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IMPROVEMENT OF THE SOLUBILITY AND DISSOLUTION RATE OF NIFLUMINIC ACID TO ACHIEVE RAPID DRUG RELEASE

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Publications

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ABBREVIATIONS

BA	Bioavailability
BCS	Biopharmaceutical Classification System
BE	Bioequivalence
CDs	Cyclodextrins
DSC	Differential scanning calorimetry
FT-IR	Fourier transform infrared spectroscopy
HP-β-CD	2-Hydroxypropyl-β-cyclodextrin
HSM	Hot-stage microscopy
K	Drug release rate
K_d	Diffusion constant
K_p	Partition coefficient
KPs+PVP	Ternary kneaded products
KPs	Kneaded products
M_w	Molecular weight
NIF	Nifluminic acid
PMs+PVP	Ternary physical mixtures
PMs	Physical mixtures
PVP	Poly(vinylpyrrolidone)
SD	Standard deviation
SGM	Simulated gastric medium
SIM	Simulated intestinal medium
SPDs C-15	Spray-dried products
SPL	Simulated plasma
SPs K-25	Solvented products
T_g	Glass transition temperature
USs	Ultrasonicated systems
UV	Ultra violet spectroscopy
XPRD	X-ray powder diffractometry

1. INTRODUCTION

Efforts to innovate existing medication include the development of medicines with higher selectivity of action, less toxicity and side-effects, higher stability, a more favourable pharmacokinetic profile and improved patient compliance. Modern pharmaceutical technology is concentrated on new drug forms which are targeted to the exact site at the appropiate time, with maximum efficiency and with reduced side-effects.

The solubility properties of drugs and the dissolution of the active substance from dosage forms have a basic impact on the bioavailability of the product. Generally, only the dissolved pharmacon is able to absorb and the dissolution rate greatly affects the rate of transport processes if the dissolution is the slowest step in the LADMER system. Enhancement of the solubility of poorly-soluble drug substances is one of the most important tasks in pharmaceutical technology. With new material drug carriers and new technological processes, it is possible to achieve this.

Complexation is one of several ways to favourably enhance the physicochemical properties of pharmaceutical compounds. It may loosely be defined as the reversible association of substrate and ligand to form a new species. Cyclodextrins (CDs) are classical examples of compounds that form inclusion complexes. These complexes are formed when a ”guest„ molecule is partially or fully incorporated into the cavity of a ”host„ molecule. When inclusion complexes are formed, the physicochemical parameters of the guest molecule are disguised or altered, and improvements in the solubility, stability, taste, safety and bioavailability of the molecule are commonly seen.

Pharmaceutical solid dispersion technology is generally accepted as a technique with which to enhance the dissolution characteristics of drugs with poor water solubility. For this purpose, the drug substance is dispersed in a water-soluble inert polymer matrix; at the higher surface area due to the presence of the polymer, sometimes the drug solubility and dissolution rate may increase.

This thesis is based on investigations of an anti-inflammatory drug nifluminic acid (NIF), [1]with poor water solubility, to apply these technological procedures so as to increase its solubility and dissolution rate.

References to the author's own articles are gave by Roman numbers.

2. BIOPHARMACEUTICAL DESIGN AND EVALUATION OF DRUG PRODUCTS

2.1. LADMER system

A drug product is a substance-carrier system subjected to a special manufacturing process in order to achieve the required therapeutic effect with the necessary dose of the drug incorporated in a useful dosage form. It is qualified by its physicochemical properties and the resulting concentration in the fluids of the body. It is very important that the drug should be liberated, absorbed, distributed and eliminated at the correct time. As shown in Fig. 1, this process is characterized by the LADMER system **[1]**.

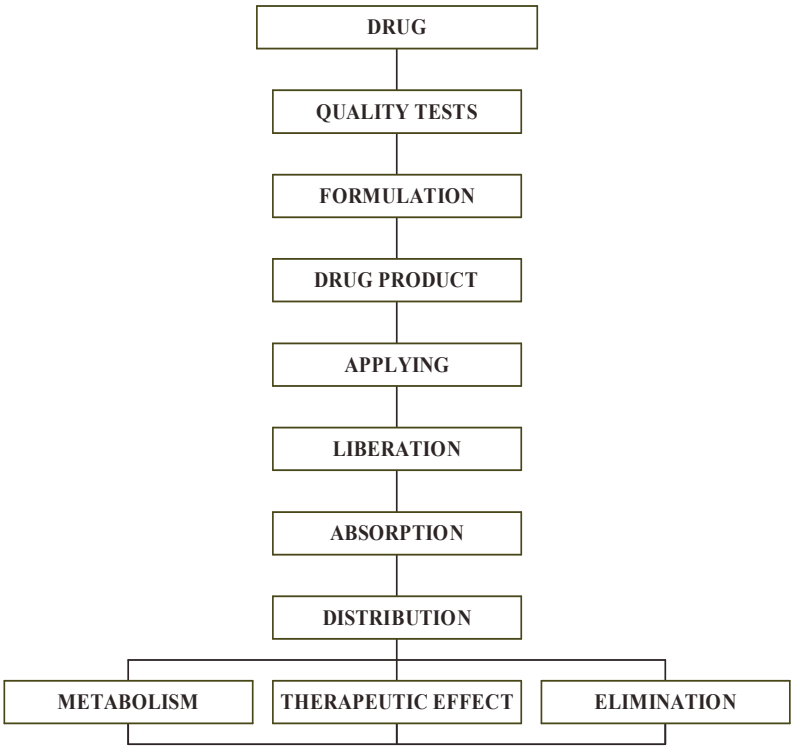

Fig. 1. Scheme of the drug's life during the LADMER system

2.2. Bioavailability, bioequivalence and biopharmaceutical classification system

The bioavailability (BA) and bioequivalence (BE) of drug products, and drug product selection, have emerged as critical issues in pharmacy and medicine during the last three decades **[2, 3]**. The availability of different formulations of the same drug substance given at the same strength and in the same dosage form poses a special challenge to health-care professionals. These issues are very relevant to pharmacists, as they play an important role in

product-selection decisions, making it necessary to know the principles and concept of BA and BE.

The regulatory BE requirements of drug products have recently undergone major changes. The introduction of the biopharmaceutical classification system (BCS) into the guidelines of the Food and Drug Administration (FDA) was a major step forward to classification of the biopharmaceutical properties of drugs and drug products. Today, many molecules are classified through screening processes, and promising candidates enter the drug pipeline for further *in vitro* and *in vivo* tests. Drug absorption from a solid dosage form after oral administration depends on the release of the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance.

On the basis of their solubility and permeability, drugs can be divided into high/low solubility-permeability classes, as shown in Table I.

Table I. Biopharmaceutical classes [4]

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

The BCS defines three dimensionless numbers. The dose number is defined as the ratio of dose concentration to drug solubility. The dissolution number is the ratio of the residence time to the dissolution time. The absorption number is the ratio of the permeability and the gut radius times the residence time in the small intestine. In the case of class II, there are drugs for which the dissolution profile must be most clearly defined and reproducible. More precisely, this is the class where the absorption number is high and the dissolution number is low. Drugs in class IV present significant problems for effective oral drug delivery. The BCS is utilized to set drug product dissolution standards so as to reduce the *in vivo* BE requirements. The knowledge of the BCS characteristics of a drug in formulation can also be used to develop a more optimized dosage form based on fundamental mechanistic features **[4–6]**.

2.3. The main effects of pharmaceutical technology factors on bioavailability and bioequivalence

Before the therapeutic effect of an orally administered drug can be achieved, the drug must be absorbed. The systemic absorption of an orally administered drug in a solid dosage form is comprised of three basic steps:

- disintegration of the medicine;
- dissolution of the drug in the fluids at the absorption site;
- transfer of the drug molecule across the membrane lining the gastrointestinal tract into the systemic circulation.

The physical and chemical characteristics of a drug, and its formulation, are of prime importance as concerns its BA, because they can affect not only the absorption characteristics of the drug, but also its stability **[7]**.

Formulation factors that affect BA and BE may be broadly classified into three main categories (Table II) **[8]**.

Table II. Technological factors affecting BA/BE

Technological factors		
Properties	**Processing stresses**	**Manufacturing procedures**
Particle size Surface area Solubility Dissolution Partition coefficient Ionization constant Crystal properties Stability Organoleptic properties Others	Pressure Mechanical Radiation Exposure to liquids Exposure to gases and liquid vapours Temperature	Precipitation Filtration Emulsification Mixing Milling Granulation Drying Compression Autoclaving Crystallization Handling Storage Transport

Alteration of the physicochemical properties of the active ingredient is one of the most useful possibilities to improve its BA.

2.4. Dissolution process

The solubility properties of drugs and the dissolution of the active substance from dosage forms are of basic importance as regards the BA of the product. Generaly only, the dissolved

pharmacon is able to absorb, and the dissolution rate greatly affects the rates of transport processes if the dissolution is the slowest step in the LADMER system.

2.4.1. In vitro *drug release model*

As it is shown in Fig. 2, the drug-release property of a solid dosage form may be characterized by two subprocesses: the liberation of the drug particles from the dosage form and the dissolution of the drug from the liberated drug particles. It is assumed that the dissolution of the drug from the surface of the intact dosage form is negligible.

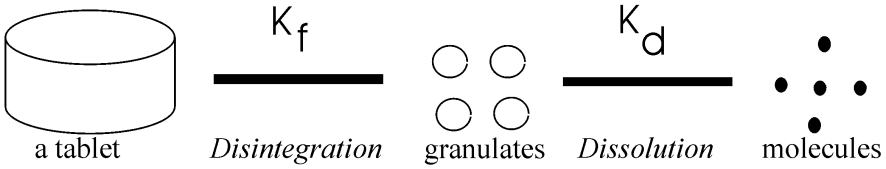

Fig. 2. Scheme of disintegration and dissolution processes

The disintegration is considered to be a first-order process, and the dissolution from the drug particles is proportional to the concentration difference between the particle surface and the bulk solution. The factors influencing the liberation of drug particles from dosage forms include formulation and processing factors. The disintegration involves the effects of the formulations and manufacturing process variables, whereas the dissolution from the drug particles mainly involves the effects of solubility and particle size.

During preformulation studies, it is possible to change the characteristics of the dosage form and/or alter the physicochemical properties of the drug. As a general rule, if a drug substance has an aqueous solubility of less than 10 mg/ml, dissolution is the rate-limiting step in the process of drug absorption. The *Noyes-Whitney* equation describes the factors influencing drug release:

$$\frac{dC}{dT} = \frac{A \cdot D \cdot (C_s - C)}{h} \qquad \textbf{(Eq. 1)}$$

where dC/dT is the rate of dissolution; A is the surface area accessible to the dissolution medium; D is the diffusion coefficient of the drug; C_s is the solubility of the drug in the dissolution medium; C is the concentration of the drug in the medium at time t; and h is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving drug.

According to this equation, the main possibilities for *improving the dissolution rate* are to increase the surface area by decreasing the particle size of the solid drug; *to increase* the drug

diffusion coefficient by selecting a suitable carrier; and *to increase its solubility* by changing its physical and/or chemical structure. The molecules of a crystalline drug form a compact crystal with high lattice energy and consequently low solubility. However, amorphous drugs have high solubility **[9]**.

2.4.2. Technologies improving the solubility and dissolution rate of poorly water-soluble drugs

Aqueous solubility is one of the most important physicochemical properties. It is believed that a drug has to be in solution to be absorbed. From the aspect of pharmaceutical development, the solid state form is another important factor that affects the solubility, the dissolution rate and eventually the developability. The solid state form to some extent determines the physicochemical stability, properties and formulation stability; these factors should be carefully examined and optimized. In some cases, changes in crystallinity due to different chemical processes result in large differences in BA when the drug is delivered by a solid dosage formulation **[10]**.

There are several technological procedures via which to increase the solubility and dissolution rate of substances poorly soluble in water (Table III).

