparatebau, Heusenstamm, Germany). 100 g of the granules was tested for 4 min at 25 rpm. Loose dust particles were removed from the granules with air pressure before and after the test. The granules friability was calculated from the loss of mass and expressed as the percentage of the initial mass.

4.4 In vitro drug release

The dissolution experiments were carried out with a Stricker (1969) flow-through cell dissolution apparatus (Sartorius, Göttingen, Germany) (Figure 4.7).

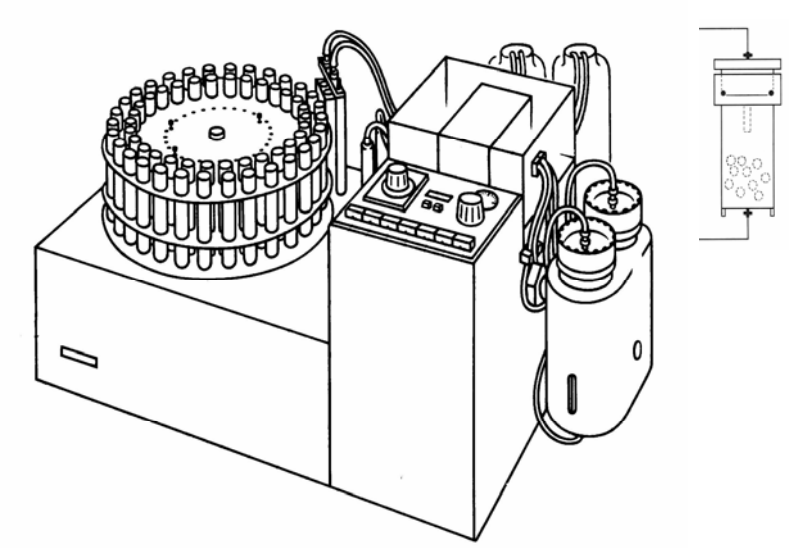


Figure 4.7 Stricker dissolution apparatus.

A quantity sample of ibuprofen or equivalent amount of their products containing the same content of ibuprofen (Table 4.3 and Table 4.4) was added to the dissolution vessel containing 100 ml HBSS buffer and rotating at 1.2 rpm and temperature of 37 °C. Samples of 4 ml were taken from the dissolution vessel and replaced with an equal volume of the dissolution fluid. The withdrawn samples were filtered through a membrane filter (45 µm pore size; Cellulose Nitrate, Sartorius, Göttingen, Germany). The filtrates were assayed spectrophotometrically (UV-VIS spectrophotometer 550 S, Perkin Elmer, Überlingen, Germany). The dissolution coefficient (K_w) was calculated according to the Weibull equation (Heinrich *et al.*, 1986) in order to describe the kinetic parameter of the curve.

$$K_W = \frac{1}{t_{63.2\%}} \qquad \text{Eq.4.5}$$

$t_{63.2\%}$: Time needed to resale 63.2 % of the drug

This equation was used earlier by Loth and Hemgesberg (1986) or Charoenchaitrakool *et al*. (2000 and 2002) for the characterisation of ibuprofen dissolution kinetic. The specific parameters of the dissolution test are given in table 4.3 and table 4.4.

Table 4.3 Dissolution parameters of ibuprofen and their binary systems with β-CD.

Sample weight	3 mg of ibuprofen or equivalent amount[+] of their n-Hexane washed binary systems.
Dissolution fluid	HBSS buffer pH 5
Withdrawn samples	8 samples were taken from the dissolution vessel at 0, 5, 10, 15, 30, 45, 60 and 75 min
Assaying method	UV photometry at 221 nm

Table 4.4 Dissolution parameters of ibuprofen, their ternary systems and ibuprofen-loaded granules.

Sample weight	25 mg of ibuprofen or equivalent amount[+] of their ternary systems and ibuprofen-loaded granules
Dissolution fluid	HBSS buffer pH 6
Withdrawn samples	12 samples were taken from the dissolution vessel at 0, 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min
Assaying method	UV photometry at 264 nm

[+] Equivalent amounts of products contain adequate amount of drug was estimated according to the drug content measurements.

4.5 Error propagations and statistic analysis

The statistical method for determining a value with its uncertainty is to repeat the measurement several times thus allowing an average, the average deviation, the standard deviation (SD) or the confidence interval (CI) to be calculated by the following equations:

$$\bar{\chi} = \frac{\chi_1 + \chi_2 + \ldots + \chi_n}{n}$$

n: Number of measurement x E.q.4.6

$$SD = \sqrt{\frac{d_1 + d_2 + \ldots + d_n}{n}}$$

d_1: $(x_1 - \bar{x})$, $d_2 = (x_2 - \bar{x})$, … E.q.4.7

$$CI = \bar{x} \pm Z \frac{SD}{\sqrt{n}}$$

Z: Value determined by the probability E.q.4.8

The statistical evaluation of data was analysed by Student's T-test and multiple comparisons by one-way analysis of variance (ANOVA) followed by Tukey posterior test for multiple comparisons using GraphPad Prism software, version 4.0 for Windows (California, USA, http:// www.graphpad.com). A value of $p<0.05$ was considered significant.

CHAPTER 5

RESULTS

5.1 Analytical methods results

5.1.1 HPLC analytic of ibuprofen in their binary systems with β-CD

The calibration curve of ibuprofen provided a linear relationship between the peak area (Y) and the concentrations of ibuprofen injected (X) in mg/100 ml with a regression equation of Y = 164800 X – 4808 (r^2 = 0.9944) in the concentration range of 0.033 to 3.33 mg/100 ml (Figure 5.1).

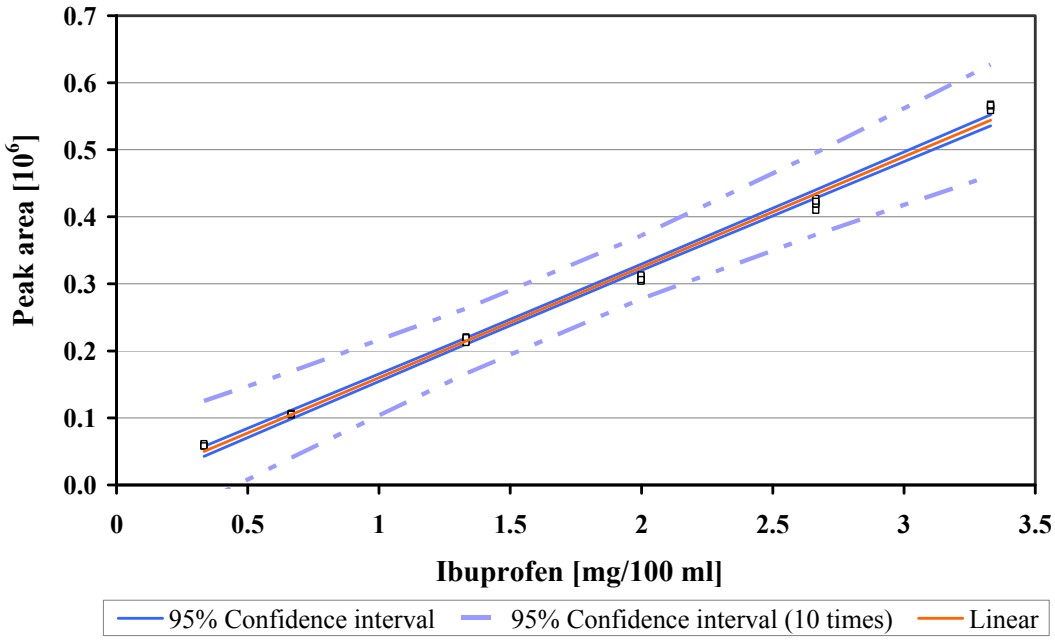


Figure 5.1 HPLC calibration curve of ibuprofen.

Validity of the analytical method can be verified by establishing several analytical and statistical parameters (Table 5.1). The linearity of the detector response for ibuprofen was confirmed between 0.333 - 3.33 mg/100 ml. In addition, the

calibration curve (Figure 5.1) was inspected to assess linearity. The calibration curve was analysed using a linear regression model and correlation coefficients (Table 5.1).

The calculated limits of detection (LOD) and limits of quantification (LOQ) according to the Eq.4.2 and Eq.4.3, respectively, were found to be 0.27 and 0.82.

The system precision in the range of 0.5 - 3.0 mg/100 ml was 1.19 % and the recovery of ibuprofen was found to be 98.69 %. Raw data of the validation are given in table 9.1.

Table 5.1 HPLC calibration data of ibuprofen.

Parameter	Calibration of ibuprofen
Concentration range [mg/100 ml]	0.333 - 3.33
Number of concentration	6
Number of calibration samples	36
Univariant	34
Correlation coefficient	0.9944
Calibration equation	Y = 164800 X – 4808
LOD [mg/100 ml]	0.27
LOQ [mg/100 ml]	0.82
System precision	1.19 % (n = 4)
Recovery	98.69 %

5.1.2 UV analytic of ibuprofen

The calibration curve of ibuprofen in HBSS buffer pH 5 provided a linear relationship between the peak area (Y) and the concentrations of ibuprofen measured (X) in mg/100 ml with a regression equation of Y = 0.4131 X – 0.0112 ($r^2 = 0.9991$) in the concentration range of 0.525 to 1.837 mg/100 ml (Figure 5.2). The calibration data are given in table 5.2.

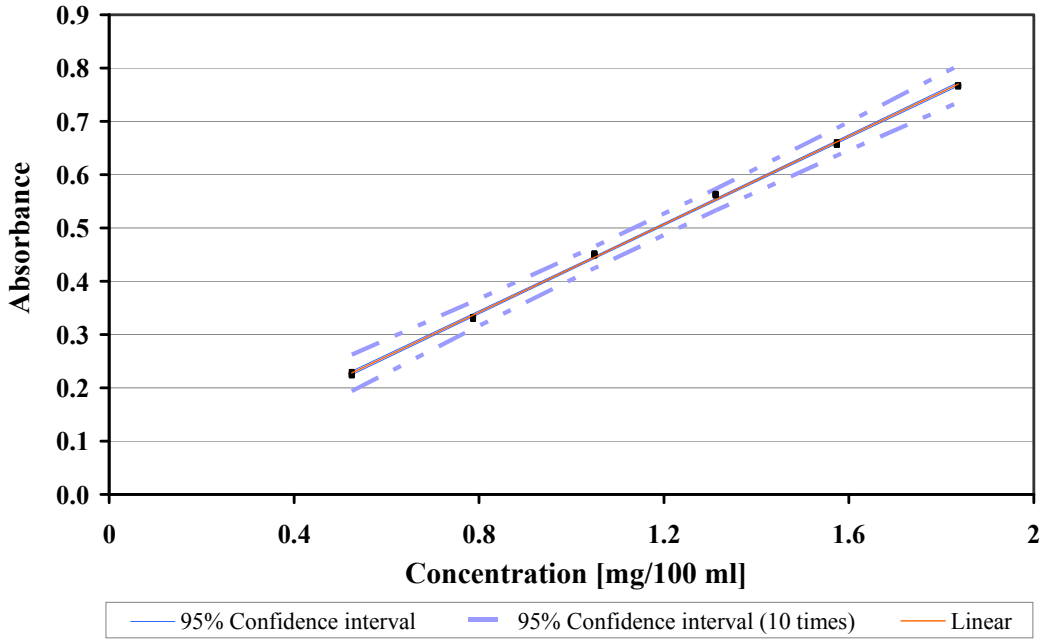


Figure 5.2 UV calibration of ibuprofen in HBSS buffer at pH 5.

The calibration curve of ibuprofen in HBSS buffer pH 6 provided a linear relationship between the peak area (Y) and the concentrations of ibuprofen measured (X) in mg/100ml with a regression equation of Y = 0.0182 X – 0.0039 ($r^2 = 0.9999$) in the concentration range of 5.00 to 40.01 mg/100 ml (Figure 5.3). The calibration data are given in table 5.2.

The calibration curve of ibuprofen in HBSS buffer pH 7.4 provided a linear relationship between the peak area (Y) and the concentrations of ibuprofen measured (X) in mg/100 ml with a regression equation of Y = 0.0185 X – 0.0073 ($r^2 = 0.9997$) in the concentration range of 7.27 to 36.36 mg/100 ml (Figure 5.4). The calibration data are given in table 5.2.

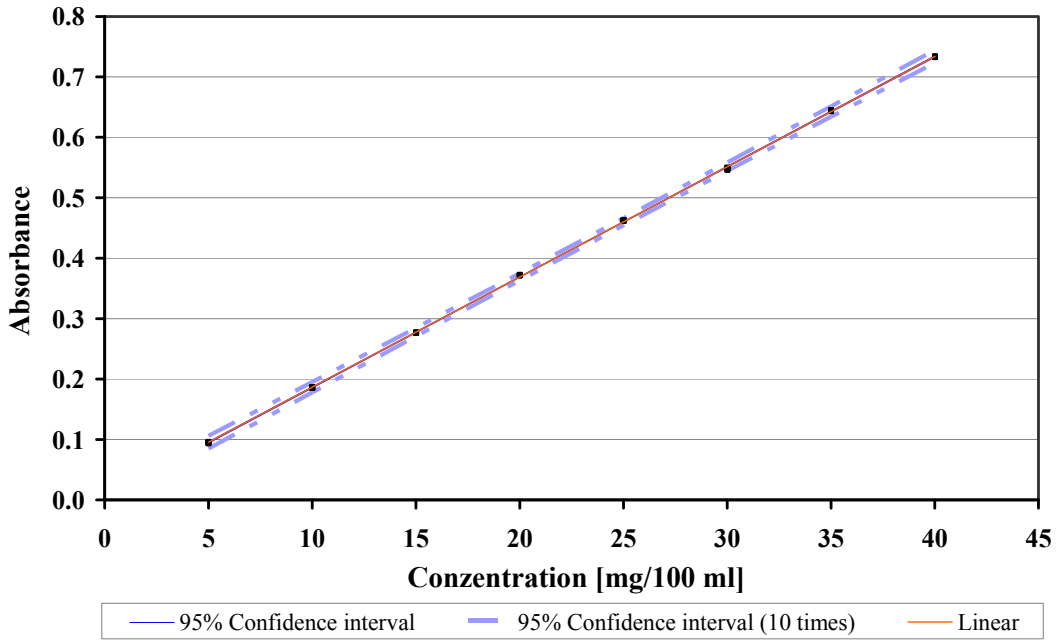


Figure 5.3 UV calibration of ibuprofen HBSS buffer at pH 6.

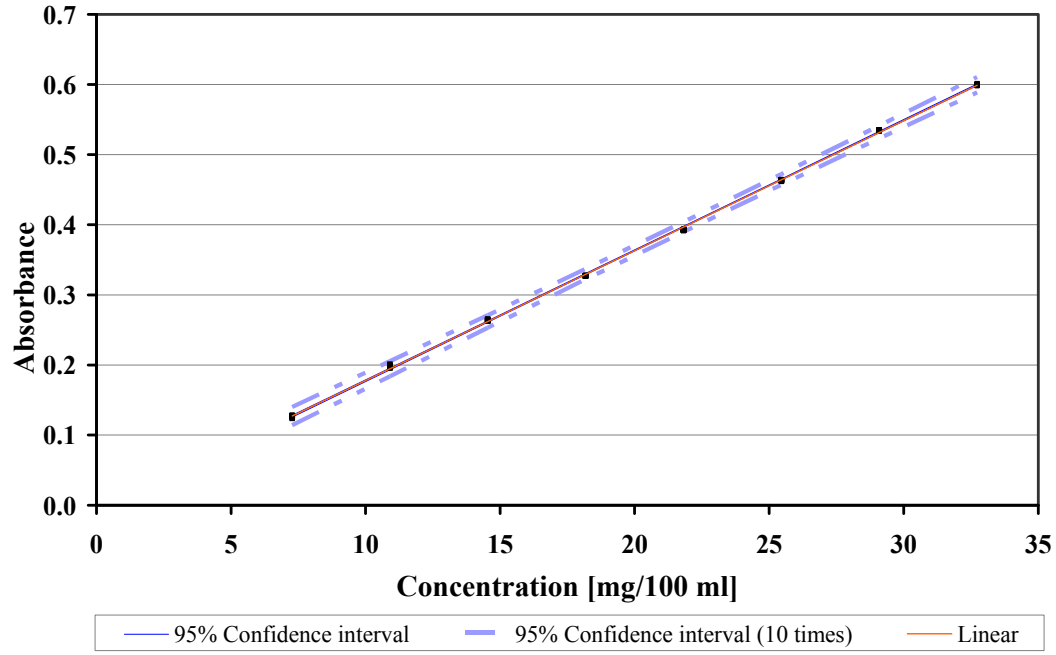


Figure 5.4 UV calibration of ibuprofen in HBSS buffer at pH 7.4.

Table 5.2 Calibration data of ibuprofen in HBSS buffer pH 5, 6 and 7.4 determined by UV-VIS spectrometry.

Parameter	Calibration of ibuprofen		
HBSS buffer	pH 5	pH 6	pH 7.4
Conc. range [mg/100 ml]	0.525 - 1.837	5.00 - 40.01	7.27 - 36.36
Number of conc.	6	8	9
Number of calibration samples	36	48	54
Univariant	34	46	52
Calibration equation	Y = 0.4131 X – 0.0112	Y = 0.0182 X – 0.0039	Y = 0.0185 X – 0.0073
Correlation	0.9991	0.9999	0.9997
Wavelength (nm)	221	264	264

5.2 *Preparation of ibuprofen/β-CD complex using CPD process*

5.2.1 Phase behaviour study

The phase behaviour of β-CD was investigated as described in chapter 4 (4.1.2) and published previously by Türk *et al*. (2007). β-CD exhibits stability in $scCO_2$ under the experimental parameters. Thereby, no melting or solvation of β-CD in $scCO_2$ was visually observed at temperature of 40 °C and pressure up to 30 MPa for 24 h.

For ibuprofen, the melting point decreases with increasing pressure from 75.45 °C at 0.1 MPa to 47.15 °C at 15.1 MPa, whereas in the pressure range between 15 and 28 MPa, the melting temperature is nearly constant.

In parallel to the SLG behaviour of CO_2/ibuprofen, the solubility of ibuprofen in $scCO_2$ was studied, since the solubility data are essential parameters for an accurate experimental design in the CPD process. The solubility of ibuprofen was investigated at 40 °C and a pressure range of 20.5 - 25.5 MPa (Figure 5.5). Our measured solubility data, however, supplement solubility data measured in a range of 9.5 - 22 MPa and reported previously by Charoenchaitrakool *et al*. (2000). The measured solubility data agree with those calculated by Türk *et al.* (2007) using the Peng-Robinson equation (Peng and Robinson, 1979) of state and Van der Waal's mixing and combination rules (PR–EoS). As shown in figure 5.5, the solubility of ibuprofen in $scCO_2$ increases by increase in the pressure. When the pressure increases, carbon dioxide density increases and the intermolecular mean distance of carbon dioxide molecules decreases; therefore, the specific interaction between the solute and solvent molecules increases (Gurdial and Foster, 1991).