Table III. Possibilities of technological methods

Chemical modification	prodrugs
	salts
Physical modification	particle size
	crystal habit
	polymorphs
	pseudopolymorphs/solvates
	complexation/solubilization
	dispersion into carriers
Manufacturing methods	precipitation
	emulsification
	mixing
	milling
	granulation
	drying
	compression
	crystallization

The methods can be classified in three main groups: chemical, physical and manufacturing methods. The production of soluble prodrugs or salts involves chemical methods which lead to governed solubility **[11]**. A frequently used physical method is micronization, but different

polymorphic forms of drugs, complexation with CDs **[12]** and other auxiliary materials or the preparation of solid dispersion systems with inert, water-soluble carriers, *etc*. may also be used **[13, 14]**. Most of the processing steps depend at least indirectly upon the physicochemical properties of the drug. Particle size, shape and morphology are often determined by the solid form of the drug and the conditions under which the drug is crystallized. Particle formation and the design of solid particles and powdery composites with unique properties are currently areas of major development for the application of supercritical fluids **[IV]**. At the focus of supercritical fluid technology, particle size reduction results in an increase in the specific surface of powders **[15]**. The drug dissolution rate, absorption rate, dosage form, content uniformity and stability are all dependent to varying degrees on the particle size, the size distribution and the interactions of solid surfaces. The fine material obtained by nanotechnology dissolves a higher rate, which can lead to improved drug absorption by passive diffusion **[16–18]**. Processing can also result in changes in the form of the drug. Manufacturing (*e.g.* milling, lyophilization, granulating and drying) may introduce a certain level of amorphous structure to an otherwise highly crystalline material **[19, 20]**. The amorphous state may also be introduced deliberately to enhance the biopharmaceutical properties of the product. For example, for a crystalline drug with very poor aqueous solubility, the formation of a coamorphous mixture with a water-soluble additive can provide an opportunity to enhance dissolution and perhaps BA **[21, 22]**.

3. CYCLODEXTRINS AND COMPLEXATION PHENOMENA

The CDs are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose containing a relatively hydrophobic central cavity and a hydrophilic outer surface. They were discovered in 1891 when *Villiers* observed crystallization occurring in a bacterial digest of starch **[23]**. Fifteen years later, *Schardinger* **[24]** studied those microorganisms which play a role in the deterioration of foods, and isolated *Bacillus macerans*, which reproducibly produced two distinct crystalline substances when cultivated on starch-containing medium. As most of their properties were similar to those of the already known partial degradation products of starch, the dextrins, he named them α- and β-dextrin **[25–27].** The first patent was taken out composed by *Freudenberg*, *Cramer* and *Pleninger* in 1953 **[28]**. The drug-solubility-increasing effects of the CDs were investigated systematically by *Lach* and coworkers **[29–31]** and the first publications on formulation experiments were those of *Frömming* **[32, 33]**.

3.1. Properties of crystalline cyclodextrins and cyclodextrin derivatives

The parent or natural CDs consist of 6, 7 or 8 glucopyranose units and are referred to as α-CD, β-CD and γ-CD, respectively. Of the higher homologues, only the nine-membered ring, δ-CD, has been characterized satisfactorily, but it is not produced industrially and nothing is known about its practical utility. As a consequence of the C1 conformation of the glucopyranose units, all the secondary hydroxy groups are situated on one of the two edges of the ring, and all the primary hydroxy groups on the other. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges. The nonbonding electron pairs of the glycosidic oxygen bridges are directed towards the inside of the cavity, producing a high electron density and lending it some *Lewis*-base character. Owing to the lack of free rotation around the bonds connecting the glucopyranose units, the CDs are not perfectly cylindrical molecules, but are toroidal or cone-shaped **[34]**. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity. No covalent bonds are formed or broken during drug-CD complex formation, and in aqueous solution the complexes readily dissociate and free drug molecules remain in equilibrium with the molecules bound within the CD cavity **[35]**. Table IV lists the characteristics of α-CD, β-CD, γ-CD and hydroxypropyl-β-CD (HP-β-CD).

Table IV. Characteristics of CDs [35]

	α-CD	**β-CD**	**γ-CD**	**HP-β-CD**
No. of glucose units	6	7	8	7
Mol. wt. (M_w)	972	1135	1297	1193
Solubility in water (g/100 ml)	14.5	1.85	23.2	>50
Cavity diameter (nm)	0.47–0.53	0.60–0.65	0.75–0.83	0.78
Height of torus (nm)	0.79±0.01	0.79±0.01	0.79±0.01	–
Crystal water contant (%)	10.2	13.2–14.5	8.13–17.7	1–2
Diffusion constant (40 ºC)	3.44	3.22	3.00	–
pK (25 ºC)	12.33	12.20	12.08	–

The main reasons for the chemical modification of basic CDs are to the alter of undesirable physicochemical properties and to reduce parenteral toxicity in order to extend the pharmaceutical applications. Suitable derivatives have to satisfy the following requirements: high aqueous solubility accompanied by a lack of toxicity, while retaining or improving the complexing ability. The derivatives can be prepared by chemical or enzymatic reactions, and accordingly the substitution group can be classified as alkylated, hydroxyalkylated, esterified, branched, ionic or polymerized derivatives **[35, 36]**.

On the reaction of β-CD in alkaline solution with propylene oxide, a 2-hydroxypropyl group will be connected to one or more hydroxy groups of the β-CD, or to the hydroxy groups of the 2-hydroxypropyl groups already linked to the β-CD molecule (Fig. 3). HP-β-CD is very soluble in water. Substitution of the hydroxy groups of β-CD disrupts the network of hydrogen bonding around the rim of β-CD. As a result of this disruption, the hydroxy groups interact much more strongly with water, resulting in an increased solubility as compared with β-CD **[35, 37]**.

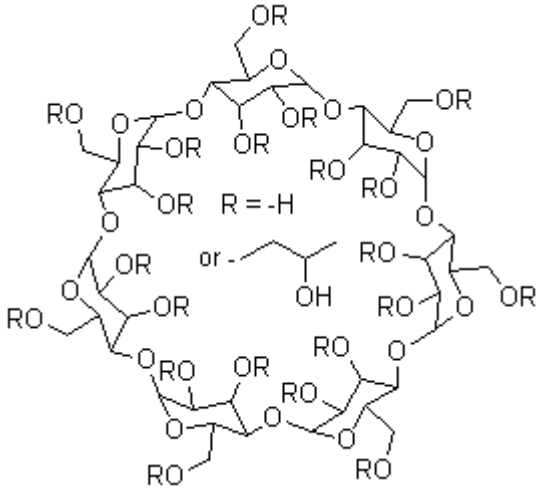

Fig. 3. Structure of HP-β-CD

3.2. Inclusion complexation

Inclusion complexes are entities comprising two or more molecules, in which one of the molecules, the host includes a guest molecule, totally or in part, only by physical forces. CDs are typical host molecules and may include a great variety of molecules having the size of one or two benzene rings, or even larger ones which have a side chain of comparable size, to form crystalline inclusion complexes (Fig. 4)**[35, 38]**. A variety of non-covalent forces, such as *van der Waals* forces, hydrophobic interactions, dipole moments and other forces, are responsible for the formation of stable complexes.

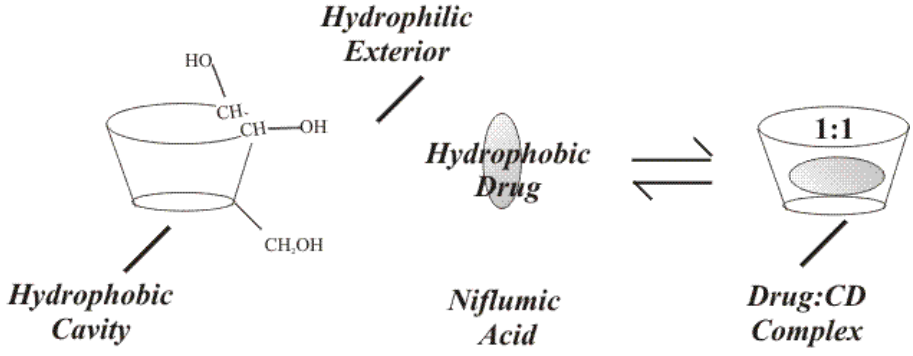

Fig. 4. Complexation of drug inside the hydrophobic cavity **[39]**

The included molecules are normally oriented in the host in such a position as to achieve the maximum contact between the hydrophobic part of the guest and the apolar CD cavity. The hydrophilic part of the guest molecule remains, as far as possible, at the outer face of the complex. This ensures maximum contact with both the solvent and the hydroxy groups of the host.

There are several characterization methods to demonstrate the complexation phenomena. Thermoanalytical methods such as thermal gravimetry, differential thermal gravimetry, differential thermal analysis and differential scanning calorimetry (DSC) can be applied in the cases of complexes which contain a guest substance having a melting or boiling point below the thermal degradation range of the CD or which are volatile in the temperature range 60–250 °C because CDs lose their water content below 100 °C, and begin to decompose over 250 °C. Complex formation may be demonstrated by solid-state spectroscopic methods too, such as infrared (IR), spectroscopy, nuclear magnetic resonance and X-ray powder diffractometry (XRPD). The IR spectroscopy may be used in some cases, but this method is of limited use in the investigation of CD inclusion complexes. The results relating to the particle size distribution, wettability and dissolution properties reveal the morphological and physical changes between the pure drugs and their inclusion complexes **[40, 41]**.

3.3. Factors influencing inclusion complex formation

CDs and their derivatives have received considerable attention in the pharmaceutical field and an increased number of reviews have been dedicated to their industrial and pharmaceutical applications (Fig. 5)**[42–46]**.

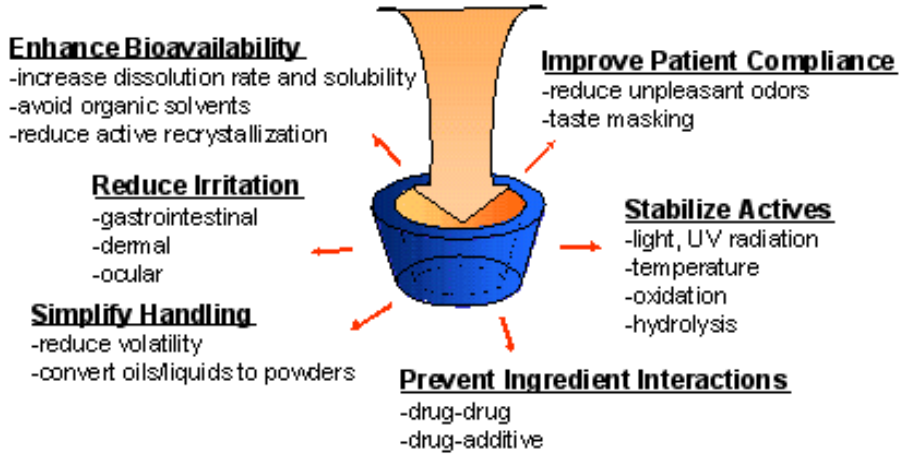

Fig. 5. Multiple benefits exist for CD complexes in pharmaceutical formulations **[47]**

A number of factors play important roles in inclusion complex formation. The type of the CD can influence the formation and also the performance of drug/CD complexes. In many cases, there are differing effects of CDs on the formulation, depending on the cavity size, the degree of substitution, M_w and the solubility **[48–52]**. In the case of ionizable drugs, the presence of the charge may play a significant role in complexation and hence a change in the solution pH can vary the complex constant. In general, ionic forms of drugs are weaker complex-forming agents than their non-ionic forms **[53–55]**.