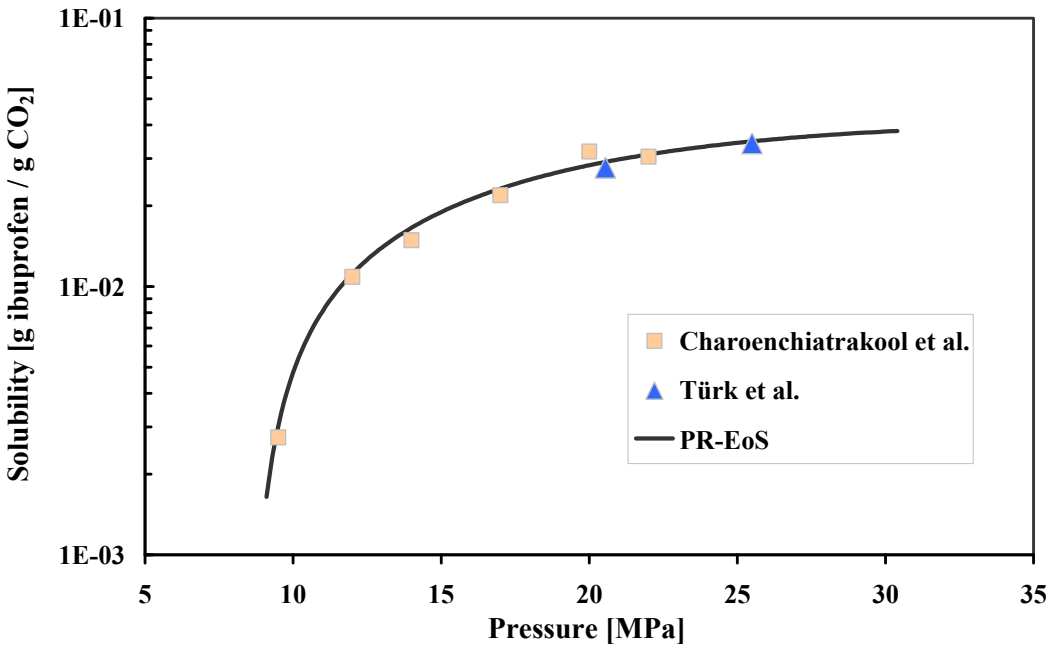


Figure 5.5 Solubility of ibuprofen in $scCO_2$ at 40 °C measured by Charoenchiatrakool et al. (2000), and measured / calculated solubility from PR-EoS equation by Türk, M.

5.2.2 Optimisation of CPD experimental parameters for preparation of ibuprofen/β-CD complex

5.2.2.1 Influence of the CPD experimental parameters on the ibuprofen/β-CD complex formation

The optimisation process of the CPD experimental parameters for preparing ibuprofen/β-CD complex was investigated in the CPD I apparatus (Figure 4.2 and Figure 4.3) using the method reported in chapter 4 (4.1.1). This process was conducted at the Institut für Technische Thermodynamik und Kältetechnik, Universität Karlsruhe (TH) and some data of this optimisation process published previously by Löwenberg (2003).

The experiments were carried out by using constant amounts of ibuprofen (6.5 g) and β-CD (8.5 g) and at 39.5 °C and a pressure up to 24 MPa (24, 25.26 and 27.4 MPa) to avoid melting point depression of ibuprofen when contacted with $scCO_2$. The time of exposure was varied at each pressure level in a range between 6 and 114 h. The

experiments were evaluated by HPLC determination of the un- and complexed ibuprofen fraction in the obtained materials and by calculating the inclusion yield using different experimental conditions (Table 5.3).

Table 5.3 Influence of the CPD experimental parameters (pressure and exposure time) on the total ibuprofen content and the inclusion yields in the ibuprofen/β-CD complex.

Exp.	Pressure [MPa]	Exposure time [h]	Total ibuprofen [% wt.]	Complexed ibuprofen [% wt.]	Inclusion yield [% wt.]
P	24.6	12	0.57	0.51	88.44
O		24	0.51	0.22	42.42
T		48	0.56	0.21	36.70
S		48	0.84	0.04	47.22
B		114	0.72	0.48	66.92
R	25.26	6	2.84	2.47	87.04
V1		13.5	1.56	1.37	87.83
Q		48	0.66	0.30	46.25
Z		114	3.88	2.52	65.01
V2	27.8	13.5	0.62	0.20	32.96
V4		13.5	1.29	0.80	62.12
A		48	3.09	1.59	51.59
AA		48	1.72	0.08	43.92

The effect of the pressure on the complex formation was investigated over a range between 24.7 and 27.8 MPa. The treatment time and the temperatures were kept at 48 h and 39.5 °C, respectively.

As illustrated in figure 5.6, an increase in the pressure resulted in a higher total ibuprofen content, whereas the inclusion yield increased in the rage of 24.7 - 25.28 MPa and was found to be similar by increasing the pressure form 25.28 MPa to 27.8 MPa. In other words, by increasing the pressure up to 27.8 MPa, increase the drug transfer but did not seem to affect on the inclusion yield.

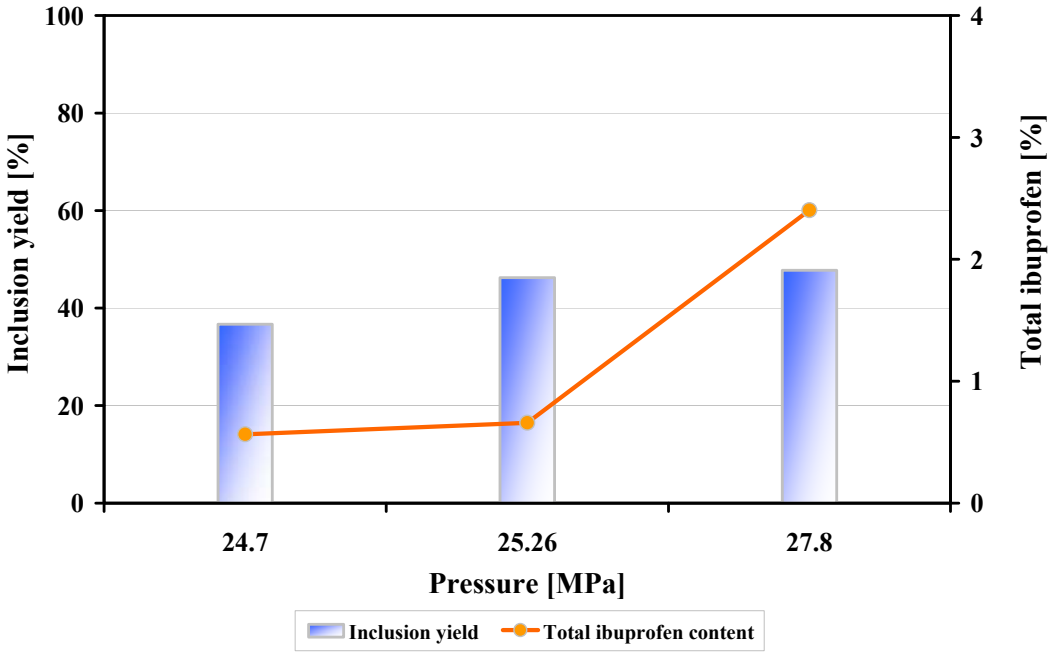


Figure 5.6 Effect of pressure on the inclusion yield and the total ibuprofen content loaded in β-CD at temperature of 39.5 °C and time of exposure of 48 h.

The effect of the contact time on the total ibuprofen content and the inclusion yield was investigated by varying the contact time from 6 to 114 h at three pressure levels (Table 5.3).

As shown in figure 5.7, the total loaded amount of ibuprofen at constant temperature (39.5 °C) and pressure of 24.6 MPa is almost independent on the loading time ≤ 1 % wt. The optimum of the inclusion yield at this pressure level was at a contact time of 12 h.

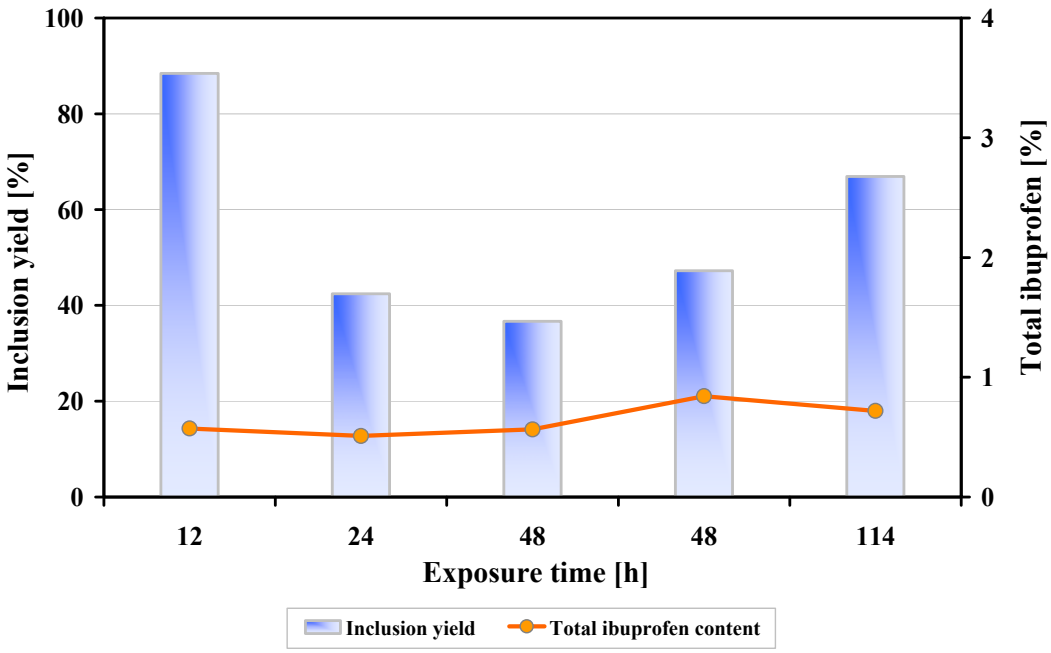


Figure 5.7 Influence of the exposure time on the total ibuprofen content and the inclusion yield at pressure 24.6 MPa and temperature 39.5 °C.

At a higher pressure of 25.26 MPa the total ibuprofen loaded decreases by increasing the contact time from 6 to 48 h, but this amount increased drastically at an exposure time of 114 h (Figure 5.8). The inclusion yield optimum was seen at a contact time of 6 and 13.5 h.

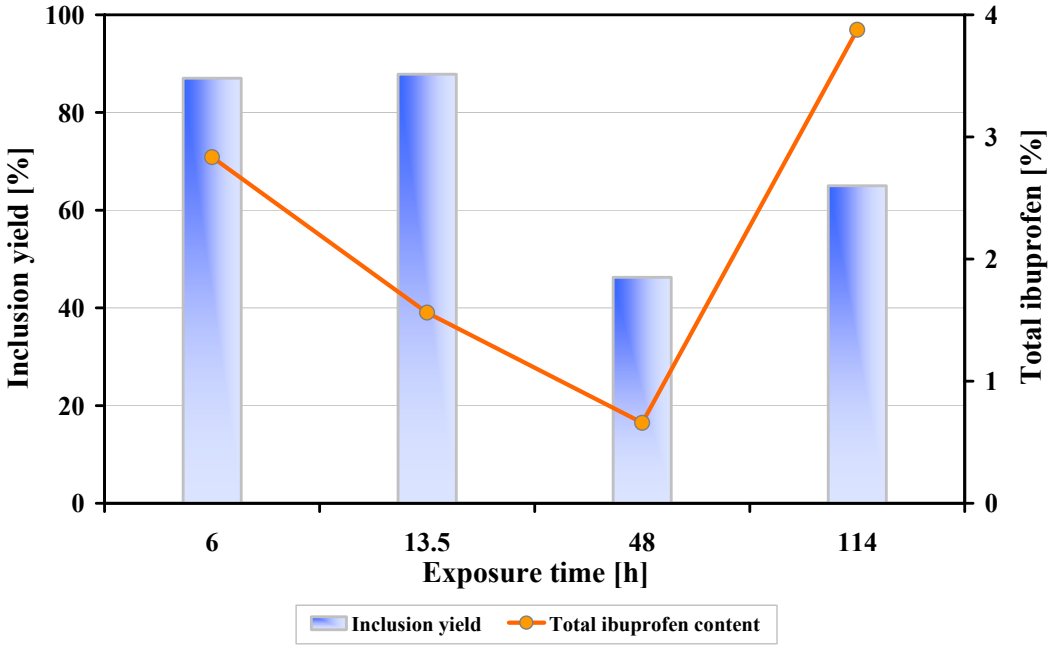


Figure 5.8 Influence of the exposure time on the total ibuprofen content and the inclusion yield at pressure 25.26 MPa and temperature 39.5 °C.

By increasing the pressure to 27.8 MPa, the total amount of ibuprofen and the inclusion yield decreased compared to the experiments performed at 25.26 MPa (Figure 5.9). Moreover, experiments performed at the same conditions, show quite variable results with regard to the total ibuprofen amount and inclusion yield.

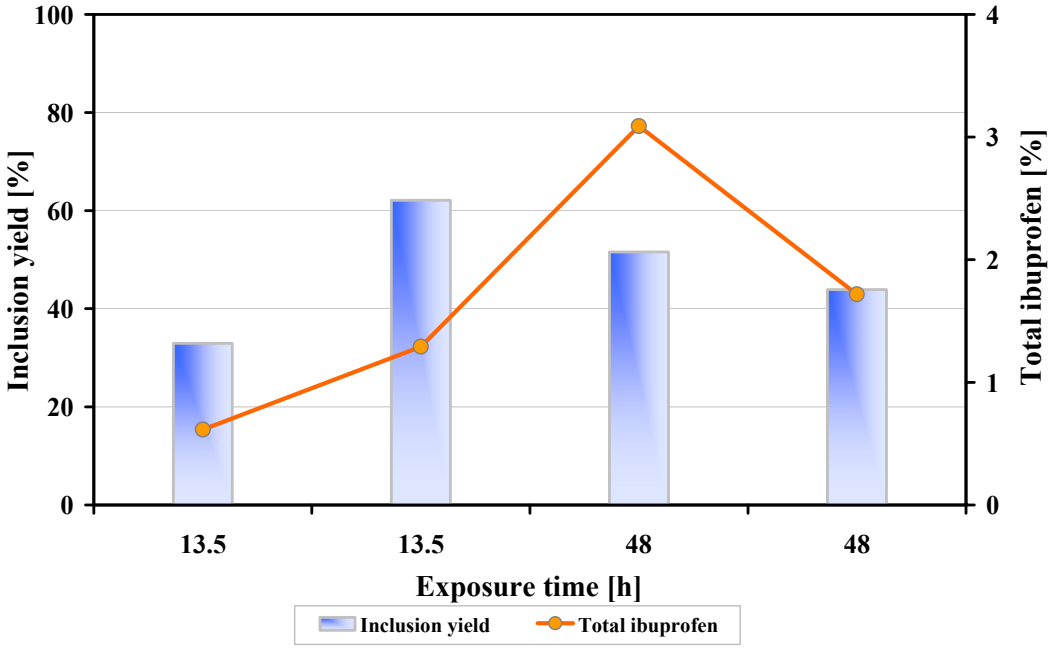


Figure 5.9 Influence of the exposure time on the total ibuprofen content and the inclusion yield at pressure 27.8 MPa and temperature 39.5 °C.

The preliminary study shows that the optimum of the CPD experimental conditions were at a temperature of 39.5 C°, a pressure of about 25 MPa and a contact time about 14 h. on the other hand, reproducibility of the experiments in the CPD was not achieved in the CPD apparatus in this stand (Figure 4.2 and Figure 4.3). A modification in the apparatus was required. As a reason for the varying results was the vertical attitude of the drug and the carrier. We observed in some experiments that the process obviously occurred in a liquid CO_2 phase at the bottom of the high pressure cell during the depressurisation step. To overcome this disadvantage, the drug/carrier cartridges were placed in a horizontal attitude in the upper part of the high pressure cell to be sure that the CPD process occurs exclusively in the supercritical CO_2 phase (Figure 4.4 and Figure 4.5).

5.2.2.2 Investigation of reproducibility

After the modifications on the CPD set-up, at first, reproducibility of ibuprofen/β-CD complex formation was investigated by performing several experiments using the same defined experimental parameters.

In this, adjusted amounts of ibuprofen (8 g) and β-CD (13 g) were placed in separate cartridges inside the high pressure cell. Then the high pressure cell was immersed in a temperature-controlled water bath (39.5 °C) and pressurized to 24.7 MPa. The contact time was kept at 15.5 h. The repeatability of the process was evaluated by HPLC determination of the un- and complexed ibuprofen fraction and the inclusion yield (Table 5.4 and Figure 5. 10).

Table 5.4 Evaluation of CPD reproducibility for preparing ibuprofen/β-CD.

	Total ibuprofen [% wt.]	Complexed ibuprofen [% wt.]	Inclusion yield [% wt.]
V35	2.73	1.45	52.97
V36	2.99	1.49	50.01
V37	2.91	1.36	46.86
V90	2.50	1.26	50.30
V91	2.62	1.17	44.54
V92	3.06	1.66	54.29
Mean	2.802	1.398	49.83
SD	0.220	0.174	3.7

The results show that the preparation of ibuprofen/β-CD using the CPD method under these experimental conditions is reproducible; since the determined amount of total ibuprofen and the complexed part were repeatable, significant variation was not observed.

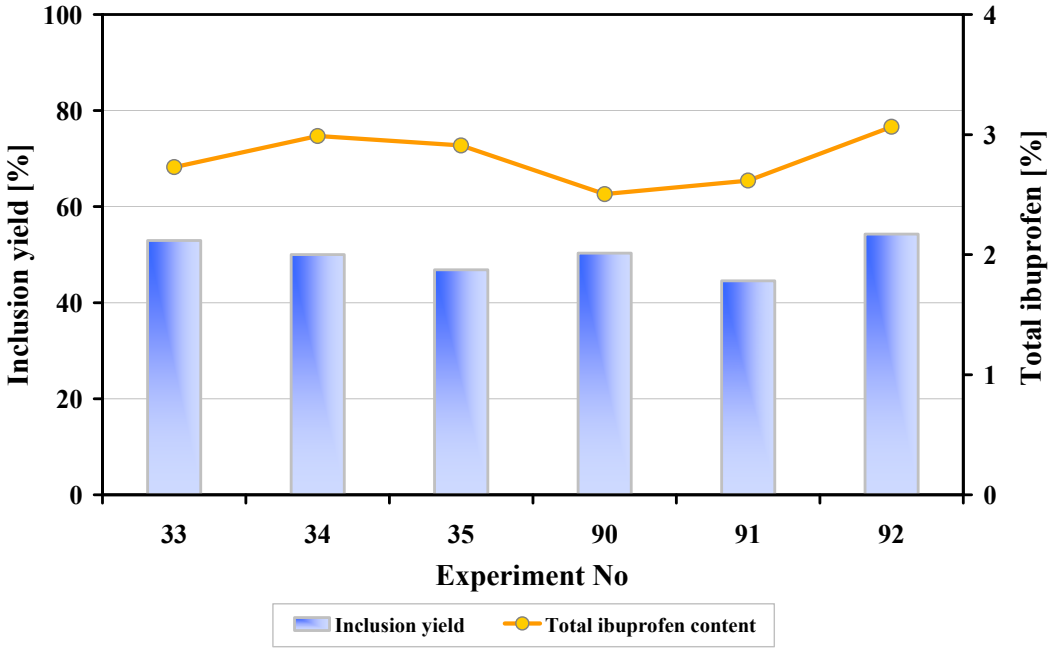


Figure 5.10 CPD reproducibility for preparing ibuprofen/β-CD complex.

5.3 Physicochemical characterisation and comparative evaluation of ibuprofen/β-CD complexes obtained by CPD and other conventional methods

In order to evaluate the effectiveness of the CPD as new developed supercritical carbon dioxide processing for preparation of β-CD-complexes, the physicochemical characteristics of the material produced by the CPD are compared to material obtained by different conventional methods (co-precipitation and freeze-drying). In addition, the dissolution properties of the products have been investigated.

5.3.1 Preparation of ibuprofen/β-CD binary systems

The solid complexes of ibuprofen and β-CD were prepared by different processes by the CPD using $scCO_2$ and by the freeze-drying and the co-precipitation, according to Kurozumi *et al*. (1975) as well known standard methods for comparison. A physical mixture of the drug and β-CD was prepared as a control.

5.3.1.1 CPD process

Ibuprofen/β-CD complexes (n = 6) were obtained by the CPD according to the method and experimental procedures described in section 5.3.2.2.

5.3.1.2 Co-precipitation method

The co-precipitation complexes (n = 3) were prepared with a molar ratio of 1:3 ibuprofen/β-CD. Ibuprofen and β-CD were dissolved/suspended in diethyl ether and water, respectively, and mixed together. The mixture was stirred at room temperature for 24 h. After that, the suspension was kept at 0 °C and finally the microcrystalline precipitate was filtered, washed with a small amount of water and dried at 50 °C.

5.3.1.3 Freeze-drying method

The freeze-dried complex (n = 3) was prepared by dissolving ibuprofen and β-CD (1:3 molar ratio) in 2.34 % aqueous ammonium solution. After 15 min of agitation

at room temperature, the resulting solution was frozen and lyophilised (Lyovac GT 2 freeze-dryer, Finn Aqua Santasalo-Sohlberg Co., Tuusula, Finland) using a 24 h drying program: -30 °C for 8 h; 0 °C for 4 h; 10 °C for 6 h and 20 °C for 6 h.

5.3.1.4 Preparation of the physical mixture

A physical mixture was prepared as a reference by mixing of previously sieved ibuprofen and β-CD with 1:3 molar ratio in a Turbula T2C mixer for 15 min at 42 rpm.

5.3.2 Characterisation of ibuprofen/β-CD binary systems

5.3.2.1 Determination of ibuprofen content

The HPLC determination of the ibuprofen content in the ibuprofen/β-CD binary systems shows the highest inclusion yield in the freeze-dried material, a medium efficiency in the CPD and the co-precipitated, and as expected, almost no effect in the physical mixture. Significant amounts of the loaded drug were incorporated in the CD though a portion of the physical mixture was present in all preparations except the freeze-dried product (Table 5.5).

Table 5.5 Inclusion data of ibuprofen/β-CD systems prepared by the CPD (n=6) and the other methods (n=3).

Product	Total ibuprofen content [% wt. ± SD]	Inclusion yield [% wt. ± SD]
CPD	2.8 ± 0.22	49.83 ± 3.65
Co-precipitation	5.39 ± 0.07	51.91 ± 1.73
Freeze-drying	5.34 ± 0.22	97.01 ± 1.70
Physical mixture	5.37 ± 0.28	3.25 ± 1.89

5.3.2.2 Infrared spectroscopy

The FTIR-ATR spectrum of pure ibuprofen (Figure 5.11, A) shows all the characteristic bands of the drug, including the carbonyl stretching at 1706 cm^{-1}. A shift to higher frequency in the characteristic acid carbonyl stretching in the physical mixture to 1714 cm^{-1} (Figure 5.11, C), the CPD to 1714 cm^{-1} (Figure 5.11, D) and the co-precipitated material to 1731 cm^{-1} (Figure 5.11, E) was seen. In addition the C=O stretching was weak in the CPD (Figure 5.11, D) and was completely absent in the freeze-dried material (Figure 5.11, F), indicating the inclusion of this part of the drug molecule in the β-CD. The disappearance or shift of the carbonyl stretching was reported earlier as evidence for the inclusion of ibuprofen inside the β-CD cavity (Cabral-Marques, 1994; Mura *et al.*, 1998; Bratu *et al*., 2005).

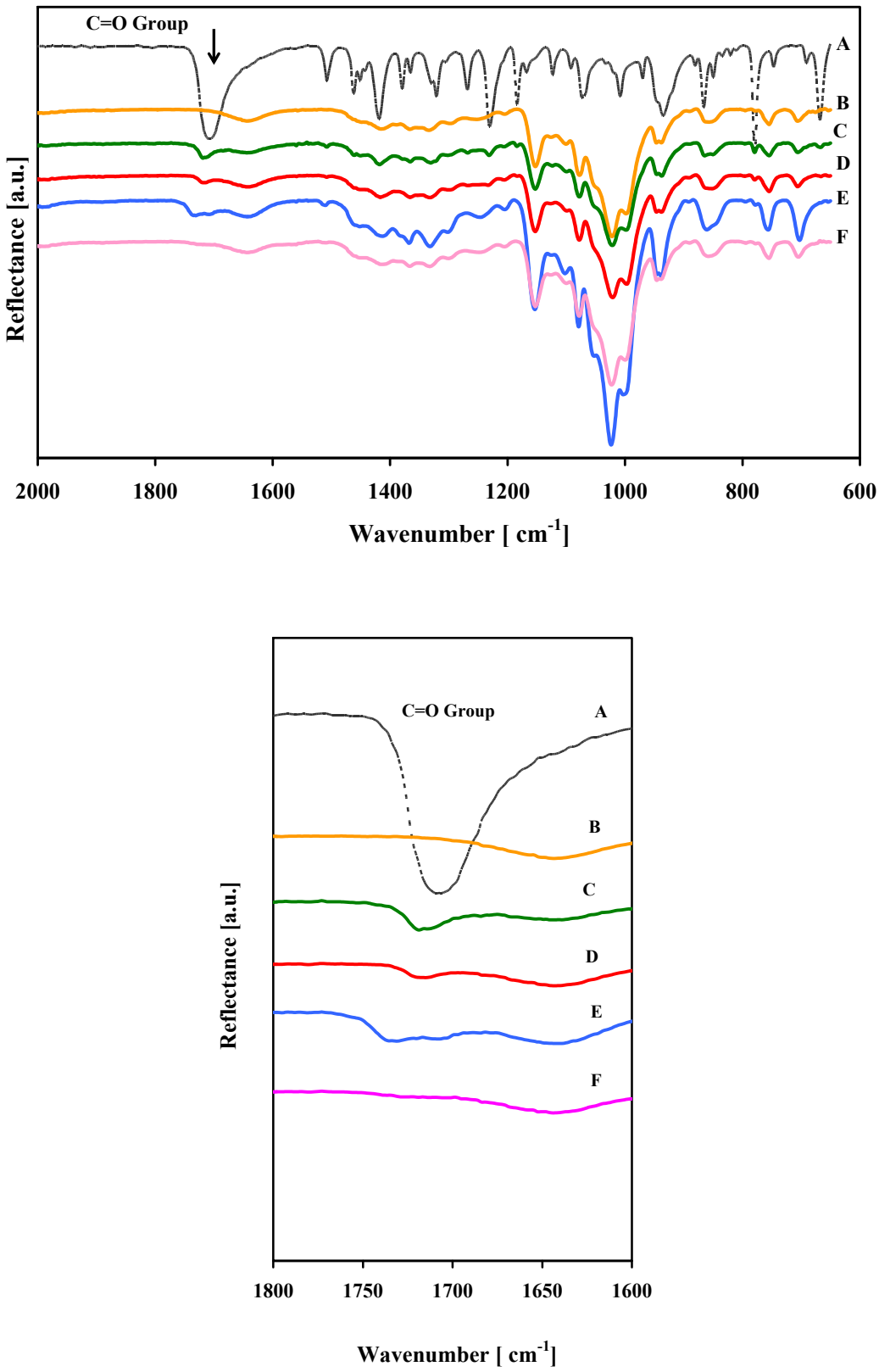


Figure 5.11 FTIR-ATR spectrums of pure ibuprofen (A), β-CD (B), ibuprofen/β-CD physical mixture (C) CPD (D), co-precipitation (E) and freeze-drying (F) products.

5.3.2.3 Thermal behaviour

The thermal behaviour of the ibuprofen/β-CD binary systems was studied with DSC to confirm the formation of a solid complex by the disappearance of the endothermic melting peak of crystalline ibuprofen. Pure ibuprofen exhibits an endothermic melting peak at about 77 °C (Figure 5.12, A). The DSC thermograph of β-CD (Figure 5.12, B) shows a broad endothermic peak around 105 °C, corresponding to the release of water. In the thermal curve of the physical mixture (Figure 5.12, C) the characteristic endothermic melting peak of ibuprofen was observed. The thermograms of the ibuprofen/β-CD complex prepared by the CPD (Figure 5.12, D) and the co-precipitation (Figure 5.12, E) displayed a melting peak at 77 °C, which still reflects the presence of drug crystals not incorporated in the carrier in this preparation. A complete disappearance of the endothermic peak of free ibuprofen was in contrast observed for the complex obtained by the freeze-drying method (Figure 5.12, F).

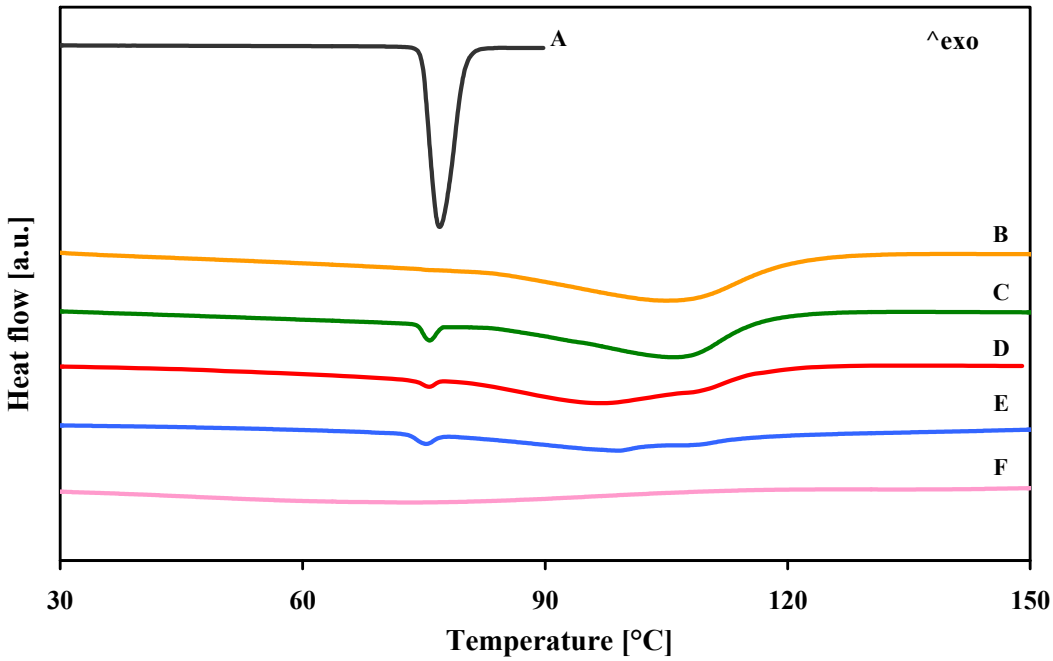


Figure 5.12 DSC thermograms of pure ibuprofen (A), β-CD (B), ibuprofen/β-CD physical mixture (C), CPD (D), co-precipitation (E) and freeze-drying (F) materials.

5.3.2.4 X-ray diffractometry

A change in the crystallinity of the drug (PXRD) indicates complex formation by appearance of a new or at least a deviation from the original pattern (Frömming and Szejtli, 1994; Cabral-Marques, 1994; Mura *et al*., 1998). The PXRD of ibuprofen (Figure 5.13, A) shows the characteristic pattern of the drug. A significant reduction of the X-ray diffraction pattern of the drug was observed in all complex formation methods, especially in the freeze-dried product indicating a complete loss of crystallinity. In addition, new peaks were observed at 6.82°, 7.3° and 7.35° in all diffractograms of CPD (Figure 5.13, D), co-precipitation (Figure 5.13, E) and freeze-dried (Figure 5.13, F) materials, respectively. These peaks may suggest the formation of ibuprofen/β-CD complexes.

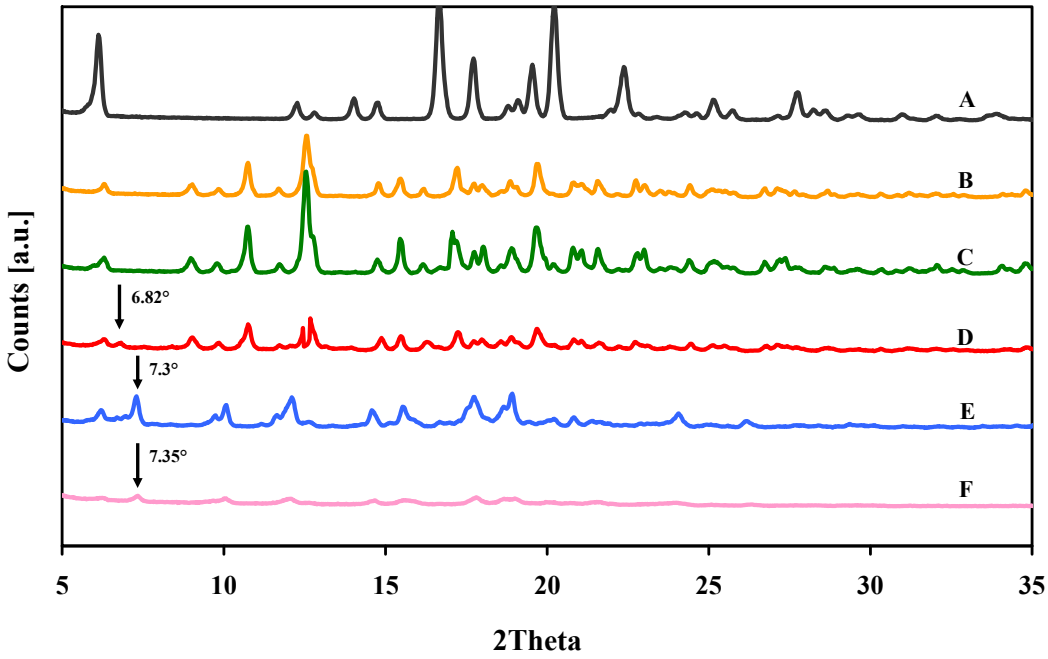


Figure 5.13 PXRD patterns of pure ibuprofen (A), β-CD (B), ibuprofen/β-CD physical mixture (C), CPD (D), co-precipitation (E) and freeze-drying (F) products.

5.3.2.5 Morphological investigation

The morphological changes of obtained materials compared to the pure drug, β-CD and the physical mixture were investigated by scanning electron microscopy

(SEM). Pure ibuprofen (Figure 5.14, A) appeared as needle-shaped crystals with a rough surface and β-CD (Figure 5.14, B) with a parallelogram shape. The ibuprofen/β-CD physical mixture (Figure 5.14, C) shows bulky particles (β-CD) with small needles (ibuprofen) adhered to its surface. In all materials obtained by complex formation methods, a new population of particles with a typical shape occurred, which was not seen in the pure material and the physical mixture. The CPD material (Figure 5.14, D) showed crystals looking different from those of ibuprofen and β-CD and having a smaller crystal size. Agglomerates of multiple crystals appeared in the co-precipitation material (Figure 5.14, E), and no crystals from the pure drug or pure β-CD could de distinguished. The freeze-dried material (Figure 5.14, F) appeared in a typical morphology with a soft and fluffy structure. The occurrence of the new form of crystals in all the produced materials gives evidence for the formation of the solid inclusion complex.

In addition to the SEM study, the different morphological state of ibuprofen/β-CD complex obtained by different methods was depicted using digital pictures (Figure 5.15). In this, the CPD material is shown as free-flowing powder, the co-precipitation, the freeze-dried materials in contrast were in aggregate form.