The methods of preparation (*e.g.* co-grinding, kneading, solvent evaporation, spray-drying or freeze-drying), temperature changes and the effects of various additives are important factors in complexation processes **[56–64]**. The addition of small amounts of water-soluble polymers to an aqueous CD solution, together with heating, increases the CD complexation of lipophilic water-insoluble drugs. The polymers not only increase complexation, but also increase the drug availability in the CD-containing drug formulation **[65–68]**. The effects of HP-β-CD and poly(vinylpyrrolidone) (PVP)-K30 on the solubility of naphthoquinone were investigated by *Granero at al.* **[69]**, where PVP-K30 increased the solubilizing effect of HP-β-CD by enhancing the apparent stability constant of the drug:HP-β-CD complex. The addition of 0.5% (w/v) PVP-K30 to the complexation medium resulted in an 83% increase in the stability constant of the complex. *Patel* and *Vavia* **[70]** combined the polymer and the CD, and the combination was clearly more effective in enhancing the aqueous solubility of fenofibrate in comparison with the corresponding drug-cyclodextrin or drug-polymer binary systems. Hydrophilic polymers increased the complexation efficacy of CD towards fenofibrate (as shown by the increased stability constants of the complexes). PVP was found to be most effective in enhancing the solubilization of fenofibrate by β-CD. The best results were obtained in the ternary system with β-CD in presence of 1% (w/v) PVP. The formulated ternary system with an optimized drug:CD:polymer ratio of 1:3.5:1 (w/w) resulted in a significant improvement in the rate of dissolution of fenofibrate and showed 90% dissolution efficiency as compared with around 15% and 83% for the plain drug and the binary system, respectively. The combined effect of HP-β-CD and PVP on the solubility of naproxen was studied by *Mura et al.* **[71]**. Equimolar naproxen:HP-β-CD solid systems, in the presence or the absence of 15% (w/w) PVP, were prepared by cogrinding, kneading, coevaporation or freeze-drying. The combined use of PVP and HP-β-CD resulted in a synergistic increasing effect on the aqueous solubility of the drug (120 times that of the pure drug).

Skiba et al. set out to investigate the possibility of improving the dissolution rate of progesterone/β-CD binary systems via formation of ternary complexes with the hydrophilic polymer, poly(ethylene glycol) (PEG) 6000 **[72]**. The results proved that progesterone was

diffused into the CD cavity, replacing the water molecules, and in the ternary system, the progesterone β-CD was well dispersed into the PEG, this improving the BA of progesterone for subsequent oral delivery in the same way as derivatized CDs.

3.4. Effects of cyclodextrins on important drug properties in formulations

Inclusion complex formation of drugs with CDs results in alterations in physicochemical properties such as solubility, dissolution rate, membrane permeability and chemical reactivity. This ability, combined with the favourable properties of special CD derivatives with respect to aqueous solubility and toxicity, has yielded considerable pharmaceutical potential.

CDs enhance the BA of insoluble drugs by increasing the drug solubility, dissolution, and/or permeability **[73–80]**. In the case of water-soluble drugs, CDs increase the drug permeability by direct action on the mucosal membranes, and enhance the drug absorption and/or BA **[81]**. Labile drug stabilization by CDs, and their ability to ameliorate drug irritation and thus improve drug contact time at the absorption site in nasal, ocular, rectal and transdermal delivery, are other important factors that contribute to the CD-improved BA **[82, 83]**.

CDs have been used to decrease the irritation caused by drugs. The increased drug efficacy and potency (*i.e.* reduction of the dose required for optimum therapeutic activity) caused by CD-increased drug solubility may reduce drug toxicity by making the drug effective at lower doses **[84–86]**. CDs can improve the stability of various labile drugs against dehydration, hydrolysis, oxidation and photodecomposition, and thus increase the shelf-life of these drugs. The stabilizing effect of CDs depends on the nature and effect of the included functional group on the drug stability and the nature of the vehicle **[87–90]**.

4. SOLID DISPERSION SYSTEMS

4.1. Solid dispersion technology: an attractive alternative

The concept of preparation of solid dispersions to improve the dissolution rate of sparingly soluble drugs has been widely explored since 1961 **[91]**. *Chiou* and *Riegelman* defined the term solid dispersion as “a dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting, solvent or melting-solvent method” **[92]**. There are several approaches to formulate solid dispersions; the effects of the processing conditions on the physicochemical properties of the overall formulation and the exact physical state of

the drug that is dispersed throught the carrier are yet to be ascertained. The nature of the drug dispersion, *i.e.* molecular, phase segregation, amorphous, crystalline and the presence of polymer, can influence the overall free energy and the physical stability of the product. To understand the mechanisms of release of a drug from a solid dispersion, it is important to know their physicochemical structure.

Six representative structures of interactions between the carrier and the drug have been outlined:

1. simple eutectic mixtures
2. solid solutions
3. glass solutions and glass suspensions
4. amorphous precipitations in a crystalline carrier
5. compound or complex formation
6. combinations **[93]**.

The formulation of solid dispersions is beneficial for a number of reasons: the drug substance is often obtained in the amorphous state, meaning that crystal lattice forces have already been overcome; in cases in which a solid solution or molecular dispersion is formed, the theoretical drug particle size is reduced to the smallest size possible, *i.e.* to the isolated, dissolved molecules; and based on an appropriate selection of the drug carrier, the wettability is increased and a microenvironment conducive to dissolution is generated **[94–97]**.

4.2. Preparation of solid dispersions

The two basic procedures used to prepare solid dispersions are the melting and solvent techniques. *Sekiguchi* and *Obi* **[91]** reported first the melting method, when a physical mixture (PM) of an active agent and a water-soluble carrier is heated until it is melted. The melt is solidified rapidly in an ice bath, pulverized and then sieved. An important prerequisite for the manufacture of solid solutions by the hot melt method is the miscibility of the drug and the carrier in the molten form. When there are miscibility gaps in the phase diagram, this usually leads to a product that is not molecularly dispersed. Another important limitation to the hot melt method is the thermostability of the drug, which may decompose or evaporate. Of course, oxidative reactions can be avoided by processing in an inert atmosphere or under vacuum, while evaporation can be avoided by processing in a closed system.

Tachibana and *Nakamura* were the first to dissolve both the drug and the carrier in a common solvent and then evaporate the solvent under vacuum to produce a solid dispersion **[98]**. An important prerequisite for the manufacture of a solid dispersion using the solvent

method is that both the drug and the carrier are sufficiently soluble in the solvent. The solvent can be removed by any one of a number of methods. Temperatures used for solvent evaporation usually lie in the range 23–65 °C and the solvent can also be removed by freeze-drying or by spray-drying **[99–101]**. With the discovery of the solvent method, many of the problems associated with the melting method were solved. Many polymers that could not be utilized for the melting method due to their high melting range (*e.g.* PVP) could now be considered as carrier possibilities. As a result, for many years the solvent method was the method of choice for polymer-based systems.

4.3. Polyvinylpyrrolidone as carrier

The polymerization of vinylpyrrolidone leads to polyvinylpyrrolidone (PVP) with M_w ranging from 2 500 to 3 000 000. These can be classified according to the *K* value. The glass transition temperature (T_g) of a given PVP is dependent not only on its M_w, but also on the moisture content. In general, the T_g is high and for this reason PVPs have only limited application for the preparation of solid dispersions by the hot-melt method. Due to their good solubility in a wide variety of organic solvents, they are particularly suitable for the preparation of solid dispersions by the solvent method. They have good water solubility and can improve the wettability of dispersed compounds in many cases **[102]**. The chain length of the PVP has a very significant influence on the dissolution rate of the dispersed drug from the solid dispersion. The aqueous solubility of the PVPs becomes poorer with increasing chain length, and a further disadvantage of the high M_w PVPs is their much higher viscosity at a given concentration **[103]**. Solid dispersions prepared with high proportions of PVP tend to exhibit higher drug solubilities and release rates than those with high proportions of drug **[104, 105]**. Most studies of PVP solid dispersions reported in the literature have used PVPs of M_w of 2 500–50 000. Above 50 000, the aqueous solubility of PVP decreases and it has a much higher viscosity at a given concentration, which can be used for controlled release. The aim of the preparation of PVP solid dispersions is generally to transform the drug into the amorphous form and thus to achieve faster dissolution. Dispersions containing crystalline areas exhibit biphasic release profiles, with the amorphous areas dissolving quickly and the crystalline areas more slowly. The amorphous state may also be introduced deliberaterly to enhance the biopharmaceutical properties of the product. For example, for a crystalline drug with a very poor aqueous solubility, the formation of an amorphous mixture with a water-soluble additive can provide an opportunity to enhance the dissolution and perhaps BA **[106]**.

4.4. Characterization of solid dispersions

Whether or not a solid dispersion results in an improved dissolution rate can be investigated by using the dissolution test. Thermal analysis methods such as DSC can be used to investigate the thermal stability and T_g of the materials used. Lack of a melting peak in the DSC recording of the solid dispersion indicates that the drug is present in an amorphous form rather than a crystal form. This result can be confirmed by the X-ray pattern. Crystalline molecules of a drug form a compact crystal with high lattice energy and consequently low solubility as compared with the amorphous drug. IR spectroscopy is used to investigate the drug and polymer interaction, which influences the drug release mechanisms. Physical adsorption is due to electrostatic interactions, hydrogen bonding or *van der Waals* forces and is usually reversible, while in chemical bonds, including ion exchange, protonation and complexation, it is irreversible. The extent of adsorption depends on the physicochemical properties of both the drug and the excipient **[107]**.

5. EXPERIMENTAL AIMS

The aims of the present work were as follows.

In view of the poor water-solubility of a pharmaceutical ingredient, NIF, my aim was to increase its solubility and dissolution rate by applying different formulation methods.

The first aim of the study was to prepare *binary* and *ternary CD complexes* at several mole and mass ratios and via three complexation methods.

The second aim was to examine *solid dispersion systems* with solvent evaporation processes, such as vacuum- and spray-drying technologies.

The third aim was to investigate the characteristic physicochemical properties, biopharmaceutical behaviour for solubility and dissolution rate and structural characteristics of the samples on the basis of the following:

1. Preformulation studies on the products
    a. Recording the contact angles to establish their wettability
    b. Determination of the *n*-octanol/water partition coeffitient to predict their permeability features
    c. Investigation of the saturation concentration in water to determine their solubility
    d. Analysis of the particle size
2. *In vitro* investigations
    a. Determination of the solubility and the rate of dissolution of the drug in simulated media
    b. Determination of the membrane diffusion
3. Structural evaluations
    a. Study of the *Fourier* transform-IR spectra, XRPD and thermal analysis (DSC and hot-stage microscopy (HSM))

6. MATERIALS

6.1. Active substance: Nifluminic acid (NIF)

NIF: 2-[[3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid
(G. Richter Ltd., Hungary)
Chemical structure (see Fig. 6)

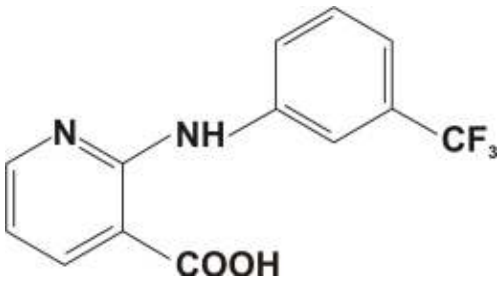

Fig. 6. Chemical structure of NIF

Molecular formula: $C_{13}H_9F_3N_2O_2$

M_w: 282.23

Melting point: 205 ºC

Original name: Donalgin®

Description: a yellow, fine powder **[108]**

NIF, an anthranilic acid derivative, is a frequently used anti-inflammatory drug, which also has a weak analgetic effect. It is primarily used to treat different forms of rheumatism, *e.g.* rheumatoid arthritis and arthrosis, and to decrease other inflammatory phenomena. The usual single dose is 250 mg of NIF for adults, generally in capsules (*e.g. Donalgin® capsule*, G. Richter Ltd., Budapest, Hungary). It has some side-effects, such as nausea or vomiting. In cases of stomach ulcer, it may only be used under medical control **[109]**. Three h after a 250 mg dose administered to 6 male volunteers as the first dose on day 10 of a 14-day 250 mg, 4 times daily dosage regimen, the mean peak plasma concentration of radiolabelled NIF was 123 µg/ml **[110, 111]**. According to the BCS, NIF can be considered a class II compound, *i.e.* a water-insoluble, lipophilic and highly permeable compound **[112]**. Since NIF is also widely prescribed for mild illnesses, the safety aspect becomes central and efforts should be made to optimize the overall drug pharmacological profile. *Iervolino* and co-workers applied CDs in 1:1 ratio, using three methods to reduce the gastric toxicity of NIF and could improve its safety profile **[113].** This thesis focused on the other possibilities of formulation of the drug.

6.2. Auxiliary materials

Cyclodextrins:

- α-CD, β-CD, γ-CD, hydroxybutenyl-β-CD (HB-β-CD), 2-hydroxypropyl-β-CD (HP-β-CD), heptakis-2,6-di-*O*-methyl-β-CD (DIMEB), and randomly methylated-β-CD (RAMEB) (Cyclolab R&D Laboratory Ltd., Hungary); Captisol® (Cydex, Inc., USA).

Other materials:

- PVP: C-15 (M_w ~ 8 000), K-25 (M_w ~ 34 000), C-30 (M_w ~ 58 000) (C/o ISP Customer Service GmbH, Germany); K-90, M_w: 1 300 000 (Pharmacopoeia Hungarica 7th Edition)
- Other chemicals, such as acetone, ethanol and methanol, are official in the Ph.Hg. VIII (Spektrum 3D Ltd., Budapest, Hungary).

7. METHODS

7.1. Preliminary experiments

The effects of the different CD derivatives on the solubility properties of NIF were determined. For this purpose, a mixture of 30 mg of NIF and 50 mg of CD derivative (α-CD, β-CD, γ-CD, DIMEB, RAMEB, HB-β-CD, HP-β-CD or Captisol®) was mixed with water to 20.0 g and stirred for 20 min with a magnetic mixer. The suspension systems were filtered through filter paper, and after suitable dilution their UV spectra were recorded by Unicam UV2/VIS spectrometer (Unicam Ltd., England). A system without the CD was used as control. HP-β-CD exerted the highest solubility-increasing effect on NIF. This CD derivative was therefore chosen for further examinations (Table V).

Table V. Influence of CD derivatives on the UV spectrum of NIF

NIF+CD derivative	Absorbance	Increase (fold)
NIF	0.461	1.00
NIF + α-CD	0.526	1.14
NIF + β-CD	0.568	1.23
NIF + γ-CD	0.774	1.67
NIF + RAMEB	0.861	1.86
NIF + DIMEB	0.949	2.05
NIF + HB-β-CD	1.018	2.20
NIF + HP-β-CD	1.124	**2.43**

To determine the effects of water-soluble polymers on the complexation phenomena, a mixture of 0.03 g of NIF, 0.20 g of HP-β-CD and 0.20 g PEG or 0.20 g PVP was mixed with water to 20.0 g and stirred for 20 min with a magnetic mixer. The suspension systems were filtered through filter paper and (after suitable dilution) their UV spectra were recorded in the range 230–400 nm. The best solubility properties were detected in the case of the NIF–HP-β-CD–PVP system.

7.2. Preparation of the sample

7.2.1. Cyclodextrin binary complexes

The products were prepared in four different mole ratios (NIF:CD mole ratio = 2:1, 1:1, 1:2 and 1:3). *Physical mixtures* (**PMs**): The plain drug and CD were mixed in a mortar and sieved through a 100 μm sieve. Two types of solvent method were applied (kneading and ultrasonication). *Kneaded products* (**KPs**): **PM**s of the drug and HP-β-CD were mixed with the same quantity of a solvent mixture of ethanol + water (1:1). They were kneaded until the bulk of the solvent mixture had evaporated. The *ultrasonicated systems* (**USs**): the **PMs** were dissolved in 50% ethanol, placed in the Grant ultrasonic bath XB2 (England) for 1 h, dried and pulverized. After this, they were dried at room temperature and then at 105 ^{o}C, and were pulverized and sieved through a 100 μm sieve.

7.2.2. Cyclodextrin ternary systems

The three-component products were prepared in four different mole ratios (NIF:HP-β-CD mole ratio = 2:1, 1:1, 1:2 and 1:3), in all cases containing 15% (w/w) PVP K-90. *Ternary physical mixtures* (**PMs+PVP**): NIF, HP-β-CD and PVP were mixed in a mortar and sieved through a 100 μm sieve. *Ternary kneaded products* (**KPs+PVP**): PMs+PVP were mixed with the same quantity of a solvent mixture of ethanol + water (1:1), and were kneaded until the bulk of the solvent mixture had evaporated. After this, they were dried at room temperature and then at 105 ^{o}C, pulverized and sieved through a 100 μm sieve.

7.2.3. Solid dispersions

The two-component products were prepared in four different mass ratios (NIF:PVP mass ratio = 1:1, 1:2, 1:4 and 1:6). The **PMs C-15, C-30** and **K-25** were mixed, pulverized in a mortar and sieved through a 100 μm sieve.

Solid dispersions were prepared by using a vacuum dryer and spray dryer for solvent evaporation.

The *solvented products* were prepared with drug:PVP K-25 mass ratios of 1:1, 1:2, 1:4 and 1:6 (**SP K-25**). To a solution of NIF (1 g) in 30 ml of acetone, the appropriate amount of PVP K-25 was added. The minimum amount of methanol was added to solubilize the polymer. The solvents were removed under reduced pressure at 30 ^{o}C and the residue was dried under

vacuum at room temperature for 3 h **[114]**. The samples were pulverized and sieved through a 100 μm sieve.

The *spray-dried samples* (SPDs) were prepared by using a Büchi Mini Dryer B-191 (Switzerland), at 165 ^{o}C inlet and 86 ^{o}C outlet temperature with a compressed air flow of 600 l/min and a nozzle diameter of 0.5 mm as solvent evaporator. The aspirator rate was 80% and the pump rate was 10%. According to some references **[114]**, the M_w of the polymer might play a role in the performance of a solid dispersion and better results can obtained with a lower M_w. However, at a higher ratio of PVP, the solubilization process may be neutralized by the diffusion process by increasing the viscosity of the solution around the particle **[114]**. For these reasons, by the preparation of the spray-dried products were prepared in mass ratios of 1:5 and 1:10 with PVP C-15 (**SPD C-15**) – because of its low M_w – and were dissolved in 30% ethanol.

All of the products were stored under normal conditions at room temperature (22 ^{o}C).

7.3. Physicochemical characterization of the products

7.3.1. Study of contact angles

The OCA Contact Angle System (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany) was used for studies of the wettability of NIF and its products. 0.15 g of powder was compressed under a pressure of 1 ton by a Specac hydraulic press (Specac Inc., USA). The wetting angles of the pressings were determined after 4.3 μl of distilled water had been dropped onto the surface of the pressings. The change in the wetting angle was registered from 1 to 25 s (minimum of 5 parallel numbers), using the circle fitting method of the OCA System.

7.3.2. Determination of n*-octanol/water partition coefficient*

The partition coefficient (K_p) is defined as the ratio of the drug concentration in the oil phase (usually represented by *n*-octanol) divided by the drug concentration in the aqueous phase measured at equilibrium under specified temperature *in vitro* in an oil/water two-layer system **[115]**. The diffusion ability across biological membranes is determined in two separate solutions: *n*-octanol saturated with water, and water saturated with *n*-octanol **[116]**. NIF or the products containing NIF were added to these solvent systems during continuous stirring until the excess drug appeared in suspended form for 72 h, at 25±2 °C. After filtration, the

saturated solutions were diluted with *n*-octanol-saturated distilled water or distilled water-saturated *n*-octanol, and the drug content was determined spectrophotometrically:

$$K_p = \frac{c_0}{c_w} \qquad \textbf{(Eq. 2)}$$

where c_0 = concentration of NIF in *n*-octanol and c_w = concentration of NIF in water **[117, 118]**.

7.3.3. Saturation concentration

Saturation concentrations of solid dispersion systems were determined at 25 °C. NIF and its products were added to distilled water during continuous stirring until the excess drug appeared in suspended form. After filtration, the saturated solution was diluted and the drug concentration was determined spectrophotometrically.

7.3.4. Particle size analysis

The LEICA Image Processing and Analysis System (LEICA Q500MC, LEICA Cambridge Ltd., England) was used to measure the particle size distribution of the solid dispersion. We determined and compared the products with the pure drug, using 350 particles per sample. The particles were described by their length, breadth, surface area, perimeter and roundness.

7.4. *In vitro* investigations

7.4.1. Methods of in vitro *dissolution*

The modified paddle method with the USP dissolution apparatus (USP rotating-basket dissolution apparatus, type DT) was used to examine 20 mg samples of pure NIF or products containing 20–200 mg of drug according to the dissolved drug quantity in 100 ml of simulated gastric medium (SGM) (pH = 1.1 ± 0.1; 94.00 g of 1 M HCl, 0.35 g of NaCl, and 0.50 g of glycine to 1000 ml with distilled water), simulated intestinal medium (SIM) (pH= 7.0 ± 0.1; 14.4 g of $Na_2HPO_4 \cdot 2H_2O$, and 7.1 g of KH_2PO_4 to 1000 ml with distilled water). The basket was rotated at 100 rpm and sampling was performed up to 120 min (sample volume 5.0 ml). After filtration and dilution, the NIF contents of the samples were determined spectrophotometrically ($\lambda_{(SGM)}$ = 256 nm, $\lambda_{(SIM,\ SPL)}$ = 288 nm).

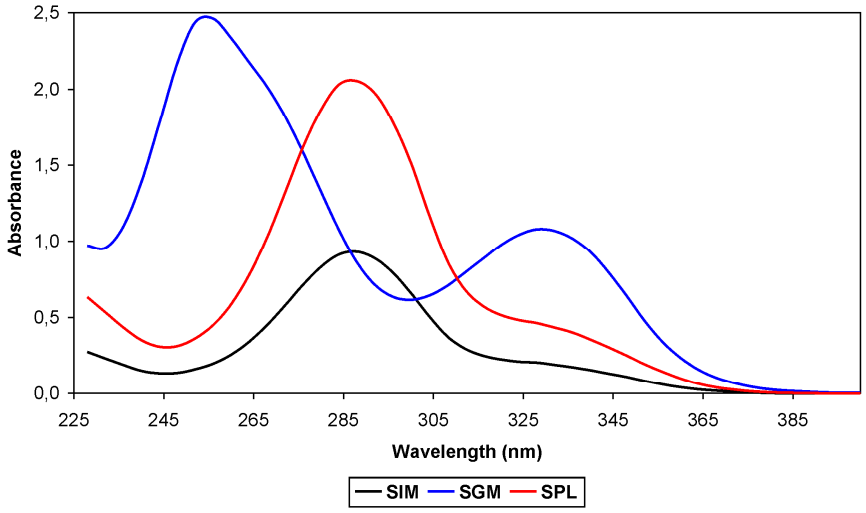

Fig. 7. The effect of the pH on the NIF absorbance

7.4.2. Mathematical models of in vitro dissolution

In vitro dissolution has been recognized as an important element in drug development. The nature of the drug, and its polymorphic form, crystallinity, particle size, solubility and amount in the pharmaceutical dosage form can influence the release kinetics. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. The following mathematical models have been used to evaluate the results of the dissolution as concerns the dissolution profiles of NIF and its products **[119]**.

Higuchi **[120]** developed several theoretical models to study the release of water-soluble and low-soluble drugs incorporated in semi-solid and/or solid matrixes. *Higuchi* described drug release as a square root time-dependent diffusion process based on *Fick*'s law:

$$m = 100 - k_d\sqrt{t} \qquad \textbf{(Eq. 3)}$$

where m is the quantity (%) of the drug not released in time t, and k_d (mg/min$^{1/2}$) is the *Higuchi* dissolution constant, treated sometimes in a different manner by different authors and theories.