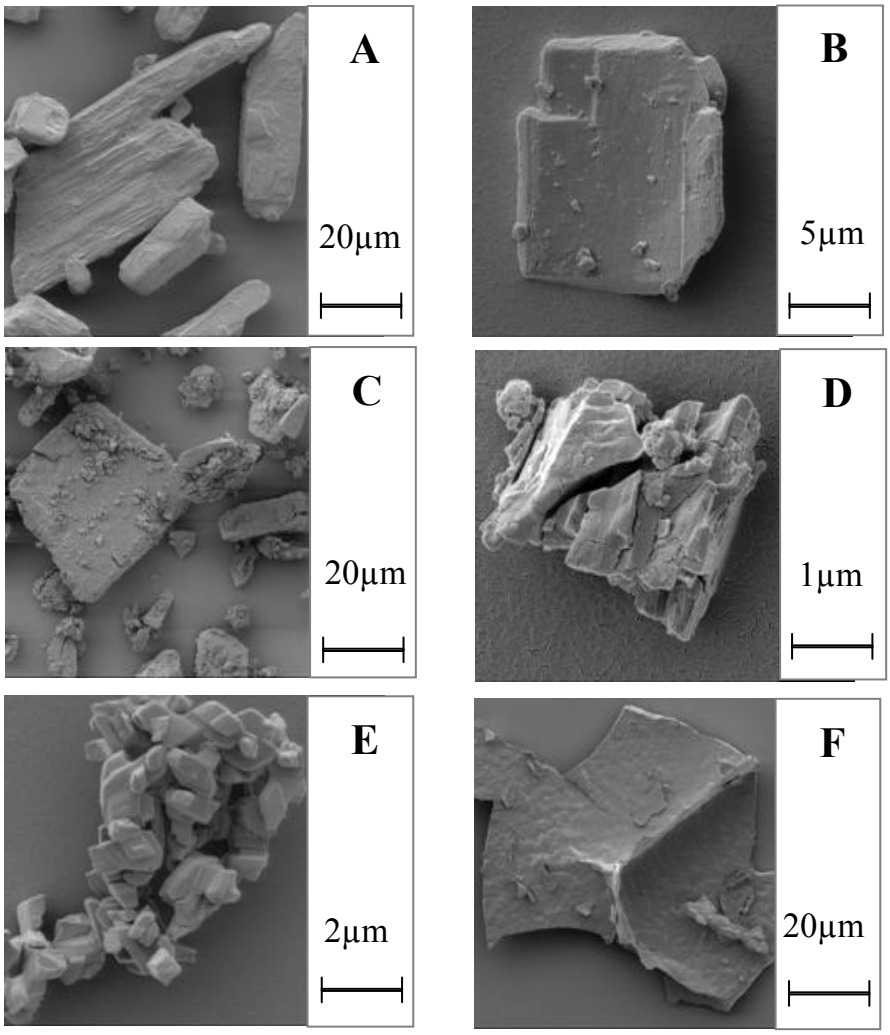


Figure 5.14 SEM micrographs of pure ibuprofen (A), β-CD (B), ibuprofen/β-CD physical mixture (C), CPD (D), co-precipitation (E) and freeze-drying (F) products.

A **B** **C**

Figure 5.15 Pictures of freeze-drying (A), co-precipitation (B) and CPD materials.

5.3.2.6 *Determination of drug release*

In order to evaluate the ibuprofen/β-CD complexes obtained using different techniques in improving the water solubility of ibuprofen, *in vitro* dissolution testing was performed at pH 5, where pure ibuprofen (Figure 5.16, E) shows low dissolution with only about 60 % of drug dissolving after 75 min (Table 5.6).

The presence of β-CD in the physical mixture (Figure 5.16, C) improved the dissolution of ibuprofen significantly; this is likely to be due to the wettability-enhancing effect of β-CD (Govindarajan and Nagrsenker, 2004; Charumanee, 2004) or the complex formation with drug in a solution.

Although the drug dissolution of the co-precipitation material (Figure 5.16, D) after the first 15 min was well expressed and significant, this improvement was rather limited after 75 min because of agglomerates found in this product (Table 5.6). The highest enhancement of the drug dissolution was seen for the CPD product and the freeze-dried materials with about 90 % of ibuprofen released within 75 min (Figure 5.16, B and A) respectively.

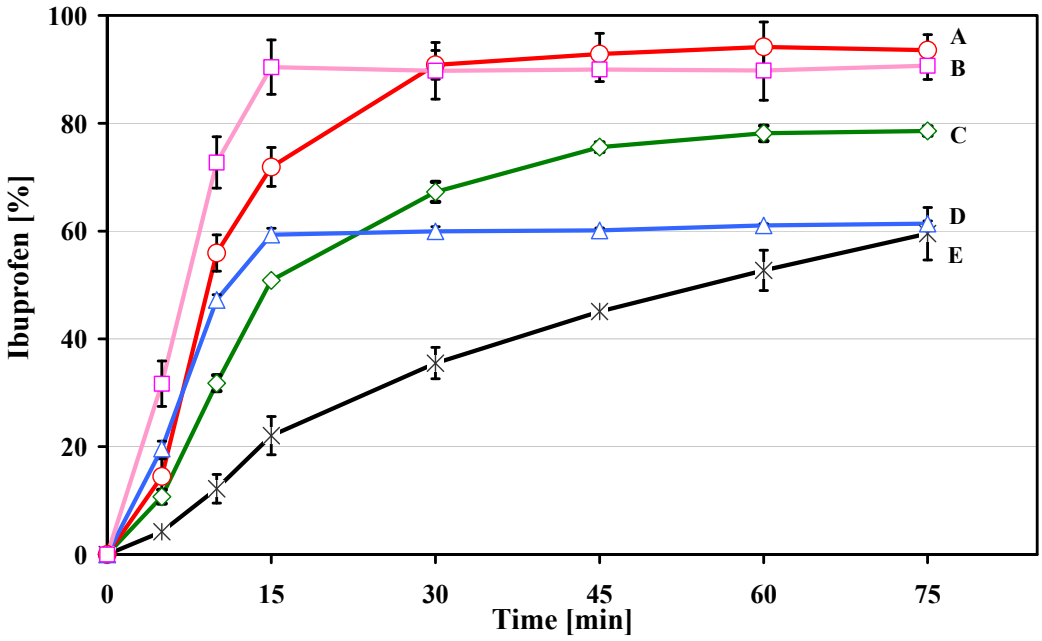


Figure 5.16 Dissolution profiles of ibuprofen (E), ibuprofen/β-CD physical mixture (C), CPD (A), co-precipitation (D) and freeze-drying (B) products in HBSS buffer pH 5 at 37 °C.

The calculated Weibull-coefficient (K_w) confirmed these results. Here, the CPD and the freeze-dried materials show the highest dissolution rate compared to the pure drug, the physical mixture and the co-precipitation material. For pure ibuprofen and the co-precipitated material, this coefficient could not be calculated because of their slow dissolution (less than 63.2 % after 75 min).

Table 5.6 Dissolution rate coefficient ($K_w \pm SD$) and amount of ibuprofen dissolved after 15 and 75 min (% ± 95 % CI).

Product	Dissolution rate coefficient [min^{-1}]	Dissolved amount of drug after 15 min	Dissolved amount of drug after 75 min
CPD	0.086 ± 0.002 (A)	71.9 ± 3.62 (A)	93.5 ± 2.89 (A)
Co-precipitation	<0.013 $^{+}$	59.3 ± 1.17 (B)	61.3 ± 0.52 (B)
Freeze-drying	0.114 ± 0.007 (B)	90.4 ± 5.05 (C)	90.6 ± 2.54 (A)
Physical mixture	0.039 ± 0.002 (C)	50.8 ± 0.19 (D)	78.5 ± 0.85 (C)
Ibuprofen	<0.013^{+}	22.0 ± 3.56 (E)	59.5 ± 4.86 (B)

$^{+}K_w < 0.013$ means that ibuprofen dissolution did not reach 63.2 % within 75 min. These values were excluded from statistical analysis.

Values marked with different letters differ significantly (ANOVA, Tukey-test, $p<0.05$; n=3).

5.4 Preparation and characterisation of ibuprofen/β-CD/PVP ternary system

5.4.1 Preparation of ibuprofen/β-CD/PVP ternary system using CPD method

In this part of the study, the addition of water-soluble polymer to increase the complexing and solubilizing efficiencies was investigated. Ibuprofen/β-CD/PVP ternary system was carried out in the modified set-up CPD II in two different procedures, either by using two cartridges, one for the drug and the other for β-CD/PVP mixture (separate) or only one cartridge which was filled with ibuprofen/β-CD/PVP physical mixture (physical mixture). Experiments were carried out at a pressure of 24.5 MP, a temperature of 39.5 °C and an exposure time of 15.5 h. More details about the experimental conditions are given in table 5.7.

Table 5.7 CPD experimental parameters for preparation of ibuprofen/β-CD/PVP ternary systems.

	Ibuprofen [g]	β-CD [g]	PVP [%] wt.	
V75	2.67	14.72	1 %	Physical mixture
V76	2.66	14.61	10 %	Physical mixture
V77	2.67	14.43	1 %	Separate
V78	2.67	14.43	10 %	Separate
V95	16.0	14.30	1 %	Separate
V96	16.0	3	1 %	Separate
V97	8.0	13	10 %	Physical mixture
V98	8.0	13	10 %	Physical mixture

5.4.2 Characterisation of the ibuprofen/β-CD/PVP ternary system

5.5.2.1 Drug content

The HPLC determination of the un- and complexed ibuprofen fraction was not possible due to presence of PVP in the ternary system, Therefore the total drug content was determined using UV photometry according to the method described in section 4.2.1. The uncomplexed drug amount was estimated using DSC by integrating the melting peak of the drug, which appeared in the thermograms of the obtained ternary system according to the method described in section 4.3.2.

As shown in the table 5.8, the entire drug loaded in experiments performed with a low ibuprofen amount (about 2.6 g) and a high carrier amount (about 14.4 g) was complexed with β-CD/PVP mixture, since crystalline drug was not observed in the DSC measurement (V75 - V78). No significant different in the total loaded ibuprofen content was observed by variation of the PVP amount from 1 % to 10 % (V75 - V76 and V77 - V78). On the other hand, the total ibuprofen content was higher in experiments preformed by placing drug and carrier in separate cartridges (V77 and V78) compared to keeping the drug and carrier as a physical mixture (V75 and V76). Increasing of the drug amount to 16 g and keeping the carrier amount at 14.4 g (V95) leads to an increase in the loaded amount of ibuprofen to 9.79 % wt. Only 1.73 % wt. of this amount was found outside the complex (free drug). The highest amount of drug being outside the complex was found in experiment V96, due to the use of high drug concentration but a low amount of the carrier.

Experiments conducted with the same amount of ibuprofen and β-CD as used in the preparation of the binary complex (5.2.2.2) but with addition of PVP 10 % wt. (V97 and V98) showed a loaded drug content of ~ 4.7 % wt., whereas only about 0.5 % wt. was found as free drug outside the complex.

Table 5.8 Total and free ibuprofen content in ibuprofen/β-CD/PVP ternary systems.

Product	Total ibuprofen [% wt.± SD]	Free ibuprofen [% wt.± SD]
V75	1.00 ± 0.011	-
V76	1.01 ± 0.011	-
V77	1.96 ± 0.011	-
V78	1.93 ± 0.003	-
V95	9.76 ± 0.053	1.73 ± 0.044
V96	14.13 ± 0.133	13.39 ± 0.16
V97	4.90 ± 0.109	0.68 ±0.02
V98	4.47 ± 0.115	0.31 ±0.03

5.4.2.2 Thermal behaviour

Products obtained by the same experimental conditions (V97 and V98) were chosen for more detailed characterisation.

As shown in (Figure 5.17, A), the melting peak of ibuprofen appears at about 77 °C. The β-CD/PVP mixture exhibits an endothermic melting peak around 105 °C, corresponding to the release of water (Figure 5.17, B). In the thermal curve of the ibuprofen/β-CD/PVP physical mixture (Figure 5.17, C) the characteristic endothermic melting peak of ibuprofen was observed; this peak disappeared because of the complexation with β-CD/PVP after the treatment with $scCO_2$ in the CPD process (Figure 5.17, D).

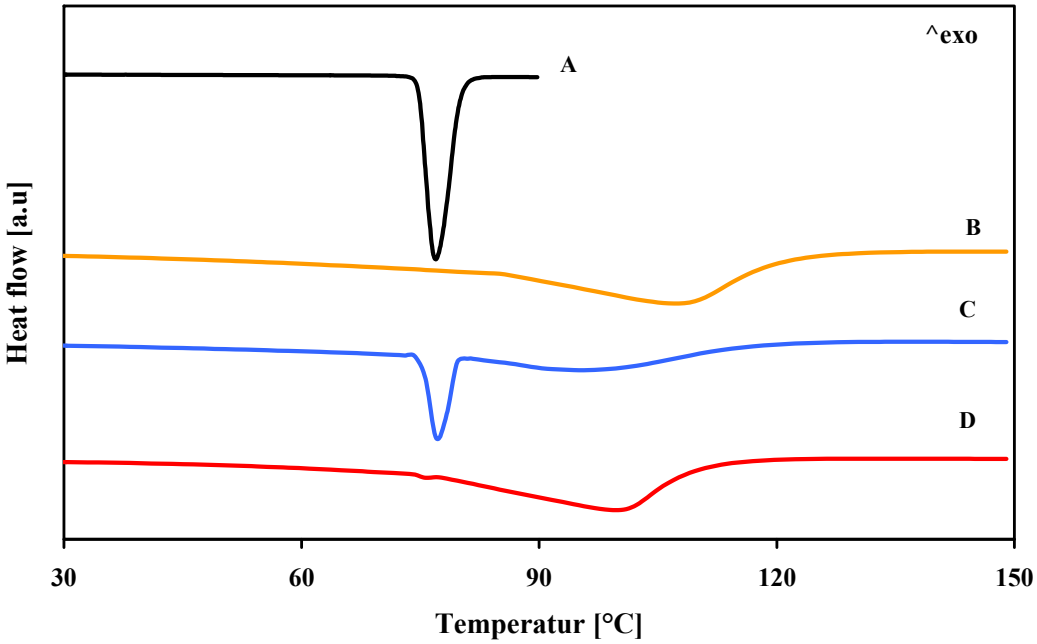


Figure 5.17 DSC thermograms of pure ibuprofen (A), β-CD/PVP physical mixture (B), ibuprofen/β-CD/PVP physical mixture (V98) before the treatment with $scCO_2$ (C) and ibuprofen/β-CD/PVP physical mixture (V98) after the treatment with $scCO_2$ in the CPD (D).

5.4.2.3 Dissolution study

The dissolution rate of ibuprofen at pH 6 (Figure 5.18) was low with 80.89 ± 0.61 % wt. dissolving at the end of 120 min. The percentage of ibuprofen dissolved (85.41 ± 3.32 % wt.) from the physical mixture (ibuprofen/β-CD/PVP) was not statistically different from pure ibuprofen. The CPD processing physical mixture significantly enhanced the dissolution rate of ibuprofen with 101.01 ± 2.36 % wt. dissolved after 120 min. The Weibull-coefficient (K_w) calculated confirmed this results, whereas the dissolution rate coefficient of the physical mixture was significantly different from pure ibuprofen. The Weibull-coefficient was in the order: ibuprofen < physical mixture < CPD ternary system (Table 5.9).

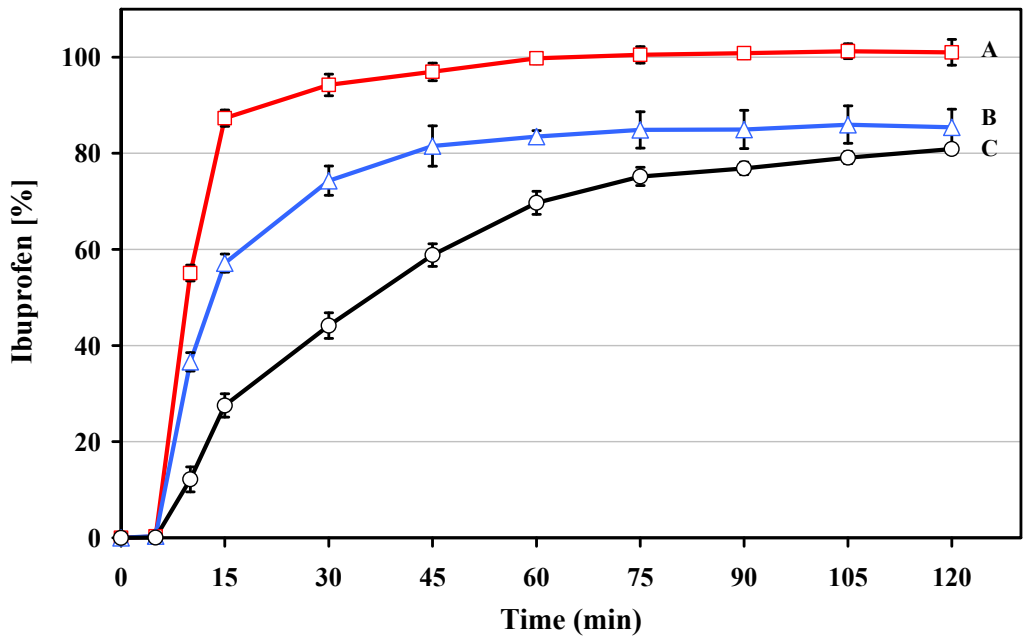


Figure 5.18 Dissolution profiles of ibuprofen (A), ibuprofen/β-CD/PVP physical mixture (V98) before the treatment with $scCO_2$ (B) ibuprofen/β-CD/PVP physical mixture (V 98) treated with $scCO_2$ in the CPD(C) in HBSS buffer pH 6 at 37 °C.

Table 5.9 Dissolution rate coefficient according to Weibull ($K_w \pm SD$) and amount of ibuprofen dissolved after 120 min (% wt. ± 95 % CI).

Product	Dissolution rate coefficient [min^{-1}]	Dissolved amount of drug after 120 min
CPD-V98	0.092 ± 0.001 (A)	101.01 ± 2.36 (A)
Physical mixture	0.053 ± 0.003 (B)	85.41 ± 3.32 (B)
Ibuprofen	0.020 ± 0.001 (C)	80.89 ± 0.61 (B)

Values marked with different letters differ significantly (ANOVA, Tukey-test, $p<0.05$; n = 3).

5.5 Preparation and characterisation of ibuprofen/M-β-CD complex

5.5.1 Preparation of ibuprofen/M-β-CD complex using CPD process

The ability to prepare a solid dispersion in the CPD technique in a single-step process was tested by marking a complex of ibuprofen with M-β-CD. This complex was produced previously by Charoenchaitrakool *et al*. (2002) using a multi-stage dynamic system in a two step process when at the first, the drug was extracted with $scCO_2$ and thereafter, the $scCO_2$/ibuprofen mixture was passed through the M-β-CD packed bed.

The authors showed that the maximum of the drug loading was 10.8 % wt. at 19 MPa, 35 °C and 24 h static contacting time with $scCO_2$. In this procedure, it should be considered that under these conditions M-β-CD melted, since M-β-CD starts to transform into a liquid at 14.7 MPa and 35°C when contacted with $scCO_2$.

In our study, the ibuprofen/M-β-CD complex was produced in the modified set-up CPD II using the method described in chapter 4 (4.1.1). In this, a fixed amount of ibuprofen (13 g) and M-β-CD (5 g) were placed in the same cartridge and were separated with a metal screen. The conditions used for preparing the ibuprofen/M-β-CD complex were temperature (39.5 °C), pressure (24.5 MPa) and exposure time of 15.5 h.

5.5.2 Characterisation of ibuprofen/M-β-CD complex

5.5.2.1 Drug content

The HPLC assay indicated the total ibuprofen content loaded into M-β-CD during the CPD process was found to be 7.60 ± 0.07 % wt. This entire amount was complexed with M-β-CD, since any free drug was not detected in the n-Hexane wash solution.