A general empirical equation described by *Weibull* was adapted to the dissolution/release process; it is applied to almost all kinds of dissolution curves. This equation is very frequently used and therefore some modifications have been made according to the different dissolution profiles. When applied to drug dissolution or release from pharmaceutical dosage forms, the *Weibull* equation expresses the accumulated fraction of the drug, m_d, in solution at time t, by the RRSBW model:

$$m_d = 1 - \exp(-(t - T_i)^{b/a}) \qquad \textbf{(Eq. 4)}$$

In this equation, the scale parameter *a* defines the times scale of the process. The location parameter T_i represents the lag time before the onset of the dissolution or release process and in most cases will be zero. The shape parameter *b* characterizes the curves.

The *Langenbucher* equation is very frequently used with modifications depending on the different dissolution profiles:

$$\sqrt[3]{\frac{m_t}{m_0}} = 1 - \frac{t}{T} \qquad \textbf{(Eq. 5)}$$

Modified Langenbucher equation:

$$\sqrt[3]{1 - \frac{m_t}{m_0}} = \ln t \qquad \textbf{(Eq. 6)}$$

$(Bt)^a$ model:

$$\sqrt[3]{\frac{m_t}{m_0}} = 1 - (bt)^a \qquad \textbf{(Eq. 7)}$$

where m_0 is the mass of the drug at time $t=0$, and m_t is that at time t and T is the total time of the dissolution **[121]**.

The kinetic analysis were carried out with an *in vitro - in vivo* kinetic computer program. To compare the kinetic models in preliminary calculation, it was concluded that the dissolution of NIF from products is described most precisely by the Langenbucher equation.

7.4.3. In vitro membrane diffusion

Stricker's Sartorius apparatus (Sartorius-Membranfilter GmbH, Germany) was used **[122, 123]**. Measurements were performed on 100.0 ml of SGM or SIM into simulated plasma (SPL) (pH = 7.5 ± 0.1; 20.5 g of $Na_2HPO_4 \cdot 2H_2O$ and 2.8 g of KH_2PO_4 to 1000 ml with distilled water). 20 mg samples of drug or products containing 40 mg of NIF were placed in the donor phase in all cases. The artificial membrane was made of cellulose acetate (Schleicher & Schuell ME 29, Dassel, Germany: pore size 3 μm, diffusion surface 40 cm^2). The temperature was 37.5 ± 1.5 ^oC. 5.0 ml samples were taken five times (after 30, 60, 90, 120 and 150 min) and their NIF contents were determined spectrophotometrically after filtration and dilution. The amount of diffused active agent and the diffusion constant K_d were calculated:

$$K_d = \frac{c_{II2} - c_{II1}}{T_2 - T_1} \cdot \frac{1}{c_{I0}} \cdot \frac{V_{II0}}{F} \text{ [cm min}^{-1}\text{]} \qquad \textbf{(Eq. 8)}$$

where c_{IIx} is the corrected drug concentration in phase II at time T_x (mg/ml); V_{II0} is the volume of aqueous phase II at time T_0 (100 ml); F is the surface area of the membrane (cm^2); T_x is time (min); and c_{I0} is the theoretical initial drug concentration in phase I (mg/ml).

7.5. Structural evaluation

7.5.1. Methods of thermal analysis

7.5.1.1. Hot-stage microscopy (HSM)

Microscopic observations of morphological features and their changes during heating were carried out with a LEICA Thermomicroscope (LEICA MZ 6, Germany). The samples were observed under the microscope by using a scanning speed of 2 °C/min. The magnification in the photographs was 59.7x.

7.5.1.2. Differential scanning calorimetry (DSC)

The DSC measurements with a Mettler Toledo DSC 821^e thermal analysis system with the STAR^e thermal analysis program V6.0 (Mettler Inc., Schwerzenbach, Switzerland), approximately 2–5 mg of pure drug or product was examined in the temperature range between 25 ^oC and 300 ^oC. The heating rate was 5 ^oC min^{-1}. Argon was used as carrier gas, at a flow rate of 10 l/h during the DSC investigation.

7.5.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were measured on an AVATAR 330 FT-IR apparatus (Thermo Nicolet, USA), in the interval 400–4000 cm^{-1}, at 4 cm^{-1} optical resolution. Standard KBr pellets were prepared from 150 mg of KBr pressed with 10 ton and the calculated amount of the samples containing 0.5 mg of NIF.

7.5.3. X-ray powder diffraction (XRPD)

The physical state of the NIF in the different samples was evaluated by XRPD. Diffraction patterns were obtained on a Philips PW 1710 diffractometer, where the tube anode was Cu with $K\alpha$ = 1.54242 Å. The pattern was collected with a tube voltage of 50 kV and 40 mA of tube current in step scan mode (step size 0.035, counting time 1 s per step).

8. Results and discussion

8.1. Investigation of cyclodextrin binary and ternary complexes

Inclusion complexation with **CD**s affords a possibility for the increase of solubility properties. For this purpose, primarily HP-β-CD was used to prepare products by powder mixing, kneading and ultrasonication in four molecular ratios (2:1, 1:1, 1:2 and 1:3). The dissolution and *in vitro* membrane diffusion of the products were investigated. The wetting angles of pure NIF and HP-β-CD and of the products, and the *n*-octanol/water partition coefficients were determined. The interactions leading to partial complex formation between the components of the products was examined by thermoanalytical methods. For this reason, the next step was to formulate ternary complexes using 15 m/m% of PVP K-90 because the addition of small amounts of water-soluble polymers to an aqueous CD solution, together with heating, increases the CD complexation of lipophilic water-insoluble drugs. The polymers not only increase complexation, but also increase the drug availability in the CD-containing drug formulation **[I-III]**.

8.1.1. Physicochemical characterization (wettability and n*-octanol/water distribution)*

The first step of any dissolution process is the interaction of the solid and liquid surfaces, *i.e.* the wetting of the solid phase by the solvent. Wettability tests can rapidly differentiate between the hydrophilic characters of the samples and the lipophilic drug.

The contact angles of NIF, HP-β-CD, PVP K-90 and their products were determined at 5 sec, when the SD was the lowest (Table VI). The wetting angle of NIF was 71.7 °, *i.e.* it is a very hydrophobic drug. The wetting angles of HP-β-CD, 18.5°, and of PVP K-90, 35.4°, reveal their hydrophilic characters.

Table VI. Wetting angles of NIF and its products

	[°]±SD	[°]±SD	[°]±SD	[°]±SD	[°]±SD	[°]±SD
NIF	71.3±3.2					
HP-β-CD	18.5±1.6					
PVP K-90	35.4±7.6	**PMs**	**KPs**	**USs**	**PMs+PVP**	**KPs+PVP**
2:1		30.4±4.5	40.9±0.6	42.3±4.6	30.7±0.7	42.3±6.7
1:1		29.0±1.3	32.6±1.5	34.6±1.2	29.9±0.1	36.6±2.1
1:2		24.8±2.2	25.2±2.3	27.0±2.0	28.7±1.3	30.0±2.0
1:3		26.4±2.2	25.8±1.7	25.4±1.1	35.4±2.5	31.6±1.6

The wetting angles of the investigated products in all cases lay between the values for CD and NIF. The investigation of the systems produced by the different preparation methods revealed that, with increasing HP-β-CD content, the contact angles decreased (except for 1:3 PM+PVP and 1:3 KP+PVP). Usually the 1:2 and 1:3 ratios gave better results.

This provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily milieu rather than water (*i.e.* its hydrophilic/lipophilic balance). The *n*-octanol/water partition coefficient is the ratio of the concentration of a chemical in *n*-octanol and in water at equilibrium and at a specified temperature. *n*-octanol is an organic solvent that is used as a surrogate for natural organic matter. This parameter is applied in many environmental studies to help determine the fate of chemicals in the environment. The *n*-octanol/water partition coefficient has been correlated to water solubility; therefore, the water solubility of a substance can be used to estimate its *n*-octanol/water partition coefficient. To compare the results of wettability with *n*-octanol/water partition coefficient, in both study the 1:2 PM+PVP and 1:3 ratios for all methods shown similar tendency.

Table VII. *n*-octanol/water distribution

NIF and Products		$c_{n\text{-octanol}}$ [μg ml^{-1}]	c_{water} [μg ml^{-1}]	c_o/c_w
NIF		**48439.07**	**26.75**	**1810.80**
PMs	2:1	8459.21	177.27	47.72
	1:1	10876.13	311.36	34.93
	1:2	9365.55	162.76	57.54
	1:3	6646.52	243.01	**27.35**
KPs	2:1	9466.26	132.51	71.44
	1:1	9466.26	103.84	91.16
	1:2	8962.73	91.43	98.03
	1:3	10775.42	184.61	**58.37**
USs	2:1	8962.70	147.27	60.85
	1:1	10352.00	169.58	61.04
	1:2	4813.60	182.86	26.32
	1:3	5236.60	205.24	**25.51**
PMs+PVP	2:1	6747.23	422.03	15.99
	1:1	5840.88	237.76	24.57
	1:2	1309.16	220.28	**5.94**
	1:3	2076.54	251.31	**8.26**
KPs+PVP	2:1	8026.18	262.23	30.61
	1:1	7724.06	207.60	37.20
	1:2	4773.41	196.24	24.32
	1:3	2860.02	313.37	**9.13**

NIF has a high partition coefficient (1810.80), reflecting its poor water solubility and high affinity for *n*-octanol; its concentration in water at 22 ^{o}C is 26.75 μg/ml, while that in *n*-

octanol is 48439.07 μg/ml. The *n*-octanol/water partition coefficients revealed that the water solubility increase was generally better for the ternary than for the binary systems. The concentration of NIF decreased in *n*-octanol and increased in water with increasing HP-β-CD concentration (Table VII). The best partition coefficient results were 25.51 for 1:3 US; 5.94 for 1:2 PM+PVP and 9.13 for 1:3 KP+PVP. The concentration of NIF in water could be increased even 15-fold (from 26.75 μg/ml to 422.03 μg/ml).

8.1.2. Solubility study

NIF has an acidic character, and its dissolution was therefore better in SIM (14.33 mg/100 ml at 120 min) than in SGM (9.92 mg/100 ml at 120 min). The dissolution of the CD- and CD+PVP-containing products was in all cases better than that of the pure drug in both media except for the PMs in the ratios of 2:1 and 1:2 (Fig. 8).

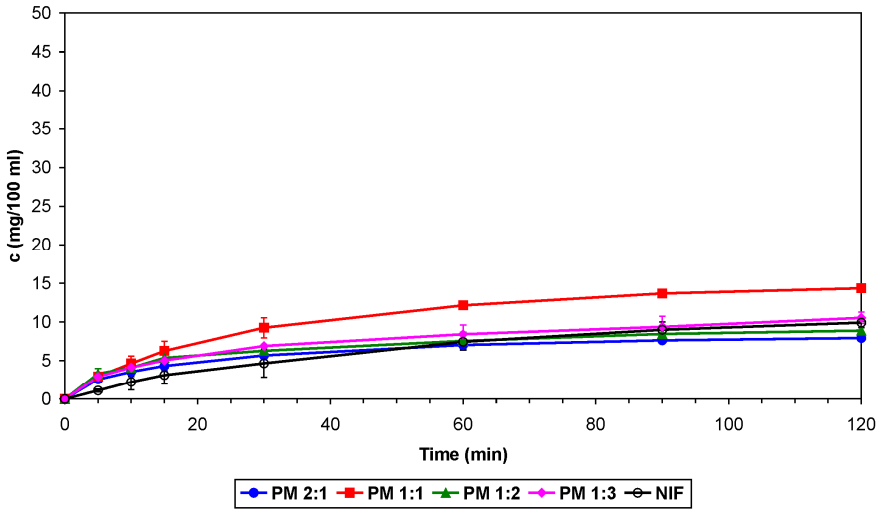

Fig. 8. Dissolution of NIF and the PMs in SGM

The dissolution behaviour of the KPs was better than that of all the products in SGM. In SGM, the dissolution increased in proportion to the CD content in all of the KPs, where the 1:3 KP gave the best dissolution result; the solubility increase was 4.4-fold as compared with the pure drug (Fig. 9). Similar phenomena were observed for KPs+PVP. Elevation of the HP-β-CD ratio exerted a slight influence in increasing the dissolution of PMs, USs and PMs+PVP in SGM. In these cases, the dissolution was prolonged, the quantity of NIF dissolved increasing only gradually. In SGM, extensive dissolution did not occur and the dissolutions of the different products were nearly the same. The solubility increase was nearly 1.5-fold.

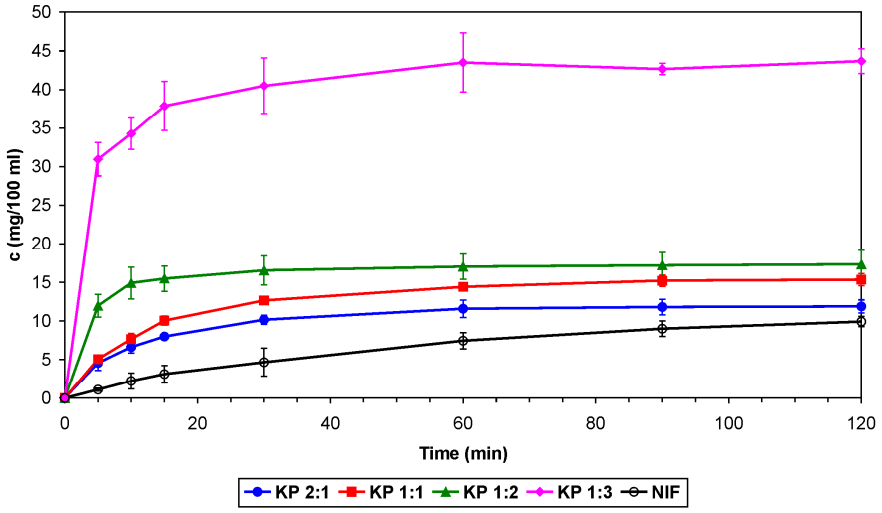

Fig. 9. Dissolution of NIF and the KPs in SGM

Table VIII. lists the summarized dissolution results for NIF and all of the products in SGM. In this period (0–10 min), similar phenomena were seen for PMs and PMs+PVP.

Table VIII. Summarized dissolution results for NIF and products (mg/100 ml).

NIF and Products		SGM			
		10th min	**±SD**	**120th min**	**±SD**
NIF		**2.23**	1.02	**9.92**	0.65
PMs	2:1	3.55	0.60	7.93	0.32
	1:1	4.64	0.96	14.36	0.41
	1:2	4.10	0.48	8.90	1.15
	1:3	4.11	0.21	10.55	0.73
KPs	2:1	6.63	0.79	11.89	0.84
	1:1	7.66	0.72	15.35	0.79
	1:2	14.91	2.07	17.33	1.86
	1:3	**34.27**	2.02	**43.69**	1.59
USs	2:1	5.94	0.24	11.63	1.69
	1:1	9.72	1.00	14.57	1.41
	1:2	12.48	1.11	15.70	0.43
	1:3	12.67	0.29	16.14	0.51
PMs+PVP	2:1	5.44	0.67	10.83	0.26
	1:1	4.87	0.07	12.47	0.58
	1:2	5.25	0.66	10.08	0.39
	1:3	5.10	1.39	10.66	0.99
KPs+PVP	2:1	13.17	1.03	17.56	0.98
	1:1	14.58	3.38	18.06	4.41
	1:2	13.43	0.27	17.35	0.36
	1:3	**30.86**	1.09	**40.91**	1.33

In the first 10 min, a 15-fold solubility increase was detected in the cases of 1:3 KP and 1:3 KP+PVP as compared with NIF. In both cases, the applied solvent and the higher CD content may help improve the solubilty. Little difference was found between products containing PVP and without PVP in SGM.

Figures 10 and 11 reveal that, in SIM use of PVP resulted in significantly higher solubility as compared with NIF and the binary products. The saturation concentration was attained in 10–15 min. The maximum solubility increase (7-fold) was observed for the 1:1, 1:2 and 1:3 ternary KPs, while a 3-fold increase was found for the 1:1 PM+PVP.

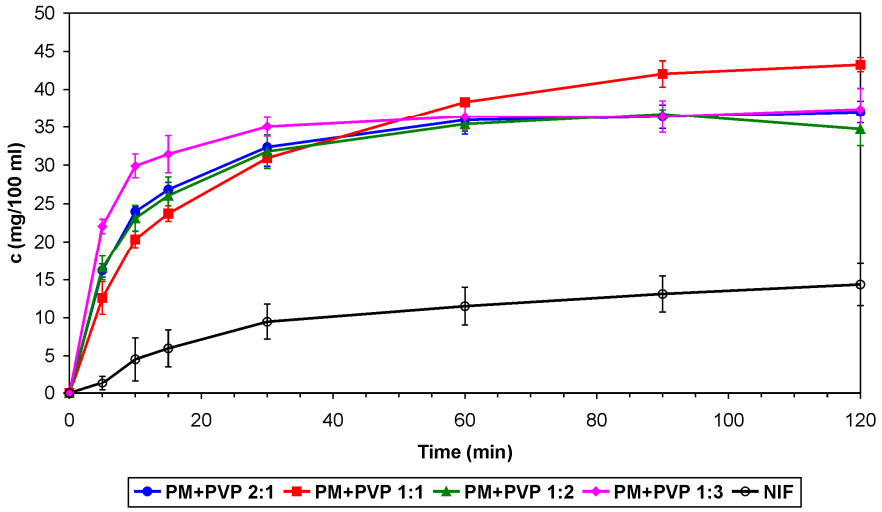

Fig. 10. Dissolution behaviour of NIF and PMs+PVP in SIM

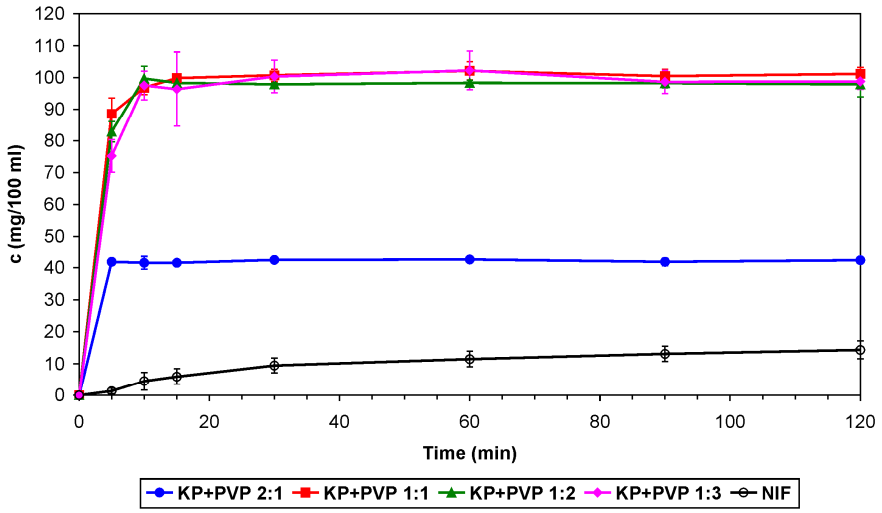

Fig. 11. Dissolution behaviour of NIF and KPs+PVP in SIM

Table IX summarized the dissolution results at 10 and 120 min in SIM. At the higher pH, the ternary samples gave favourable results: some of the KPs+PVP dissolved totally and in the first 10 min a 20-fold solubility improvement was registered as compared with NIF. There

was a great difference between the PMs and PMs+PVP. The concentrations of the PMs in SIM were nearly the same as in the case of NIF after 120 min, in contrast with the PMs+PVP, where a 3-fold increase was observed. The USs gave parallel values in the different dissolution media.

Table IX. Summarized dissolution results for NIF and products (mg/100 ml).

NIF and Products		SIM			
		10th min	**±SD**	**120th min**	**±SD**
NIF		**4.55**	2.81	**14.34**	2.78
PMs	2:1	6.17	3.15	11.86	0.33
	1:1	8.62	2.56	14.24	3.25
	1:2	7.38	1.74	13.20	1.88
	1:3	11.00	0.38	16.03	0.29
KPs	2:1	13.45	4.54	15.92	2.42
	1:1	45.50	1.85	47.08	3.06
	1:2	50.06	1.63	**50.65**	2.96
	1:3	**47.69**	1.26	**49.21**	0.60
USs	2:1	18.07	2.97	16.41	0.73
	1:1	17.49	1.65	17.78	1.71
	1:2	18.15	0.52	18.37	0.65
	1:3	18.87	0.46	18.97	0.46
PMs+PVP	2:1	23.96	0.67	36.99	1.44
	1:1	20.35	1.11	43.25	0.92
	1:2	23.11	1.67	34.72	2.16
	1:3	29.91	1.54	37.38	2.73
KPs+PVP	2:1	41.47	1.99	42.26	0.51
	1:1	**96.85**	2.18	**100.0**	2.05
	1:2	**99.74**	3.86	**97.90**	3.96
	1:3	**97.55**	4.51	**98.81**	2.44

8.1.3. Determination of dissolution rate

The kinetics of the drug release processes of the products were investigated with focus on SGM and SIM because NIF is absorbed first in 2/3 ratio from the stomach and in 1/3 ratio from the intestines [110, 111].

The kinetic studies of the release of NIF were made by using the most precise of the mathematical models, as a function of the value of the correlation coefficient of the dissolution process. The kinetic models were obtained according to the equation $y = ax+b$ (a represents K, the drug release rate), the correlation coefficients (r^2) and the residual ordering.

It was concluded that the dissolution of NIF from products in SGM and SIM is described most precisely by the *Langenbucher* equation. The analyses of the rates of dissolution of the drug and products in different periods suggest two-step dissolution kinetics for the dissolution of NIF from the products. In the first step, *i.e.* the first 30 min, the release process is fast and has a high drug release rate. According to the dissolution of the pure NIF in the first step, the release rate was low, and therefore the drug content was smaller as compared with the second step. In this period (30–120 min), *K* had a value twice as high as in the first 30 min, in contrast with the dissolution kinetics of the products.

The kinetic parameters (0–120 min)(Table X) show very well that the dissolution was dependent on the preparation methods because the kneading method and the use of PVP led to significantly higher dissolution rates as compared with NIF.

Table X. Kinetic parameters in SGM and SIM calculated by Langerbucher equation

NIF and Products		SGM		SIM	
		Correl. coeff. r^2	***K***	**Correl. coeff. r^2**	***K***
NIF		0.975	**0.0049**	0.996	**0.0069**
PMs	2:1	0.930	0.0030	0.971	0.0043
	1:1	0.992	0.0056	0.986	0.0044
	1:2	0.993	0.0031	0.985	0.0040
	1:3	0.993	0.0041	0.896	0.0043
KPs	2:1	0.962	0.0041	0.861	0.0016
	1:1	0.970	0.0057	0.559	0.0037
	1:2	0.850	0.0026	0.617	0.0025
	1:3	0.937	**0.0077**	0.700	0.0069
USs	2:1	0.947	0.0046	0.974	0.0006
	1:1	0.842	0.0043	0.823	0.0015
	1:2	0.895	0.0030	0.534	0.0009
	1:3	0.774	0.0025	0.659	0.0003
PMs+PVP	2:1	0.997	0.0037	0.951	0.0118
	1:1	0.991	0.0049	0.998	**0.0183**
	1:2	0.991	0.0033	0.933	0.0112
	1:3	0.990	0.0035	0.871	0.0079
KPs+PVP	2:1	0.891	0.0029	0.848	0.0013
	1:1	0.972	0.0033	0.627	0.0037
	1:2	0.941	0.0030	0.951	**0.0084**
	1:3	0.997	**0.0073**	0.769	0.0061

8.1.4. Results of membrane diffusion studies

7.88 mg of NIF was able to diffuse during 150 min from SGM, and 9.35 mg from SIM. CD inclusion complexes with relatively high stability constants often have decreased diffusion

properties, as in the case of the NIF complexes. Fig. 12 shows that the diffusion of all these products was poorer than that of the active ingredient itself in SIM; it was about 3–9 mg/150 min in the cases of the KPs. Similar phenomena were observed to see the PMs and USs: about 2–8 mg/150 min.