5.5.2.2 *Thermal behaviour*

The thermal behaviour of ibuprofen/M-β-CD complex obtained by the CPD was studied with the DSC to confirm the formation of a solid complex by the disappearance of the endothermic melting peak of crystalline ibuprofen. Pure ibuprofen exhibits an endothermic melting peak at about 77 °C (Figure 5.19, A). The DSC thermograph of M-β-CD (Figure 5.19, B) shows a broad endothermic peak around 60 °C, corresponding to the release of water. A complete disappearance of the endothermic peak of free ibuprofen was in contrast observed for the complex obtained by the CPD (Figure 5.19, C) confirming the HPLC results. Our results, regarding the product, are consistent with Charoenchaitrakool *et al*. (2002).

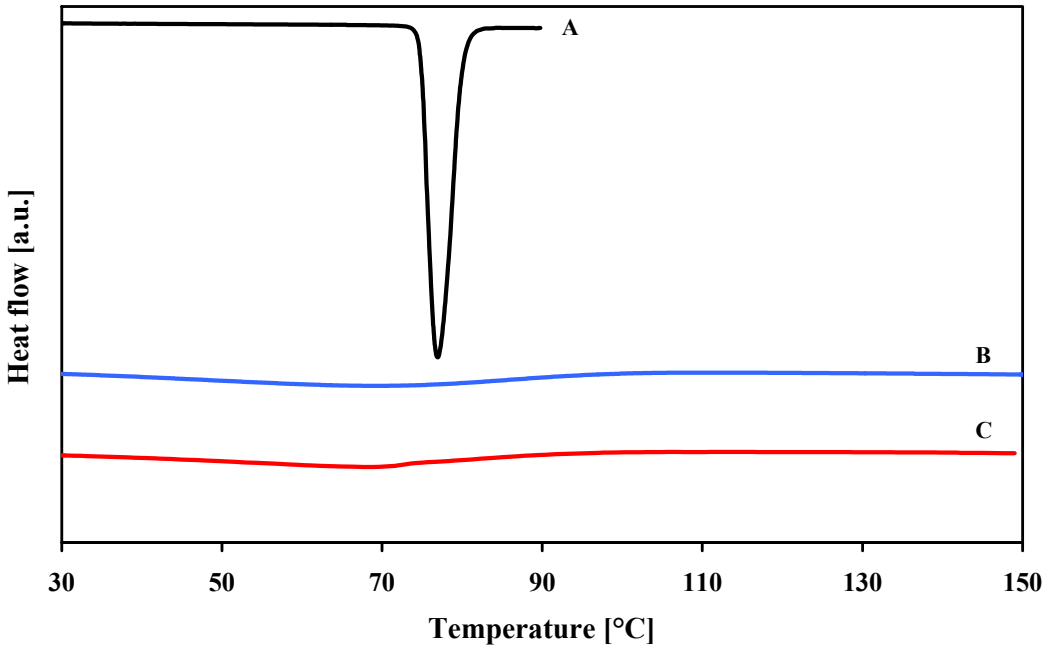


Figure 5.19 DSC thermograms of pure ibuprofen (A), M-β-CD (B) and ibuprofen/M-β-CD complex prepared by the CPD (C).

5.5.2.3 *Morphological study*

As shown in the digital picture (Figure 5.20), ibuprofen/M-β-CD complex obtained by the CPD were irregular in the size and the shape, very likely due to the melting phase transition of M-β-CD/ibuprofen/scCO_2 ternary mixture under the experimental conditions.

Figure 5.20 Picture of the ibuprofen/M-β-CD prepared by the CPD method.

5.5.2.4 Dissolution study

Due to the irregular particle size of the ibuprofen/M-β-CD complex obtained by the CPD, the dissolution kinetic of this material shows an irregular curve with a high standard deviation. However, the obtained ibuprofen/M-β-CD complex shows enhancement in the drug dissolution at pH 5 with about 90 % drug released after 75 min compared to pure ibuprofen (Figure 5.21).

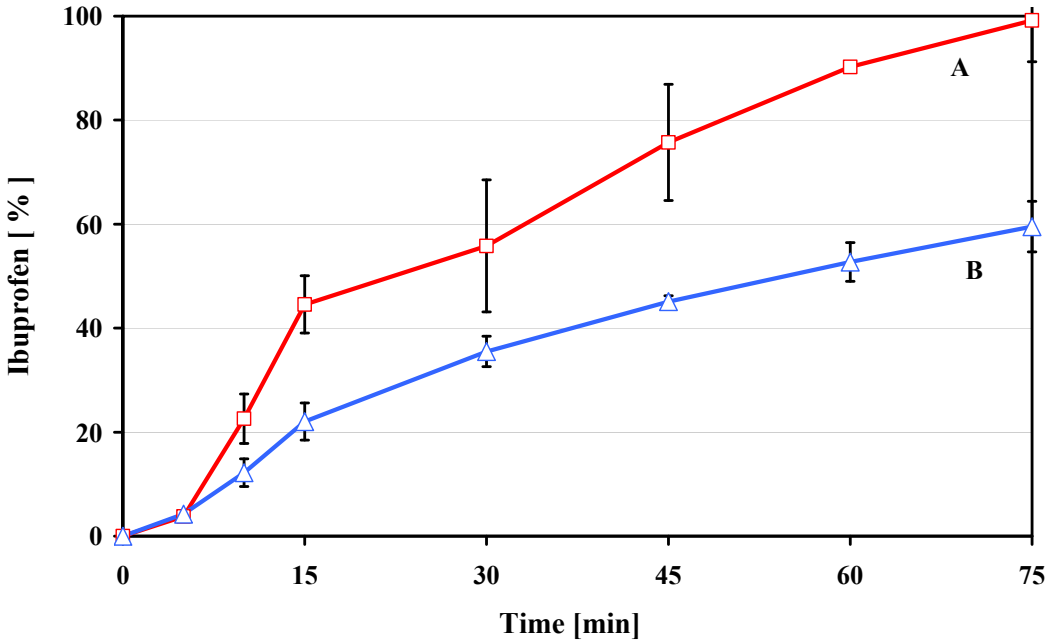


Figure 5.21 Dissolution profiles of ibuprofen (B) and ibuprofen/M-β-CD complex obtained by the CPD (A) in HBSS buffer pH 5 at 37 °C.

The calculated dissolution rate coefficient K_w according to Weibull was found to be 0.031 ± 0.006 min^{-1} for the CPD material, and less than 0.013 min^{-1} in the unprocessed ibuprofen (Table 5.10).

Table 5.10 Dissolution rate coefficient ($K_w \pm SD$) and amount of ibuprofen dissolved after 15 and 75 min (% ± 95 % CI).

Product	Dissolution rate coefficient [min^{-1}]	Dissolved amount of drug after 15 min	Dissolved amount of drug after 75 min
CPD	0.031 ± 0.006	44.56 ± 3.18 (A)	99.17 ± 9.95 (A)
Ibuprofen	<0.013 [+]	22.0 ± 3.56 (B)	59.5 ± 4.86 (B)

[+] $K_w < 0.013$ means that ibuprofen dissolution did not reach 63.2 % within 75 min. These values were excluded from statistical analysis.

Values marked with different letters differ significantly (Students T-test, $P<0.05$; n=3).

5.6 Ibuprofen loading into β-CD granules using CPD and other conventional methods

5.6.1 Preparation and evaluation of the drug-free granules

The ability of the CPD process to load drugs in solid carriers (granules) was investigated. For this aim drug-free granules depending on β-CD were produced by a wet granulation process. The powder mixture of β-CD (160 g) and MCC (40 g) was mixed in a Turbula T2C mixer for 15 min at 42 rpm. Afterward it was kneaded with 150 ml of PVP-aqueous solution (10 % wt.) using a Z-blade kneader (LK 5, Erweka, Heusenstamm, Germany). The wet mass was forced through a 3.5-mm screen by hand. The granules formed were dried at 40 °C for 20 min and sieved to obtain granules with particle size of 1-2 mm. The obtained drug-free granules give solid carriers that exhibited good mechanical properties with regard to friability (4.59 ± 0.76 % wt.). The water content in this fraction was 15.1 ± 0.47 % wt.

5.6.2 Drug loading procedures

The selected fraction (1-2 mm) of the granules was loaded with a model drug (ibuprofen) using the controlled particle deposition (CPD) method and with solution immersing (SI) as conventional method for comparison.

5.6.2.1 Drug loading by supercritical CO_2

Drug loading experiments were performed in a high-pressure cell in the modified apparatus CPD II. In this, a weighted amount of ibuprofen (13.0 g) and β-CD-granules (8.0 g) were placed into separate cartridges inside the high-pressure cell and placed in a constant temperature water bath. Prior to the loading experiments, the whole system was evacuated for 5 minutes to remove atmospheric moisture and air. Then, the required amount of liquid CO_2 (437.14 ± 3.55 g) was condensed into the high-pressure cell and heated to the desired temperature. As soon as the desired pressure in the high pressure cell was reached, the exposure time was fixed to 15.5 h. At the end of the experiments, depressurization occurred within 30 seconds. The same

conditions were used to investigate the effect of $scCO_2$ on the drug-free granules with absence of the drug.

5.6.2.2 Drug loading by solution immersing (SI)

This method is widely used for drug loading (Vallet-Regi *et al*., 2001; Charnay *et al*., 2004; Andersson *et al*., 2004; Salonen *et al*., 2005) and based on immersing drug-free carriers in an organic solution of the drug with subsequent surging. In this study, n-hexane was selected as loading solution, since ibuprofen has a good solubility in this solvent but the carrier (β-CD-granules) has not. A weighted amount of β-CD-granules (1 g) was placed in a metal basket with sieve form and immersed in 200 ml saturated solution of ibuprofen in n-hexane (54 mg/ml). The basket rotated with 50 rpm at room temperature for 2 h. At the end of the loading period, the obtained sample was washed with 10 ml n-hexane to remove unbound ibuprofen crystals and dried for 24 h at room temperature.

5.6.3 Characterisation of the drug-loaded granules

5.6.3.1 Loading efficiency

The UV determination of the total amount of ibuprofen loaded into granules (Table 5.11) shows superior loading yield in the CPD method compared to the drug adsorbed by the SI. A part of the loaded ibuprofen was found in crystalline form, which could be observed by the appearance of the endothermic melting peak of ibuprofen at about 77 °C in the drug-loaded granules (Figure 5.22). This amount was quantified by integration of the melting peak of ibuprofen; this fraction was higher in the CPD-product (Table 5.11).

Table 5.11 Total/free ibuprofen content loaded in β-CD-granules.

Product	Total ibuprofen [% wt. ± SD]	Free ibuprofen [% wt. ± SD]
CPD-granules	17.42 ± 2.06 (A)	3.8 ± 0.15 (B)
SI-granules	3.12 ± 1.14 (A)	0.9 ± 0.4 (B)

Values marked with different letters differ significantly (Student's T-test, $p<0.05$; n = 3).

5.6.3.2 Thermal behaviour

The investigation of the thermal behaviour of the pure ibuprofen by the DSC shows an endothermic melting peak at about 77 °C (Figure 5. 22, A). The drug-free granules (Figure 5.22, B) exhibit a broad endothermic dehydration peak in the range of 80 - 120 °C, the same peak can be seen in the drug-free granules treated with $scCO_2$ (Figure 5.22, C) which demonstrates thermal stability of β-CD-granules in the $scCO_2$ experimental procedure. The endothermic melting peak at 77 °C appears in granules loaded by the SI and the CPD (Figure 5.22, D and E) indicating that a part of the ibuprofen is loaded in crystalline form.

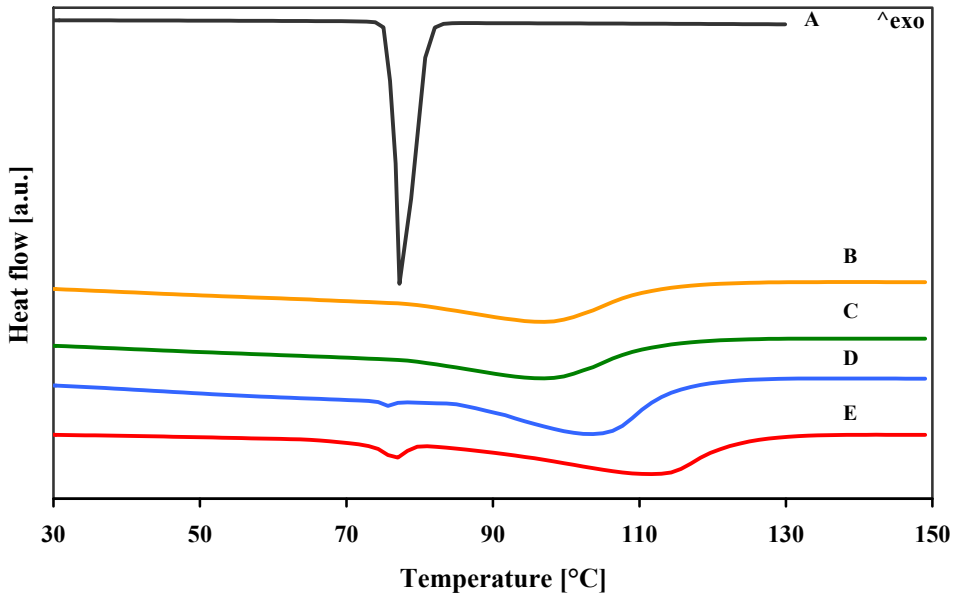


Figure 5.22 DSC thermograms of pure ibuprofen (A), drug-free granules (B), drug-free granules treated with $scCO_2$ (C), SI-granules (D) and CPD-granules (E).

5.6.3.3 *X-ray diffratrometry*

The X-ray diffraction of pure ibuprofen and the unprocessed drug-free granules (Figure 5.23, A and B) exhibited a series of intense peaks indicative of their crystallinity. Drug-free granules treated with $scCO_2$ (Figure 5.23, C) show the same intense patterns as unprocessed granules. The diffractogram of the granules loaded by the SI method (Figure 5.23, D) shows patterns of unloaded granules, but not of crystalline drug. Although these have been found in the DSC, this fraction (0.9 ± 0.4 % wt.) seems to be too small to be detected in the XRD. Patterns of ibuprofen at 14.02°, 16.65° and 20.29° were seen in the CPD material (Figure 5.23, E) indicating the presence of ibuprofen in its crystalline state, this agrees with the DSC results.

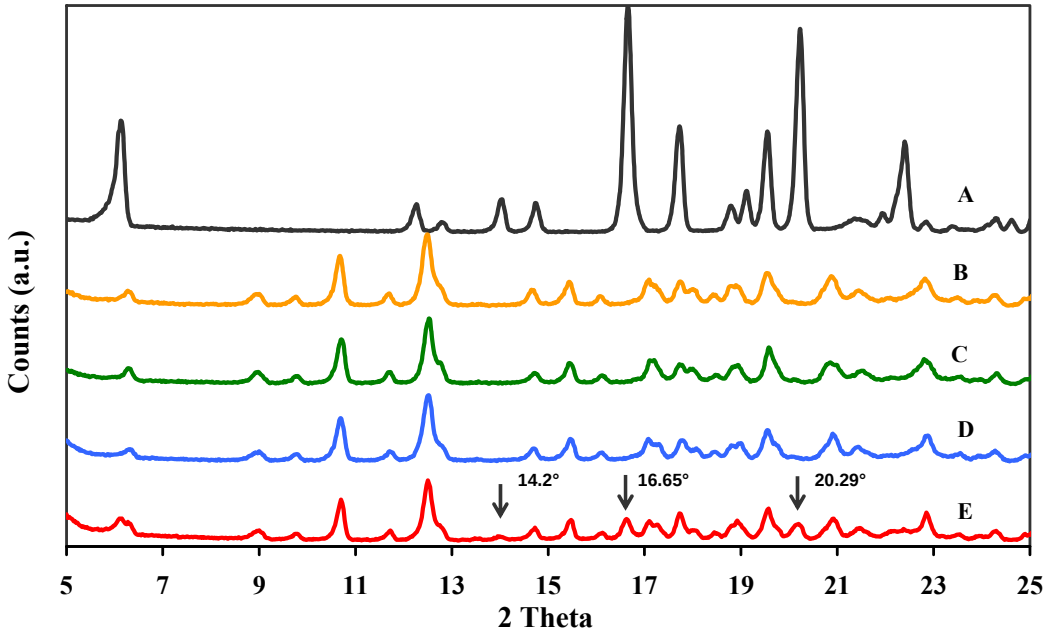


Figure 5.23 X-ray diffractograms of pure ibuprofen (A), drug-free granules (B), drug-free granules treated with $scCO_2$ (C), SI-granules (D) and CPD-granules (E).

5.6.3.4 *BET surface area*

The drug loading with different techniques into the carrier was confirmed by reduction of the BET surface area of the CPD (1.134 ± 0.070 m^2/g) and the SI (1.407 ± 0.048 m^2/g) comparing to unprocessed drug-free granules (1.533 ± 0.031 m^2/g) and

drug-free granules treated with $scCO_2$. The investigation of the BET-surface area, however, was observed supporting the drug content results since the CPD-granules have the highest drug loading.

Table 5.12 BET surface area of the unprocessed, $scCO_2$ treated and drug-loaded granules (n = 3, values are mean ± SD).

Product	Specific area BET [m^2/g]
Drug-free granules	1.533 ± 0.031 (A)
Drug-free granules treated with $scCO_2$	1.520 ± 0.164 (A)
CPD-granules	1.134 ± 0.070 (B)
SI-granules	1.407 ± 0.048 (A)

Values marked with different letters differ significantly (ANOVA, Tukey-test, $p<0.05$; n = 3).

5.7.3.5 SEM microscopy

The investigation of morphological changes by the SEM shows that the structure of β-CD-granules (Figure 5.24, A) did not alter after the drug loading in the SI and the CPD granules (Figure 5.24, C and D). In addition, no needle shaped crystals of the drug (Figure 5.24, B) were observed on the granules surface.