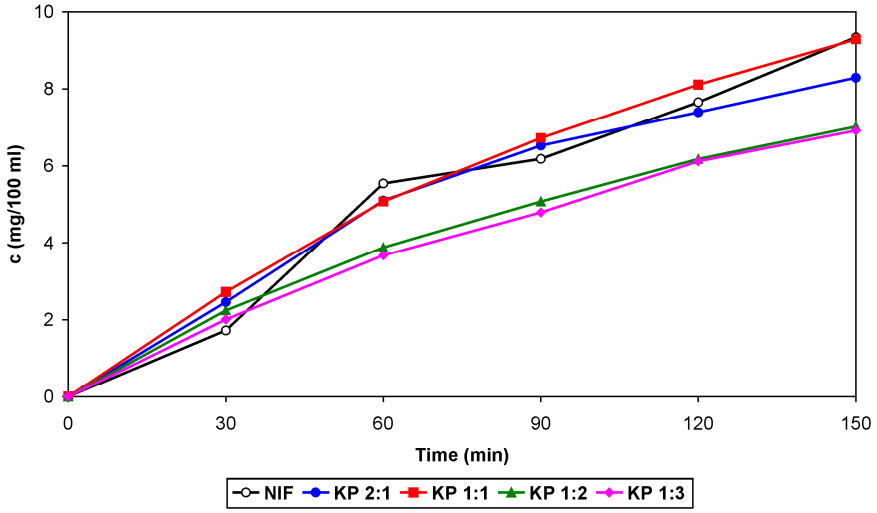

Fig. 12. Diffusion curves of NIF and KPs in SIM

In the case of the binary products, significant differences were observed from SGM. A 3-fold increase was measured for the 1:3 US after 150 min from SGM (Fig. 13) and the 2:1 ratio was better than the 1:1 ratio, as for the dissolution result.

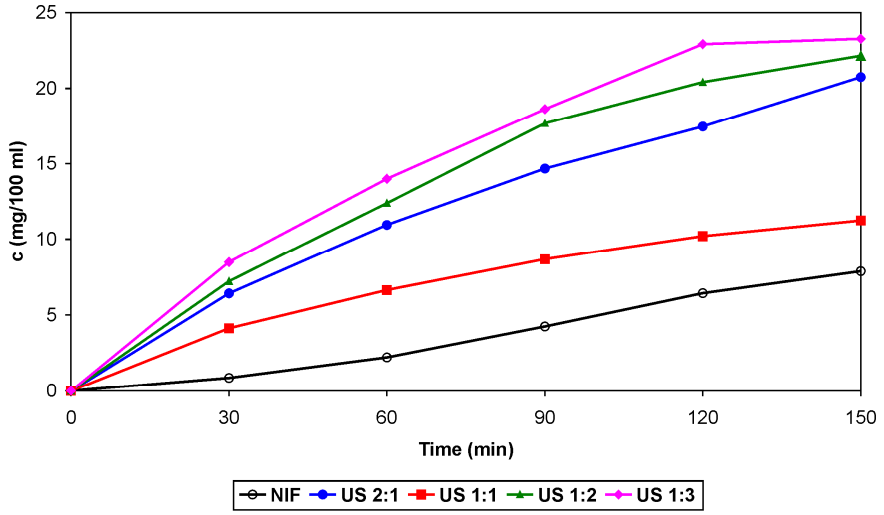

Fig. 13. Diffusion curves of NIF and USs in SGM

The diffusions of the ternary products were valuable only in the case of SGM (Fig. 13). For the PMs+PVP, the dependence of the diffusion on the mole ratio was not very appreciable. We experienced decreased diffusivity with increasing CD concent. The diffusion was improved 2-fold for the 1:2 PM+PVP. For the KPs +PVP a 1.5-2-fold improvement in

diffusion was detected. For all products except the 2:1 KP+PVP, the diffusion was more advantageous that than of NIF in SGM.

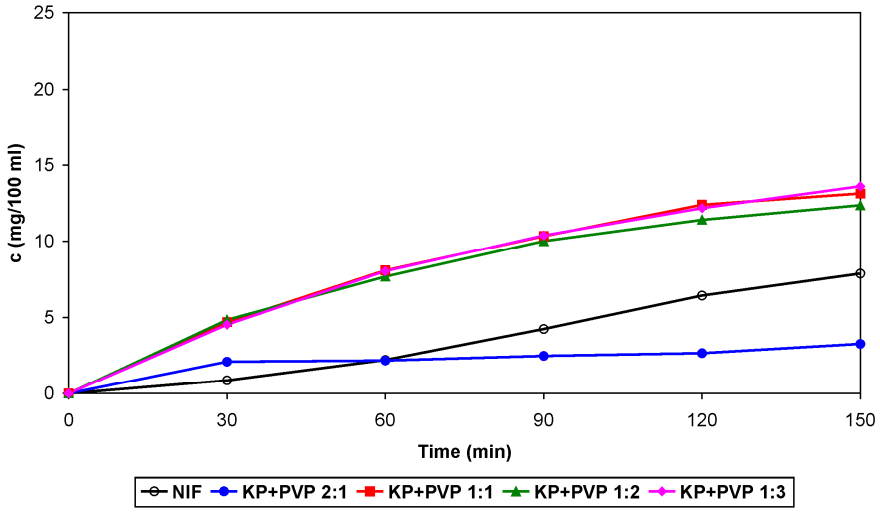

Fig. 14. Diffusion curves of NIF and KPs+PVP in SGM

The last part of the diffusion curves exhibited a saturated character, as a consequence of the increased diffused drug amount.

Table XI. The diffusion constants in SGM

NIF and Products		***Kd*** [cm min^{-1} $\times 10^{-3}$]	**SD**$\times 10^{-3}$
NIF		**2.36**	0.77
PMs	2:1	4.03	1.99
	1:1	4.72	2.56
	1:2	3.64	3.23
	1:3	2.65	1.52
KPs	2:1	4.70	4.20
	1:1	4.15	4.02
	1:2	3.89	4.13
	1:3	4.09	2.17
USs	2:1	6.22	2.82
	1:1	3.37	2.22
	1:2	6.65	4.06
	1:3	**6.98**	5.24
PMs+PVP	2:1	3.39	2.29
	1:1	4.07	1.57
	1:2	4.37	3.13
	1:3	2.93	1.71
KPs+PVP	2:1	0.98	1.40
	1:1	3.94	2.74
	1:2	3.71	2.78
	1:3	**4.08**	2.42

This is the explanation of the significant differences in the values of K_d. Table XI lists the K_d values, where NIF has a 2.36 (10^{-3}) cm/min as of K_d value compared with the K_d values of the samples, which were improved.

With increase of the diffused drug concentration, the K_d values rose. The KPs+PVP showed the lowest K_d, 0.98, and for the USs a 2–3-fold rise was observed. For the binary products (PMs and KPs), on decrease of the amount of CD, K_d increased.

8.1.5. Structural analysis

DSC thermograms of NIF, HP-β-CD and PVP K-90 alone are shown in Fig. 15. NIF gave a melting endotherm at 203.81 °C, which can be identified from the literature data as its melting point. The onset of melting was observed at 201.15 °C and the end at 203.81 °C; the normalized enthalpy was 127.50 J g^{-1}. After the melting of NIF, the total mass of the investigated sample was progressively lost, with an endotherm at about 250 °C relating to its decomposition. The CDs lose water below 100 °C and begin to decompose above 250 °C; DSC method can therefore be used if the substance melts between 100 and 250 °C. The DSC curve indicates that the melting point of NIF is at 203.81 °C, and this value also shows that DSC is useful for these products.

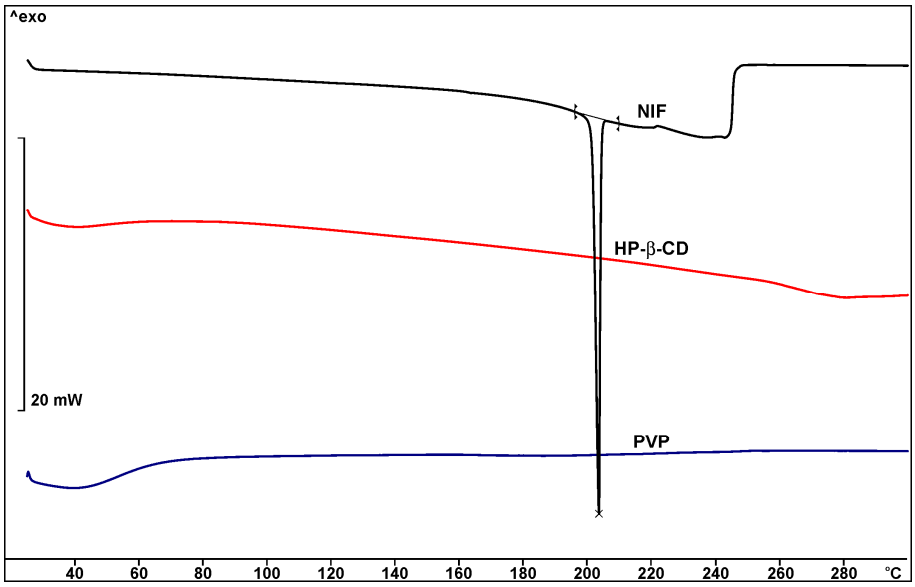

Fig. 15. DSC curves of NIF, HP-β-CD and PVP K-90

The PMs undergo initial water loss as the temperature is raised. The melting point decreases with increase of the CD concentration (189–182 °C).

For the KPs, the melting point likewise decreases on increase of the CD content (190–180 ^{o}C). In both cases, the 1:3 products give the best result (Fig. 16). Partial complexation or amorphisation are presumable. Complex formation can easily be followed by evaluation of the DSC curves of the products.

NIF melting was not observed in the DSC curves of the PMs+PVP (Fig. 17) and 1:2 and 1:3 KPs+PVP. Preliminary thermomicroscopic investigations indicated that in these cases the NIF dissolved in the melted PVP. For the 2:1 and 1:1 KPs+PVP, an endotherm of NIF melting was detected. When PVP K-90 was present, partial or total complexation could be presumed, or the active material was found in the amorphous form.