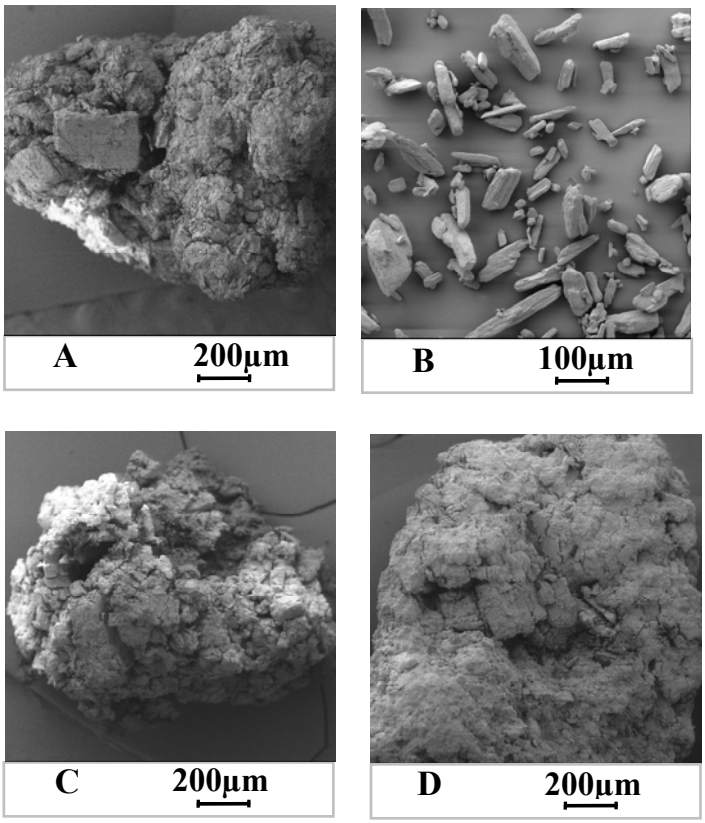


Figure 5.24 SEM micrographs of drug-free granules (A), pure ibuprofen (B), SI-granules (C) and CPD-granules (D).

5.6.3.5 Drug release studies

Dissolution studies were performed at pH 6 in order to evaluate the efficacy of the loading processes by improvement of the water-solubility of ibuprofen. The unprocessed ibuprofen shows rather slow dissolution (Figure 5.25) in this medium with a dissolution rate coefficient (K_w) of 0.02 min^{-1} and only about 80 % wt. dissolved amount after 2 h (Table 5.13).

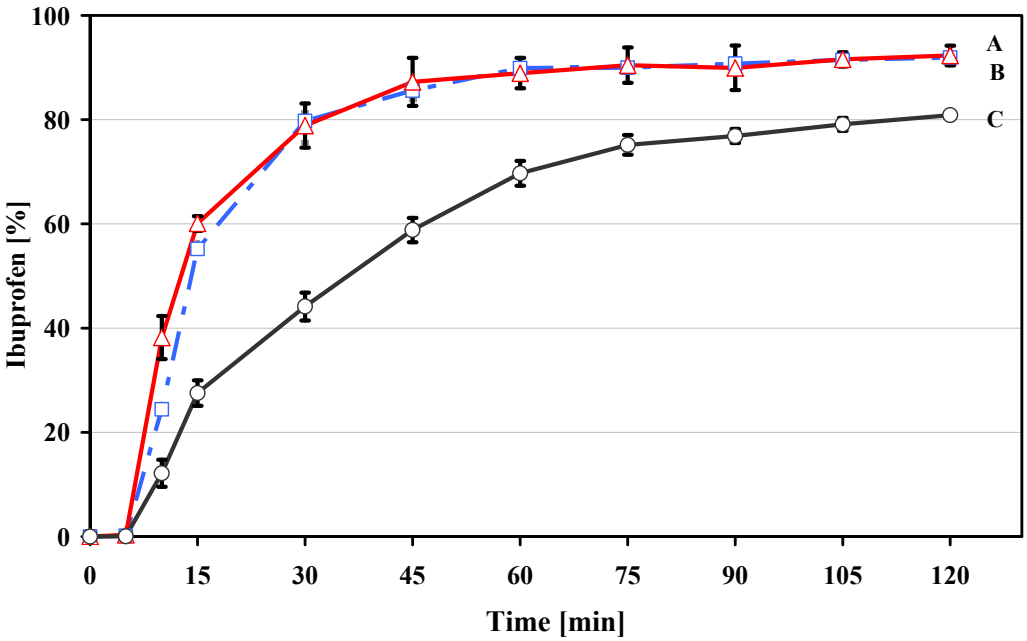


Figure 5.25 Dissolution profiles of ibuprofen (A), CPD-granules (B) and SI- granules (C) in HBSS buffer pH 6 at 37 °C.

Table 5.13 Dissolution rate coefficient according to Weibull ($K_w \pm SD$) and amount of ibuprofen dissolved after 30 and 120 min (% ± 95 %).

Product	Dissolution rate coefficient [min $^{-1}$]	Dissolved amount of drug after 120 min
CPD-granules	0.053 ± 0.002 (A)	91.94 ± 0.80 (A)
SI-granules	0.060 ± 0.002 (A)	92.35 ± 1.37 (A)
Ibuprofen	0.020 ± 0.001 (B)	80.89 ± 0.61 (B)

Values marked with different letters differ significantly (ANOVA, Tukey-test, $p<0.05$; n = 3).

CHAPTER 6

DISCUSSION

6.1 Preparation of ibuprofen/β-CD complex using CPD method

The controlled particle deposition (CPD) was developed to load drugs into solid carries in a single-step, toxic-free process (Türk *et al*., 2007; Hussein *et al*., 2007). A key element of the CPD-process is the use of separate cartridges to hold the drug and the carrier in a static incubation system. The process requires solubility of the drug but insolubility of the carrier in the $scCO_2$. Thereby the drug is transported via the supercritical phase to the carrier and precipitates into the pores of the carrier after release of the pressure. Under these conditions, no melting of the drug is obtained, in contrast to comparable methods reported in the literature (Charoenchaitrakool *et al*., 2002; Bandi *et al*., 2004). This gives a better chance for stable, crystalline or molecular dispersed products (Charumanee, 2004).

With this respect, β-CD was chosen as carrier; hence, it shows stability in $scCO_2$ at the experimental conditions. On the other hand, the employing of ibuprofen as a model drug in this work was due to its poor water-solubility (drug class II), furthermore ibuprofen is able to form an inclusion complex with β-CD using conventional methods including grinding, co-precipitation, freeze-drying and spray-drying (Kurozumi, *et al*., 1975; Nozawa *et al.,* 1994; Mura *et al.,* 1998; Khan *et al.,* 2001). Ibuprofen/β-CD complex formation was confirmed using the methods described in chapter 1 (1.3.7). The molecular modeling and the ^1H NMR studies together revealed that solution state complexation occurred by inclusion of the isopropyl group together with part of the aromatic ring of ibuprofen into the β-CD cavity, probably through its wider side (Ghorab and Adeyeye, 2001) (Figure 6.1).

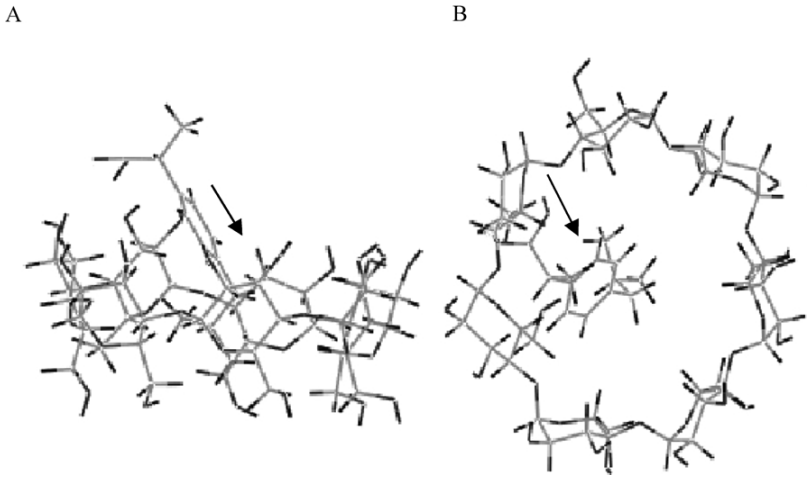


Figure 6.1 Molecular modeling of ibuprofen/β-CD complex, side view (A) and top view (B) according to Ghorab and Adeyeye (2001).

On the other side, ibuprofen is well soluble in $scCO_2$ (Figure 5.5) and this is an essential factor to load drugs into solid carriers in the CPD method. Moreover, the solubility of the drug in SCF is an important factor to form drug/CD inclusion complexes; since the SCF soluble drug gives more chance for the interaction with the carrier compared to insoluble drugs. This knowledge is reported recently by Moribe *et al*. (2007) too, who prepared the complexes of trimethyl-β-CD with different active pharmaceutical ingredients which varied in their solubility under supercritical conditions.

6.1.1 The influence of CPD process parameters on the complex formation

Upon the previous studies described in the literature, despite of possible discrepancies, some general trends become apparent regarding the influence of the process parameters on the preparation of drugs/CDs complexes in the supercritical fluids. Experimental parameters such as the temperature, the pressure, the time of exposure and the amount of the drug or the carrier and their solubility in $scCO_2$ affect the CDs complex formation, the effects of these parameters are investigated for preparing ibuprofen/β-CD complexes in the CPD process (Table 5.3).

The temperature in this study was maintained at 39.5 °C to avoid drug melting in $scCO_2$, this was based on the phase behaviour study of the drug in $scCO_2$, since ibuprofen exhibits a nearly constant melting point of 46 °C over a pressure range between 15 - 28 MPa. The experiments, however, could be performed at a temperature over 40 °C with pressure less than 15 MPa, but the use of a high temperature could damage drug stability.

On the other side, the concentration of ibuprofen in $scCO_2$ is of course a relevant parameter, since keeping the fluid phase saturated gives more chance for a high inclusion yield. Therefore, the amount of ibuprofen in this study was fixed to reach saturation under the experimental conditions.

Various effects of the pressure on the complex formation have been reported in the few studies found in the literature. In our study, therefore the effect of the pressure in a static mode was investigated in a range of 24.6 and 27.8 MPa by keeping the time of exposure and the temperature at 48 h and 39.5 °C, respectively. Within this range, increasing pressure showed commonly positive effects on the total amount of ibuprofen and the inclusion yield with β-CD, due to the increase of the penetrative effect of the ibuprofen solute in $scCO_2$ and the mass transfer. The increase of the pressure over this range has no effect on the ibuprofen solubility in supercritical solution and for this reason the influence of the pressure was not seen in the pressure range above 25 MPa. However, these results agree with others published previously (Van Hees *et al*., 1999; Charoenchaitrakool *et al*., 2002).

The preliminary investigation of the effect of the static exposure time on the ibuprofen/β-CD complex over a range from 6 to 114 h at three different pressure-levels showed that there is an optimum for the treatment time. This optimum was found to be about 14 h. Van Hees *et al.* (1999) established that the contact time between $scCO_2$ and the physical mixture of piroxicam and β-CD in the static mode had a positive influence on the complex formation. The positive effect on the exposure time, however, was confirmed in earlier findings (Hassan *et al*., 2004; Moribe *et al.,* 2007) using model drugs which have lower solubility in $scCO_2$ than ibuprofen.

In case of ibuprofen, Charoenchaitrakool *et al.* (2000) did not detect any significant difference of the ibuprofen amount loaded in M-β-CD when the static contact time was

varied from 1 - 24 h and the pressure and temperature were kept at 190 bar, 35 °C, respectively. This agrees with our results.

6.1.2 Investigation of reproducibility

One of the important factors, which were investigated in this study, was the reproducibility of the formation of the ibuprofen/β-CD complex in the CPD technique. The reproducibility was investigated by performing several experiments at defined experimental parameters (Table 5.4). The repeatability of the experiments was evaluated by determination of the un- and complexed drug amount loaded under the same experimental parameters. The results were satisfactory with the relative standard deviation of 0.22 % in the total ibuprofen loaded.

6.2 Physicochemical characterisation and comparative evaluation of ibuprofen/β-CD complexes obtained by CPD and other conventional methods

Following the investigation of the reproducibility of the CPD method for preparing the ibuprofen/β-CD complex, the CPD as a novel supercritical fluid technique was compared with conventional methods for complex formation. In particular, an inclusion complex with 2.8 ± 0.22 % wt. (n = 6) total content of ibuprofen was obtained using the CPD and compared to 1:3 molar ratio ibuprofen/β-CD (about 5.5 % wt.) complexes prepared by the co-precipitation, the freeze-drying methods or a simple physical mixture. These conventional methods, however, involve organic or toxic solvents in their preparation.

The mechanism for preparation of the complex in the CPD is comparable with those, which form the complex in suspension (co-precipitation), since this method depends on precipitation of the dissolved drug into the solid carrier; therefore, the inclusion yield in the CPD was comparable to the co-precipitation material. The freeze-drying method, however, showed a better inclusion rate because of the different mechanism for the complex formation, since the β-CD is dissolved in a solvent (aqueous

ammoniac solution) prior to form the inclusion. In contrast, the inclusion yield was very low by the simple dry mixing of ibuprofen and β-CD. Anyway, the inclusion yield results for the conventional methods and the physical mixture in this study agree with results reported previously for other systems (Veiga *et al*., 2001; Govindarajan and Nagrsenker, 2004; Reddy *et al*., 2004).

As a strategy for improving the water solubility, all the ibuprofen/β-CD binary systems obtained with different methods, due to the complex formation, enhanced the drug dissolution at pH 5 compared to the unprocessed ibuprofen (Figure 5.16). In case of the physical mixture, even though no inclusion complex was formed, the wettability of the drug is thought to be improved by cyclodextrin (Charumanee 2004; Govindarajan and Nagrsenker, 2004). The enhancement in the dissolution was quite limited in the co-precipitated material due to building aggregates after the drying step during the preparation process, whereas the CPD products were obtained in fine powder form with good flowability properties, the freeze-dried material was completely amorphous. The highest dissolution rate, however, was found in the CPD and the freeze-dried materials.

One possible reason for this improved dissolution rate in the CPD, the freeze-dried and the co-precipitation materials compared to the physical mixture is likely to be the formation of a solid inclusion drug/CD complex.

The FTIR investigation gives evidence for the interaction between the drug and β-CD by a partly disappearance of the C=O stretch at 1714 cm^{-1} and 1731 cm^{-1} in the co-precipitated and the CPD materials, respectively (Figure 5.11) or its complete absence in the freeze-dried product, indicating complete complexation obtained by this procedure. The formation of intermolecular hydrogen bond between the C=O group of ibuprofen and β-CD is a possible reason for an altered environment of the interatomic C=O bond in the IR-spectrum of ibuprofen in the obtained complexes. Our results are consistent with earlier findings (Mura *et al.*, 1998; Bratu *et al*., 2005).

The interaction between the drug and the carrier was confirmed by the complete disappearance of the typical melting peak of ibuprofen at 77 °C in the DSC of the freeze-dried product supporting the view of a partial formation of a complex in the CPD and the co-precipitation materials (Figure 5.12).

Comparison of the X-ray pattern (Figure 5.13) indicates loss of reflections in the products manufactured by CPD, freeze-drying and co-precipitation. This again stands for the formation of inclusion complexes compared to the physical mixture and the raw material, but only to an incomplete extend for the CPD and the co-precipitation methods. The formation of the inclusion complexes, however, is seen by the appearance of a new reflection band. In the freeze-dried product, a complete loss of crystallinity is likely due to the absence of reflection peaks, this may be due to amorphous structure of the complex caused by the use of ammonia during preparation (Govindarajan and Nagrsenker, 2004). The SEM micrographs (Figure 5.15) demonstrate the interaction of ibuprofen with β-CD by indicating a morphological change of the crystals in all the materials obtained, compared to the pure starting material and their physical mixture. Moreover, SEM micrographs and digital pictures (Figure 5.14 and Figure 5.15) depict the solid-state of ibuprofen/β-CD complexes obtained by the different preparation techniques; hence, the powder properties are an important factor for the pharmaceutical application of the obtained materials. The fluffy, amorphous powder obtained by the freeze-drying and the aggregate formation in the co-precipitation material gives unsatisfactory flow characteristics, which limit the formulation and the application of this material for oral administration route. In contrast, the CPD process-obtained material with a free-flowing characteristic can be easily applied in different processes in the pharmaceutical technology.

6.3 Preparation and characterisation of ibuprofen/β-CD/PVP ternary system

At the previous stage of our study, we applied successfully the ibuprofen/β-CD binary system using the CPD method to improve drug dissolution properties. But for reasons of costs, production capabilities and toxicology, only limited amount of CDs must be used in drug formulation; moreover, the fact that the isolated crystalline product obtained by the CPD contain free drug and empty β-CD in its product. Methods to improve the inclusion efficiency and to optimise the CD complexation are required. To overcome this problem, the addition of water-soluble polymers to increase CD complexation with drugs was reported previously in the literature

(Loftsson 1998; Mura *et al*., 2001; Faucci *et al*., 2001; Pose-Vilarnovo *et al*., 2002; Valero *et al.*, 2003)

A possible explanation of drug/polymer interaction with CD molecules may be found in the H-bonding between the CD molecule and OH-groups of the polymer, which produces an important change in the driving force of the complex formation. NMR data presented by Valero *et al.* (2004) revealed that in a naproxen/HP-β-CD/PVP ternary system, PVP is outside the cyclodextrin, it must be therefore wholly or partially recovering the naproxen/HP-β-CD inclusion complex acting as a bridge between both β-CD molecules and the drug. This binding, however, is based on the affinity of drug molecules to the polymer matrix.

Consequently, to the addition of a small amount of water-soluble polymer into the aqueous complexation media, an increase in the complexation efficiency of CDs towards the drug resulting in enhanced drug solubility was observed. The effect of the addition of a water-soluble polymer on the drug/CD binary system was investigated in the aqueous solution or/and in the solid, using classical methods for the complex formation e.g. freeze-drying or spray-drying (Loftsson 1998; Mura *et al*., 2001; Faucci *et al*., 2001; Pose-Vilarnovo *et al*., 2002; Valero *et al.*, 2003). However, the effect of the presence of water-soluble polymers in $scCO_2$ as complexation media is not yet reported. In our study, therefore the preparation of ibuprofen/β-CD/PVP ternary system was investigated to increase the complexation efficiency. The process was optimised by varying the amount of drug, β-CD and PVP. Moreover, the effect on the experimental mode was investigated. The evaluation of the different process parameters was achieved by determination of the total amount of the drug loaded and the fraction of ibuprofen loaded in its crystalline form.

Compared to the ibuprofen/β-CD binary system, an increase in the drug loading yield under the same experimental conditions was achieved in the CPD process.
About 9.76 ± 0.053 % wt. of ibuprofen was loaded in the ibuprofen/β-CD/PVP ternary system; moreover, most of the drug loaded was incorporated in the β-CD/PVP complex with only a small part (1.73 ± 0.044 % wt.) was found as crystalline form in the DSC measurements.

The preliminary optimisation of the CPD process parameters for the preparation of the ibuprofen/β-CD/PVP ternary system show that there is a more proportionate increase in percentage drug loading efficiency for every increase in drug concentration within the range used in our experiments; this is due to more available ibuprofen/$scCO_2$ solution for the complexation. In addition, an effective drug loading yield was obtained in experiments using two separate cartridges, one for the drug and the other for the carrier (β-CD and PVP), compared to exposure of the physical mixture of the drug and the carrier under the same experimental conditions. This may be due to the diffusivity power of ibuprofen/$scCO_2$ solutes into the carriers. No significant difference in the total loaded ibuprofen content was observed by variation of the PVP amount from 1 % to 10 % wt. Valero *et al.* (2003) investigated the effects of the water-soluble polymer on the complexation of the naproxen/β-CD. The author's demonstrate in this study that the presence of different proportions of PVP in a range of 0 - 1 % wt. does not increase the ability of drug/cyclodextrin complexation but was important to change the driving force of the complex formation. It was observed that at low polymer concentrations, the complexation process is driven entropically, while at higher PVP proportions it is enthalpically favored. The results presented by Valero *et al.* (2003) investigated the effect of the polymer quantity in aqueous medium. However, the effect of water-soluble polymer on the complex formation of drug/cyclodextrin complex in SCF as a complexation medium required more investigation.

The CPD product obtained by keeping the physical mixture of ibuprofen/β-CD/PVP under $scCO_2$ (V98) demonstrates significant improvement in drug dissolution compared to unprocessed physical mixture and pure drug. This was due to the complex formation with β-CD/PVP, verified by complete disappearance of the melting peak of the drug after the treatment with $scCO_2$ (Figure 5.17).

6.4 Preparation and characterisation of ibuprofen/M-β-CD complex

The pervious results present the CPD method as well suitable method for the preparation of the ibuprofen/β-CD complex. The mechanism of the inclusion depends on the dissolved drug in $scCO_2$ followed by permeation of the supercritical solution into the solid carrier under the high pressure deployed in this process. In this part of the study, the utilisation of the CPD process to load drugs into M-β-CD was investigated, the difference between the native β-CD and M-β-CD as drug-carriers being due to the phase behaviour of these carriers in $scCO_2$. β-CD shows stability and kept their solid-state in the SCF experimental procedures (Türk *et al.*, 2007), M-β-CD, however, was melted under the same experimental conditions. A melting point depression was reported previously by Charoenchaitrakool *et al.* (2002) and decreased to 25, 35 40, 45 °C when contacted with CO_2 at 19, 14.7, 14.3 and 9.4 MPa, respectively. The hypothesis of the complexation mechanism in the case of M-β-CD depends on the interaction between the solute drug and the molten carrier under the supercritical condition, giving a chance to form a complex, like a solid disperse.
On this basis, the product resulting from the complex formation of ibuprofen/β-CD was in powder form, this give it an significant advantage over the complex obtained from ibuprofen and M-β-CD which appeared irregular in size and shape due to the melting (Figure 5.20).

Compared to β-CD, the utilisation of M-β-CD as carrier has lead to higher drug loading yield. An amount of 7.60 ± 0.07 % wt. of ibuprofen was loaded in M-β-CD at 39.5 °C, 24.5 MPa and 15.5 h. This amount is comparable with the loaded content (7.9 ± 0.7 % wt.) reported by Charoenchaitrakool *et al.* (2002) using a multi-step process at 35 °C, 19 MPa and 12 h. However, the maximum drug content obtained by the authors at 24 h exposure time was about 10 % wt. Due to the complex formation with M-β-CD or to the transformation into the amorphous state, the endothermic melting peak of crystalline ibuprofen disappeared in the DSC thermograms (Figure 5.19).

As consequence to the formation of the complex with M-β-CD, the dissolution of drug was enhanced compared to the pure drug. About 99 % of ibuprofen was in the solution in 75 min (Table 5.10). The dissolution rate coefficient of ibuprofen/M-β-CD complex

was found to be 0.031 ± 0.006 min $^{-1}$, this value was higher (0.086 ± 0.002 min $^{-1}$) in the ibuprofen/β-CD complex prepared in the same technique.

6.5 Ibuprofen loading into β-CD granules using CPD and other conventional methods

Ghorab and Adeyeye (2001) reported the preparation of a CD complex as granules containing drugs. The authors in this study prepared ibuprofen/β-CD granules by mixing the drug and β-CD in a conventional wet granulation process. In 1998, Gazzaniga *et al.* (1998) investigated the use of β-CD as a pelletization agent with microcrystalline cellulose (MCC) in an extrusion/spherounization process. The addition of MCC conferred mechanical strength to the obtained pellets and reduced the amount of β-CD in the final formulation. In related work, Gainotti *et al*. (2004) produced drug-free pellets of β-CD and MCC using a high-shear mixer and loaded the obtained pellets with ibuprofen using powder and solution layering processes.

Our study utilise the controlled particle deposition (CPD) to load carrier in granules consists of excipients already used in the pharmaceutical industry (βCD, MCC and PVP). In contrast to Gainotti *et al*. (2004) these granules were prepared by a wet granulation process, moreover PVP was added to the formulation due to the synergistic effect to CDs in increasing the water-solubility of drugs (Loftsson, 1998; Pose-Vilarnovo *et al*., 2002; Valero *et al*., 2003).

In fact, the dug-free granules obtained according to this modified formulation exhibiting acceptable mechanical stability. In addition these granules show stability under $scCO_2$ experimental conditions, since the thermal behaviour (Figure 5.22), the crystalline state (Figure 5. 23) and the BET surface area (Table 5.12) of the unprocessed materials were not altered by treating with $scCO_2$, this demonstrates that these granules could be a good carrier for drug loading in SCF processes.

In the CPD, about 17.5 % wt. of ibuprofen was loaded into β-CD-granules, compared to about 4 % wt. of the drug loaded in the solution immersing (SI). Furthermore, the loading amount in β-CD-granules was higher than the drug loaded in β-CD powder under the same experimental conditions. This may be due to the

different mechanisms involved in the drug loading process. In particular, including the possibility to form a complex with β-CD/PVP, an adsorption of drug on the granules surface and filling of the pores are to be considered in case of drug loading in granules as carrier. The high drug loaded amount in the CPD-granules, however, was confirmed by a significant reduction in the BET surface area for the CPD-product (Table 5.12). In contrast, no reduction in the BET surface area was observed in the SI materials due to the rather small amount of drug loaded in this method.

Only a minor fraction of the drug loaded was found in the crystalline form in the DSC thermograms (Figure 5.22) and the X-ray diffractograms (Figure. 5.23); this amount could be estimated by the integration of the melting peak of ibuprofen in the loaded granules (Table 5.11). Most of the loaded drug was either in an amorphous-state or complexed with β-CD. No morphological change could be seen in the SEM micrographs of the unprocessed/ibuprofen-loaded granules (Figure 5.24), which demonstrate that the drug loading was not due to the crystallization of the drug on the surface.

With regard to the amount of drug dissolved after 120 min and the dissolution rate coefficient K_w, a significant improvement in the water-solubility of ibuprofen at pH 6 was observed by drug-loaded β-CD-granules compared to the pure drug. This was independent on the drug loading method. One should however, take in to account that the CPD-granules contain a higher amount of drug compared to the SI-granules. This reduces the mass of excipients required for a final formulation.

CHAPTER 7

CONCLUSION

This dissertation presents the controlled particle deposition (CPD) as a promising process for loading of drugs into solid carriers to improve their water solubility. The CPD process offers many advantages over the current drug loading techniques. Additionally to employ the supercritical fluid as a green technology, CPD has the advantage to be a one-step process, providing easier control over the experimental parameters. Moreover, the CPD can be considered an effective process for innovative drugs designed in the pharmaceutical development and manufacturing to improve dissolution rates of poorly water-soluble drugs for oral delivery systems.

In the CPD, a successful inclusion complex of ibuprofen/β-CD has been obtained without using any additional substance (ammoniac or organic solvents) which must be used in the traditional methods for the complex formation.

In fact, the drug loading mechanism in the CPD depends on dissolving the drug in the SCF followed by permeation of this fluid into the solid carrier. Hence, the solubility of the drug in the $scCO_2$ complex was shown to be the rate-determining factor for the formation of the inclusion complex. However, the process parameters, such as pressure and temperature contribute to the complex formation rate through their effect on the drug solubility in the $scCO_2$, whereas the processing time has no significant effect in case of ibuprofen, taking advantage of its good solubility in $scCO_2$.

The ibuprofen/β-CD complex obtained by the CPD improved the drug solubility in HBSS buffer pH 5 compared to the pure drug, ibuprofen/β-CD physical mixture and ibuprofen/β-CD complex obtained by co-precipitation method. However, the drug dissolution of the CPD product was comparable with the freeze-dried material, whereas, the CPD material still has an advantage to produce free flowing powder, which is feasible to use for the pharmaceutical applications.

One possible reason for this improved dissolution behaviour in the CPD, the freeze-dried and the co-precipitation materials compared to the physical mixture and the pure drug is formation of a solid inclusion drug/CD complex. This complex formation was further confirmed by a partly or complete disappearance of the C=O stretch in the FT-IR spectra, loss of crystalline (XRD, DSC) and the appearance of a new population in the obtained ibuprofen/β-CD complexes using different preparation methods compared to pure drug, β-CD, and their physical mixture.

A reduction of the free drug amount in the obtained CPD ibuprofen/β-CD complex and an improvement in the drug-loading efficacy was successfully achieved by the addition of a small amount of water-soluble polymer to the ibuprofen/β-CD binary system. About 9 % wt. of total ibuprofen was detected in the ibuprofen/ β-CD/PVP ternary system. The amount of free ibuprofen in this product was only 1.7 % wt. The ibuprofen/β-CD/PVP ternary system obtained by the CPD exhibited significantly enhanced dissolution rates compared to the unprocessed ibuprofen/β-CD/PVP physical mixture and the pure drug, this is due to the complex formation or/and the amorphisation during the SCF process.

Besides its application to complexes with crystalline CDs, the CPD has benefits to use amorphous CD as carrier in its process. This procedure depends upon the reduction of the melting point of the carrier under the high pressure of $scCO_2$. In this way, a higher amount of ibuprofen was loaded in the M-β-CD compared to thus loaded into the solid β-CD. The obtained martial, however, was irregular in shape and size, resulting in irregular drug dissolution rate.

The last part of this study demonstrates an elegant approach to enhance the drug solubility by loading the drug in granules consisting of excipients already used in the pharmaceutical industry. The results demonstrate that the CPD as effective method for loading drugs into granules compared to other conventional method for drug loading (Solution Immersing). Moreover, β-CD-granules loaded with a model drug (ibuprofen) show a significant improvement of drug dissolution compared to the pure drug. This demonstrates that these granules could be good vehicles for poor water-soluble drugs.

CHAPTER 8

REFERENCES

Al-Marzouqi, A., Shehatta, I., Jobe, B. and Dowaidar, A.
"Phase solubility and inclusion complex of itraconazole with β-cyclodextrin using supercritical carbon dioxide"
J. Pharm. Sci. **95** (2), 292-304 (2006)

Amidon, G. L., Lennernäs, H., Shah, V. P. and Crison, J. R.
"A theoretical basis for a pharmaceutical classification: The correlation of in vitro drug product dissolution in vivo bioavailability"
Pharm. Res. **12** (3), 413-420 (1995)

Andersson, J., Rosenholm, J., Areva, S. and Linden, M.
"Influences of material characteristics on ibuprofen drug loading and release profiles from ordered micro- and mesoporous silica matrices"
Chem. Mater. **16**(21), 4160-4167 (2004)

Arai,Y., Sako, T. and Takebayashi, Y.
"Supercritical fluids: Molecular interactions, physical properties and new applications"
Springer, Berlin; Heidelberg, New York (2002)

Avdeef., A.
"Physicochemical profiling (solubility, permeability and charge state)"
Curr. Top. Med. Chem. **1,** 277-351 (2001)

Bandi, N., Wei, W., Roberts, C. B., Kotra, L. P. and Kompella, U. B.
"Preparation of budesonide- and indomethacin-hydroxypropyl-beta-cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process"
Eur. J. Pharm. Sci. **23**, 159-168 (2004)

Bratu, I., Hernanz, A., Gavira, J. M. and Bora, G.
"FT-IR spectroscopy of inclusion complexes of β-cyclodextrin with fenbufen and ibuprofen"
Rom. Journ. Phys. **50** (9-10), 1063-1069 (2005)

Bruner, L. and Tolloczko, S.
"Über die Auflösungsgeschwindigkeit fester Körper"
Z. Phys. Chem. **35**, 283-290 (1900)

Brunner, E.
"Reaktionsgeschwindigkeit in heterogenen Systemen"
Z. Phys. Chem. **47**, 56-102 (1904)

Buttle, I.
"O/W-Emulsionen für die intravenöse Applikation von Arzneistoffen"
PhD thesis, Free University of Berlin, Germany (2004)

Cabral-Marques, H. M.
"Preparation of complexes. Evidence for complex formation"
Rev. Port. Farm. **XLIV** (4), 157-163 (1994)

Cannon, G. W. and Breedveld, F. C.
"Efficacy of cyclooxygenase-2–specific inhibitors"
Am. J. Med. 110(3A), 6-12 (2001)

Chan, H. O. and Stewart, B. H.
"Physicochemical and drug delivery consideration for oral drug bioavailability"
DDT **1** (11), 461-473 (1996)

Charnay, C., Begu, S., Tourne-Peteilh, C., Nicole, L., Lerner, D. A. and Devoisselle, J. M.
"Inclucion of ibuprofen in mesoporous templated silica: Drug loading and release property"
Eur. J. Pharm. Biopharm. **57** (3), 533-540 (2004)

Charoenchaitrakool, M., Dehghani, F., Foster, N. R. and Chan, H. K.
"Micronization by rapid expansion of supercritical solutions to enhance the dissolution rates of poorly water-soluble pharmaceuticals"
Ind. Eng. Chem. Res. **39**, 4794-4802 (2000)

Charoenchaitrakool, M., Dehghani, F. and Foster, N. R.
"Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl-β-cyclodextrin"
Int. J. Pharm. **239**, 103-112 (2002)

Charumanee, S.
"Amorphization and dissolution studies of acetaminophen-β-cyclodextrin inclusion complexes"
CMU. J. **3** (1), 13-23 (2004)

Devine, J. N., Kemmish, D. J., Wilson, B., Griffiths, I. and Seargeant, K. M.
"Biocompatible polymeric materials for medical devices"
(UK) WO 2002000275 (2002)

Diefenbacher, A. and Türk, M.
"Phase equilibria of organic solid solutes and supercritical fluids with respect to the RESS process"
J. Supercrit. Fluids **22** (3), 175-184 (2002)

Dressman, J. B., Amidon, G. L., Reppas, C. and Shah, V. P.
"Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms"
Pharm. Res. **15** (1), 11-22 (1998)

Dressman, J. B. and Reppas, C.
"In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs"
Eur. J. Pharm. Sci. **11** (2), 73-80 (2000)

Dodziuk, H.
"Cyclodextrin and their complex"
Wiley-VCH, Weinheim, Germany (2006)

Duchene, D., Wouessidjewe, D.
"Industrial application of cyclodextrin and their derivatives"
J. Coord. Chem. **27**, 223-236 (1992)

Dyas, M. A. and Shah, U. U.
"Dissolution and dissolution testing"
Encyclopedia of Pharmaceutical Technology 3th edition, 908-928 (2006)

European Pharmacopoeia 5.8th edition
Council of Europe, Strasburg (2007)

Faucci, M. T. and Mura, P.
"Effect of water-soluble polymers on naproxen complexation with natural and chemically modified beta-cyclodextrins"
Drug Dev. Ind. Pharm. **27** (9), 909-917 (2001)

FDA guidance for industry
"Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system"
U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), August 2000

FDA guidance for industry
"Bioavailability and bioequivalence studies for orally administered drug products - General considerations"
U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), March 2003

Foster, N. R., Mammucari, R. and Dehghani, F.
"Co-precipitation of pharmaceuticals using gas anti-solvent technique"
Proceedings of the 8th Meeting on Supercritical Fluids, Besnard M., Cansell F. Edts., Tome 1, 321-326 (2002)

Freiss, B., Marciacq, F., Fages, J., Sauceau, M., Lochard, H., Letourneau, J. J. and Joussot-Dubien, C.
"Procédé de préparation d'un composé d'interaction de substances actives avec un support poreux par fluide supercritique."
WO 03/043604 (2003)

Freiss, B., Marciacq, F., Fages, J., Sauceau, M., Lochard, H., Letourneau, J. J. and Joussot-Dubien, C.
"Procédé de préparation d'un composé d'interaction d'un dérivé d'anilide avec un support poreux par fluide supercritique"
WO 03/030867 (2003)

Frömming, K.-H. and Szejtli, J.
"Cyclodextrin in pharmacy"
Kluwer Academic Publishers, Netherlands (1994)

Fukushima, Y.
"Application of supercritical fluids"
R&D Review of Toyota CRDL **35** (1), 1-9 (2000)

Gainotti, A., Bettini, R., Gazzaniga, A., Colombo, P. and Giordano, F.
"Drug-β-cyclodextrin containing pellets prepared with a high-shear mixer"
Drug Dev. Ind. Pharm. **30** (10), 1061-1068 (2004)

Gazzaniga, A., Sangalli, M. E., Bruni, G., Zema, L., Vecchio, C. and Giordano, F.
"The use of β-cyclodextrin as a pelletization agent in the extrusion/spheronization process"
Drug Dev. Ind. Pharm. **24** (9), 869-873 (1998)

Ghorab, M. K. and Adeyeye, M. C.
"Enhancement of ibuprofen dissolution via wet granulation with β-cyclodextrin"
Pharm. Dev. Tech. **6** (3), 305-314 (2001)

Ghorab, M. K. and Adeyeye, M. C.
"Elucidation of solution state complexation in wet-granulated oven-dried ibuprofen and β-cyclodextrin: FT-IR and ^{1}H-NMR studies"
Pharm. Dev. Tech. **6** (3), 315-324 (2001)

Ghorab, M. K. and Adeyeye, M. C.
"Enhanced bioavailability of process-induced fast-dissolving ibuprofen cogranulated with β-cyclodextrin"
J. Pharm. Sci. **92** (8), 1690-1697 (2003)

Gottwald, W.
"Statistik für Anwender"
Wiley-VCH, Weinheim, Germany (2000)

Govindarajan, R. and Nagrsenker, S. M.
"Influence of preparation methodology on solid-state properties of an acidic drug-cyclodextrin system"
J. Pharm. Pharmacol. **56**, 725-733 (2004)

Gulati, M., Grover, M., Singh, S. and Singh, M.
"Lipophilic drug derivatives in liposomes"
Int. J. Pharm. **165**, 129-168 (1998)

Gurdial, G. S. and Foster, N. R.
"Solubility of o -hydroxybenzoic acid in supercritical carbon dioxide"
Ind. Eng. Chem. Res. **30**, 575-580 (1991)

Hassan, A., Tang, Y. M. and Ayer, J. W.
*"Itraconazole formation using supercritical carbon dioxi*de"
Drug Dev. Ind. Pharm. **30** (10), 1029-2035 (2004)

Hedges, A. R.
"Industrial application of cyclodextrins"
Chem. Rev. **98**, 2035-2044 (1998)

Heinrich, J., Chalabala, M. and Rak, J.
"Auflösungskurve und Weibull-Funktion"
Acta Pharm. Technol. **32** (2), 94-96 (1986)

Herzfeldt C. D. and Kümmel, R.
"Dissociation constants, solubilities and dissolution rates of some selected non-steroidal anti-inflammatories"
Drug Dev. Ind. Pharm. **9** (5), 767-793 (1983)

Hörter, D. and Dressman, J. B.
"Influence of physicochemical properties of drug in the gastrointestinal tract"
Adv. Drug Deliv. Rev. **46** (1), 75-87 (2001)

Hussein, K., Türk, M. and Wahl, M. A.
"Comparative evaluation of ibuprofen/β-cyclodextin complexes obtained by supercritical carbon dioxide and other conventional methods"
Pharm. Res. **24** (3), 585-592 (2007)

ICH Harmonised Tripartite Guideline
"Validation of analytical procedures: Text and methodology Q2(R1)"
http://www.analytical-services.com/domains/analytical-services_com/data/free_docs/Q2(R1)_2.pdf, November (2005)

Injoon, O., Lee, M.-Y., Lee, Y.-B., Shin S.-C. and Park, I.
"Spectroscopic characterization of ibuprofen/2-hydroxypropyl-β-cyclodextrin inclusion complex"
Int. J. Pharm. **175**, 215-223 (1998)

Jun, S.W., Kim, M.-S., Kim, J.-S., Park, H. J., Lee, S., Woo, J.-S. and Hwang, S.-J.
"Preparation and characterization of simvastatin/hydroxypropyl-b-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process"
Eur. J. Pharm. Biopharm. **66** (3), 413-421 (2007)

Junco, S., Casimiro, T., Ribeiro, N., Nunesdaponte, M. and Cabral Marques, H.
"Optimisation of supercritical carbon dioxide systems for complexation of naproxen: beta-cyclodextrin"
J. Incl. Phenom. Macrocycl. Chem. **44**, 69-73 (2002)

Junco, S., Casimiro, T., Ribeiro, N., Nunesdaponte, M. and Cabral Marques, H.
"A comparative study of naproxen - beta cyclodextrin complexes prepared by conventional methods and using supercritical carbon dioxide"
J. Incl. Phenom. Macrocycl. Chem. **44**, 117-121 (2002)

Kaiser, S. C.
"Herstellung, In-Line-Einschluss und Charakterisierung Lipophiler und Hydrophiler Kamillenextrakte unter Einsatz von verdichtetem Kohlendioxide"
PhD thesis, University of Tübingen, Germany (2003)

Kaiser, C. S., Römpp, H. and Schmidt, P. C.
"Pharmaceutical applications of supercritical carbon dioxide"
Pharmazie **56** (12), 907-926

Kasim, N. A., Whitehouse, M., Ramachandran, C., Bermejo, M., Lennernäs, H., Hussain, A. S., Junginger, H. E., Stavchansky, S. A., Midha, K. K., Shah, V. P. and Amidon, G. L.
"Molecular properties of WHO essential drugs and provisional biopharmaceutical classification"
Mol. pharmaceutics **1** (1), 85-96 (2003)

Khan, G. M., Wazir, F. and Zhu, J.-B.
"Ibuprofen-cyclodextrin inclusion complexes: Evaluation of different complexation methods"
J. Med. Sci. **1** (4), 193-199 (2001)

King, M. B. and Bott, T. R.
"Extraction of natural products using near-critical fluids"
Blackie Academic & Professional, Glasgow (1993)

Kurozumi, M., Nambu, N. and Nagai, T.
"Inclusion compounds of non-steroidal anti-inflammatory and other slightly soluble drugs with α- and β-cyclodextrin in powdered form"
Chem. Pharm. Bull. **23**, 3062-3068 (1975)

Lemert, M. R. and Johnston, P. K.
"Solid-liquid-gas equilibria in multicomponent supercritical fluid systems"
Fluid Phase Equilib. **45**, 265-286 (1989)

Löwenberg, M.
"Experimentelle Untersuchung zur Komplexbildung von Cyclodextrin und pharmazeutischen Wirkstoffen"
Diploma thesis, University of Karlsruhe, Germany (2003)

Loftsson, T.
"Increasing the cyclodextrin compexation of drugs and drug bioavailability through addition of water-soluble polymers"
Pharmazie **53**, 733-740 (1998)

Loftsson, T. and Brewster, M. E.
"Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization"
J. Pharm. Sci. **85** (10), 1017-1125 (1996)

Loftsson, T., Brewster, M. E. and Masson, M.
"Role of cyclodextrin in improving oral drug delivery"
Am. J. Drug Deliv. 2 (4), 261-275 (2004)

Loftsson, T. and Hreinsdottir, D.
"Determination of aqueous solubility by heating and equilibration: A technical note"
AAPS PharmSciTech. **7** (1), Article 4 (2006)

Loth, H. and Hemgesberg, E.
"Properties and dissolution of drugs micronized by crystallization from supercritical gas"
Int. J. Pharm. **32**, 265-267 (1986)

Martin, H.-J.
"Charterisierung von schwerlöslichen Arzneistoff-Nanopartikeln hergestellt durch das RESS-Verfahren zur Verbesserung der Bioverfügbarkeit"
PhD thesis, University of Tübingen, Germany (2003)

Martindale, The Extra Pharmacopoeia 30th edition, Reynolds J.E.F edition,
The Pharmaceutical Press, United Kingdom (1993)

McHugh, M. and Krukonis, V.
"Supercritical fluid extraction - principles and practice"
Butterworth, Stoneham, MA, USA (1986)

Merisko-Liversidge, E.
"Nanocrystals: Resolving pharmaceutical formulation issues associated with poorly water-soluble compounds"
Particles, Orlando, USA (2002)

Moribe, K., Fujito, T., Tozuka, Y. and Yamamoto, K.
"Solubility-dependent complexation of active pharmaceutical ingredients with trimethyl-β-cyclodextrin under supercritical fluid condition"
J. Incl. Phenom. Macrocycl. Chem. **57**, 289-295 (2007)

Mosher, G. and Thompson, D. O.
"Complexation and cyclodextrins"
Encyclopedia of Pharmaceutical Technology 3th edition, 624-627, 2006

Müller, R. H.
"Nanosuspensionen - eine neue Formulierung für schwerlösliche Arzneistoffe"
Pharmazeutische Technologie: Moderne Arzneiformen, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1998

Mura, P., Bettinetti, G. P., Manderioli, A., Faucci, M. T., Bramanti, G. and Sorrenti, M.
"Interactions of ketoprofen and ibuprofen with β-cyclodextrins in solution and solid state"
Int. J. Pharm. **166**, 189-203 (1998)

Mura, P., Faucci, M. T. and Bettinetti, G. P.
"The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-beta-cyclodextrin"
Eur. J. Pharm. Sci. **13** (2), 187-194 (2001)

Mura. P., Maestrelli, F., Cirri, M., Furlanetto, S. and Pinzauti, S.
"Differential scanning calorimetry as an analytical tool in the study of drug-cyclodextrin interactions"
J. Therm. Anal. Calorim. **73**, 635-646 (2003)

Nernst, W.
"Theorie der Reaktionsgeschwindigkeit in heterogenen Systemen"
Z. Phys. Chem., **47** (1), 52-55 (1904)

Noyes, A. A. and Whitney, W. R.
"The rate of solution of solid substance in their own solution"
J. Am. Chem. Soc. **19**, 930-934 (1897)

Nozawa, Y., Susuki, K., Sadzuka, Y., Miyagishima, A. and Hirota. S.
"Effects of shear rate in roll mixing on mechanical complex formation of ibuprofen with β-cyclodextrin"
Pharm. Acta Helv. **69**, 135-139 (1994)

Pace, S. N., Pace, G. W. Parikh, I. and Mishra, A. K.
"Novel injectable formulations of insoluble drugs"
Pharm. Technol. **3**, 116-134 (1999)

Pasquali, I., Bettini, R. and Giordano, F.
"Solid-state chemistry and particle engineering with supercritical fluids in pharmaceutics"
Eur. J. Pharm. Sci. **27**, 299-310 (2006)

Peng, D.-Y. and Robinson, D. B.
"A new two-constant equation of state"
Ind. Eng. Chem. Fund. **15**, 59-64 (1976)

Perrut, M., Jung, J. and Leboeuf, F.
"Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes: Part I: Micronization of neat particles"
Int. J. Pharm. **288** (1), 3-10 (2005)

Perrut, M., Jung, J. and Leboeuf, F.
"Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes: Part II: Preparation of composite particles"
Int. J. Pharm. **288** (1), 11-16 (2005)

Persson, E.
"Drug dissolution under physiologically relevant conditions in vitro and in vivo"
PhD thesis, University of Uppsala, Sweden (2006)

Pose-Vilarnovo, B., Rodríguez-Tenreiro Sánchez, C., Pérez-Marcos, M. B., and Torres-Labandeira, J. J.
"Sulphamethizole-cyclodextrin-hydroxy propylmethyl cellulose multicomponent complexes"
J. Therm. Anal. Calorim. **68**, 657-667 (2002)

Pouton, C. W.
"Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system"
Eur. J. Pharm. Sci. **29**, 278-287 (2006)

Reddy, N. M., Rehana, T., Ramakrishna, S., Chowdary, P. R. K. and Diwan, V. P.
"β-Cyclodextrin complexes of celecoxib: Molecular-modeling, characterization, and dissolution studies"
AAPS PharmSci. **6** (1), Article 7 (2004)

Rajewski, R. A. and Stella, J. V.
"Pharmaceutical applications of cyclodextrin. 2. In vivo drug delivery"
J. Phar. Sci. **85** (11), 1142-1169 (1996)

Saenger, W.
"Cyclodextrin inclusion compounds in research and industry"
Angew. Chem. Int. Ed. **19** (5), 344-362 (1980)

Salonen, J., Laitinen, L., Kaukonen, A. M., Tuura, J., Bjorkqvist, M., Heikkila, T., Vaha-Heikkila, K., M., Hirvonen, J. and Lehto, V-P.
"Mesoporous silicon microparticles for oral drug delivery: Loading and release of five of model drugs"
J. Control. Release **108**, 362-374 (2005)

Salonen, J., Paski, J., Vaha-Heikkila, K., Heikkila, T., Bjorkqvist, M. and Lehto, V-P.
"Determination of drug in porous silicon microparticles by calorimetry"
Phys. Stat. Sol. (a) **202** (8), 1629-1633 (2005)

Schneider, H.-J., Hacket, F., Rüdiger, V. and Ikeda, H.
"NMR studies of cyclodextrin and cyclodextrin complexes"
Chem. Rev. **98**, 1755-1785 (1998)

Serajuddin, A. T. M.
"Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs"
J. Pharm. Sci. **88** (10), 1058-1066 (1999)

Sharma, S., Sher, P., Badve, S. and Pawar, A. P.
"Adsorption of meloxicam on porous calcium silicate: Characterization and tablet formulation"
AAPS PharmSciTech **6** (4), Article 76 (2005)

Stegemann, S., Leveiller, F., Franchi, D., de Jong. H. and Linden, H.
"When poor solubility becomes an issue: Form early stage to poof concept"
Eur. J. Pharm. **31**(5), 249-261 (2007)

Stricker, H.
"Die in-vitro-Untersuchung der Verfügbarkeit von Arzneistoffen im Gastrointestinaltrakt."
Pharm. Ind. **31**, 794-799 (1969)

Szejtli, J.
"Introduction and general overview of cyclodextrin chemistry"
Chem. Rev. **98**, 1743-1753 (1998)

Takagi, T., Ramachandran, C., Bermejo, M., Yamashita, S., Yu, L. X. and Amidon, G. L.
"A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan"
Mol. pharmaceutics **3** (6), 631-643 (2006)

The Merck Index, 13th edition
Merck Research Laboratories, Rahway, NJ, USA (2001)

The United State Pharmacopiea, 24th edition
By authority of the United State pharmacopeial convention, Inc.; printed by national publishing: Philadelphia, PA, USA (2000)

The United State Pharmacopiea DI volume III, 24th edition
"Approved drug products and legal requirements"
Thomson Micromedex: Greenwood Village, CO (2004)

Türk, M., Hils, P., Helfgen, B., Schaber, K., Martin, H.-J. and Wahl, M. A.
"Micronization of pharmaceutical substances by the rapid expansion of supercritical solutions (RESS): a promising method to improve bioavailability of poorly soluble pharmaceutical agents"
J. Supercrit. Fluids **22** (1), 75-84 (2002)

Türk, M., Upper, G., Steuernhlater. M., Hussein, Kh. and Wahl. M. A.
"Complex formation of ibuprofen and β-cyclodextrin by controlled particle deposition (CPD) using SC-CO_2"
J. Supercrit. Fluids **39** (1), 435-443 (2007)

Uchida, H., Yoshida, M., Kojima, Y., Yamazoe, Y. and Matsuoka, M.
"Measurement and correlation of the solid–liquid–gas equilibria for the carbon dioxide + S(+)ibuprofen and carbon dioxide + S(±)-ibuprofen systems"
J. Chem. Eng. Data **50**, 11-15 (2005)

Valcarcel, M. and Tena, M. T.
"Applications of supercritical fluid extraction in food analysis"
Fresenius J. Anal. Chem. **358** (5), 561-573 (1997)

Valero, M., Perez-Revuelta, B. I. and Rodriguez, L. J.
"Effect of PVP K-25 on the formation of the naproxen:β-cyclodextrin complex"
Int. J. Pharm. **253**, 97-110 (2003)

Valero, M., Perez-Revuelta, B. I. and Rodriguez, L. J.
"Ternary naproxen:β-cyclodextrin: Polyethylene glycol complex formation"
Int. J. Pharm. **265**, 141-149 (2003)

Vallet-Regi, M., Ramila, A., del Real, R. P. and Perez-Pariente, J.
"A new property of MCM-41: Drug delivery system"
Chem. Mater. **13**, 308-311 (2001)

Van de Waterbeemd, H., Lennernäs, H. and Artursson, P.
"Drug bioavailability: Estimation of solubility, permeability, absorption and bioavailability"
Wiley-VCH, Weinheim, Germany (2003)

Van Hees, T., Barillaro, V., Piel, G., Bertholet, P., de Hassonville, S.,
Evrard, B. and Delattre, L.
"Application of supercritical carbon dioxide for the preparation of drug-cyclodextrin inclusion compounds"
J. Incl. Phenom. Macrocycl. Chem. **44**, 271-274 (2002)

Van Hees, T., Piel, G., Evrard, B., Otte, X., Thunus, L. and Delattre, L.
"Application of supercritical carbon dioxide for the preparation of a piroxicam-β-cyclodextrin inclusion compound"
Pharm. Res. **16** (12), 1864-1870 (1999)

Veiga, F., Fernandes, C. and Maincent, P.
"Influence of the preparation method on the physiochemical properties of tolbutamide/cyclodexrin binary systems"
Drug Dev. Ind. Pharm. **27**, 523-532 (2001)

Waleczek, K. J., Cabral Marques, H. M., Hempel, B. and Schmidt, P. C.
"Phase solubility studies of pure (-)-a-bisabolol and camomile essential oil with β-cyclodextrin"
Eur. J. Pharm. Biopharm. **55**, 247-251 (2003)

Wilson, C. G., Washington, N., Greaves, J. L., Kamali, F., Rees, J. A., Sempik, A. K. and Lampard, J. F.
"Bimodal release of ibuprofen in a sustained-release formulation: A scintigraphic and pharmacokinetic open study in healthy volunteers under different conditions of food intake"
Int. J. Pharm. **50**, 155-161 (1989)

Yazdanian, M., Briggs, K., Jankovsky, C. and Hawi, A.
"The "High Solubility" definition of the current FDA guidance on biopharmaceutical classification system may be too strict for acidic drugs"
Pharm. Res. **21** (2), 293-299 (2004)

York, P., Kompella, U. B. and Shekunov, B. Y.
"Supercritical fluid technology in pharmaceutical product development"
Marcel Dekker Inc, New York (2004)

CHAPTER 9

APPENDIX

9.1 Validation data of the quantitative determination of the ibuprofen by HPLC

	Conc. [mg/100 ml]	Conc. [mg/100 ml]	Conc. [mg/100 ml]	Conc. [mg/100 ml]
	0.5	1.0	2.0	3.0
	0.482	1.041	1.912	2.963
	0.483	1.048	1.912	2.970
	0.486	1.054	1.891	2.990
	0.487	1.021	1.887	2.938
	0.486	1.093	1.896	3.053
	0.486	1.033	1.890	2.957
n	6	6	6	6
Mean [mg/100 ml]	0.485	1.048	1.898	2.978
SD	0.002	0.025	0.012	0.040
RSD [%]	0.423	2.375	0.610	1.350
Mean RSD	1.19 %			
Recovery [%]	96.34	104.06	95.61	98.7
Mean recovery	98.69 %			

9.2 Index of suppliers

Air Liquide Deutschland GmbH, Hans-Günter-Sol-Str. 5, D-40235 Düsseldorf

Bandelin electronic GmbH & Co. KG, Heinrich-Str. 3-4, D - 12207 Berlin

BASF AG, BASF AG, Carl-Bosch-Str. 38, D-68056 Ludwigshafen

Beckmann Coulter GmbH, Europark Fichtenhain B13, D-47807 Krefeld

Bio-Rad Laboratories GmbH, Heidemann-Str. 164, D-80939 München

BioTek Instruments GmbH, Kocherwald-Str. 34, D-74177 Bad Friedrichshall

Carl Roth GmbH & Co. KG, Schoemperlen-Str. 1-5, D-76185 Karlsruhe

Carl-Zeiss GmbH, Carl-Zeiss-Str., D-72447 Oberkochen

Colorcon Ltd., Dartford, GB-Kent

Eppendorf AG, Barkhausenweg 1, D-22331 Hamburg

Erweka GmbH, Otto-Str. 20-22, D-63150 Heusenstamm

FINNA-AQUA Santasalo-Sohlberg GmbH, Kalscheurener-Str. 92, D-50354 Hürth

Fisher Scientific GmbH, Im heiligen Feld 17, D-58239 Schwerte

FMC Biopolymer, Wallingston, Little Island, IRL-Cork

Fluka (Sigma-Aldrich Chemie GmbH)

GraphPad Software, 11452 EI Camino Real 215, San Diego, CA, USA

Haraeus Holding GmbH, Haraeus-Str. 12-14, D-63450 Hanau

Knick Elektronische Messgeräte GmbH & Co., Beucke-Str. 22, D-14163 Berlin

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