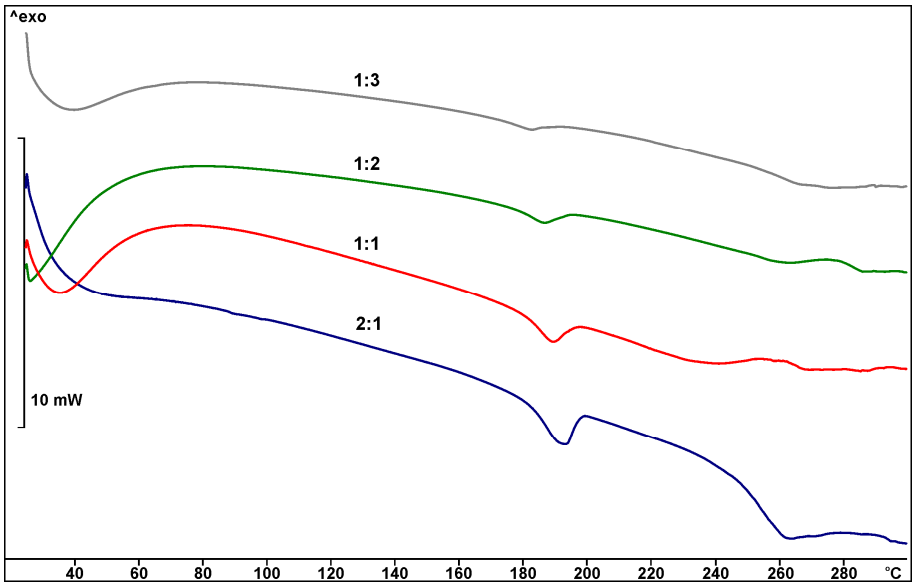

Fig. 16. DSC curves of binary KPs

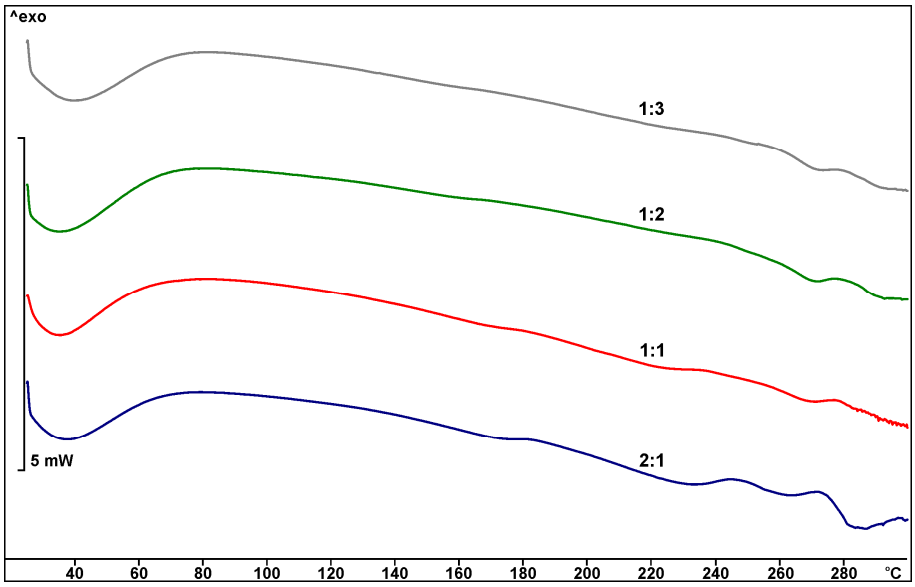

Fig. 17. DSC curves of ternary PMs+PVP

8.2. Studies of solid dispersion systems

At the begining of my work, binary systems were prepared to improve the solubility and permeability of the drug. The results were significantly better than those for compared to NIF. To study the effects of a water-soluble polymer on the complexation phenomena, ternary products were prepared and investigated. Using 15 m/m% PVP K-90 resulted in solubility and permeability increases, but the wetting angles are nearly the same; the dissolution changed for the better, especially in SIM. Among the polymers employed for formulation with slightly water-soluble active ingredients, PVP displays marked complexing and solubilizing properties. The aim of the preparation of PVP dispersions is generally to transform the drug into the amorphous form and thus to achieve faster dissolution. The drugs with poor water-solubility can provide better bioavailability in the amorphous state. Products were prepared with different types of PVP, using physical mixing and solvent evaporation methods (7.2.3). The NIF-polymer interactions in the solid state were investigated by using HSM, DSC, FT-IR and XRPD. The results suggested that PVP may inhibit the association of the drug molecule to form the crystal nucleus and thereby inhibit crystal growth. In the PMs, the NIF was dissolved in the melted PVP, whereas the solid dispersions contained NIF in the amorphous state **[V-VII]**.

8.2.1. Characterization of niflumic acid and its products

According to the particle size distribution, the characteristics (length, width, area, perimeter and roundness) of at least 350 particles were determined in the SPs and SPDs at all ratios. Table XII summarizes the average results.

The size of the most frequent NIF particles is between 30 and 60 μm. About 50% of the particles of the SPs K-25 (solvented products with PVP K-25, using vacuum dryer) and SPDs C-15 (spry-dried products with PVP C-15) had a particle size between 10 and 20 μm. Reduction of the length and width of the particles was significant for the 1:2, 1:4 SPs and 1:5 SPD (from ~ 46 μm to ~ 14 μm). According to the length's and width's reduction, the values of area and perimeter decreased significantly. This can result in an enhanced dissolution rate due to increases both in the surface area and solubilization.

Table XII. Characterization of particles of SPs K-25 and SPDs C-15						
NIF and Products		**Length [µm]**	**Width [µm]**	**Perimeter [µm]**	**Area $[\mu m]^2$**	**Roundness**
NIF	Average	**45.640**	**26.005**	**138.863**	**766.290**	1.989
	SD±	22.631	81.095	76.847	568.442	1.173
SPs K-25 1:1	Average	21.390	13.355	84.088	127.226	2.850
	SD±	13.553	8.198	63.623	115.060	1.334
1:2	Average	**13.272**	10.149	45.731	94.452	1.696
	SD±	4.005	3.003	15.896	49.602	0.493
1:4	Average	**14.647**	8.569	48.034	76.955	2.463
	SD±	5.535	3.036	20.128	49.602	0.990
1:6	Average	18.371	10.622	62.336	116.662	2.622
	SD±	7.503	3.865	28.269	76.234	1.105
SPDs C-15 1:5	Average	**14.192**	9.863	47.244	91.261	1.914
	SD±	5.178	3.593	19.955	54.646	0.766
1:10	Average	30.226	22.200	106.934	437.636	2.158
	SD±	13.629	10.399	57.081	350.497	1.017

NIF has a lipophilic character, reflecting its poor water solubility, which at 22 ^{o}C is 26.75 µg/ml. The concentration of NIF in water could be increased even 15-fold (from 26.75 µg/ml to 413.33 µg/ml).

Table XIII. Saturation concentrations and wetting angles of NIF and products			
		C_{water}	**Wetting angle**
NIF and Products		[µg/ml]	[°] ±SD
NIF		**26.75**	**71.1±0.2**
PMs C-15	1:1	50.87	44.3±4.8
	1:2	41.08	33.4±4.3
	1:4	46.15	31.3±1.6
	1:6	44.40	28.7±0.7
PMs C-30	1:1	86.36	56.8±1.9
	1:2	81.99	49.0±2.7
	1:4	74.30	43.7±1.9
	1:6	76.05	41.1±1.2
PMs K-25	1:1	92.83	69.2±2.4
	1:2	77.44	56.0±1.1
	1:4	65.04	46.0±1.9
	1:6	38.46	41.1±1.2
SPs K-25	1:1	**227.10**	**7.5±3.4**
	1:2	123.10	36.8±6.4
	1:4	134.62	44.6±2.0
	1:6	166.10	49.0±2.3
SPDs C-15	1:5	**413.33**	**26.3±2.8**
	1:10	**314.33**	**26.6±2.6**

The water solubility of NIF in the PMs was improved 2–3-fold and that of the SPs 4–8-fold. The results were outstanding in the case of the SPDs, where an 11–15-fold solubility

increase was observed. The relevant results are shown in Table XIII. The contact angles after 5 sec for NIF and its products were determined and compared with the water solubility. The contact angle for NIF was 71.1°, *i.e.* it is a very hydrophobic drug. The wetting angles of the investigated products were in all cases decreased. The investigation of the systems produced by physical mixing revealed that, with increasing PVP content, the contact wetting angles decreased: the 1:6 products were wetted 2–3 times better than NIF itself. The solvent and spray-drying methods gave the best results, except for the 1:1 SP (7.5°) and 1:5 SPD (26.3°).

8.2.2. Dissolution rate studies

NIF has an acidic character, and its *in vitro* dissolution (from 200 mg) was therefore better in SIM (14.33 mg/100 ml at 120 min) than in SGM (9.92 mg/100 ml at 120 min)**.** The dissolution behaviour of the SPs and SPDs was better than that of the PMs in both media.

It was concluded that the dissolution of NIF from the products in SGM and SIM is described most precisely by the *Langenbucher* equation. The release of NIF (dissolution rate constant, *K*) was studied kinetically by using of the precise mathematical models presented earlier, as a function of the correlation coefficient of the dissolution process (Table XIV). The processes of dissolution in SGM were prolonged and saturation was observed after 30 min. For the PMs involving the use of PVP C-15, C-30 and K-25, the quantity of NIF dissolved increased only gradually. In both media, extensive dissolution did not occur and the degrees of dissolution of the different products were nearly the same. An approximately 1.5-fold solubility increase was measured, independently of the weight ratios. A maximum solubility increase of 3.5-fold was observed for the SPs, and of 8-fold for the SPDs in SGM. NIF alone yielded the lowest initial dissolution rate (Fig. 18).

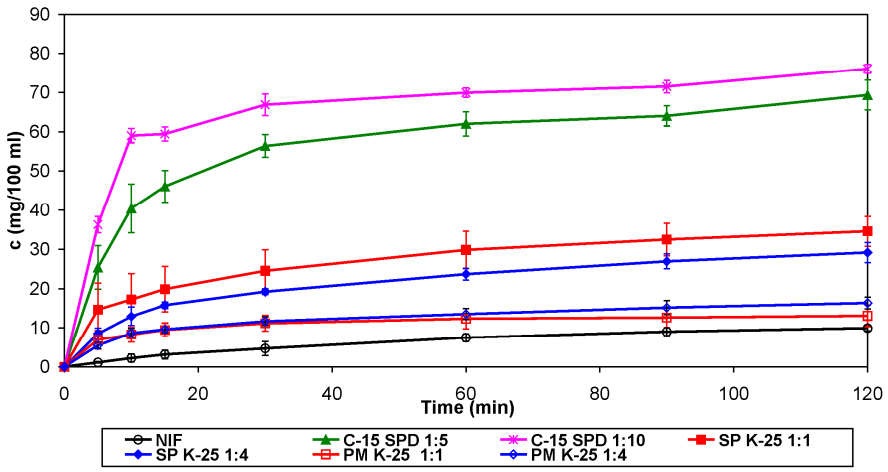

Fig. 18. Dissolution of NIF and different types of solid dispersions in SGM

All of the methods resulted in fast dissolution in SIM: the saturation concentration was reached in 5–15 min. Figure 19 presents the dissolution profiles of NIF and the 1:1 and 1:4 PMs with PVP K-25 and SPs. All of the solid dispersions resulted in 100% drug release in SIM, while for the 1:4 and 1:6 PMs with PVP K-25 resulted in 86% drug release (for this reason, K-25 was chosen at the beginning to prepare the SPs).

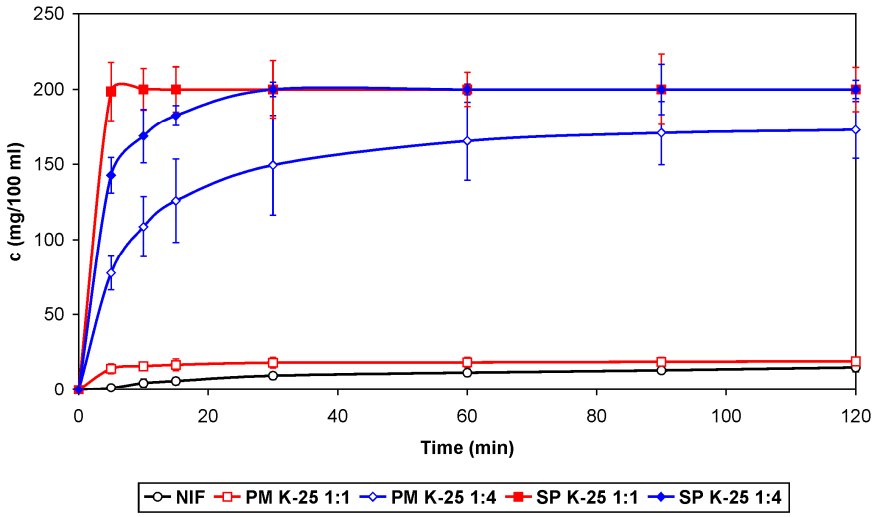

Fig. 19. Dissoultion curves in SIM

As shown in Table XIV, the *K* values of NIF from all PMs were almost the same, but in some cases higher than that for NIF alone in SGM.

This might due to the surface tension-lowering effect of PVP, resulting in wetting of the hydrophobic NIF crystalline surface **[122]**. *K* for the PMs in SIM was in all cases lower, than that for the pure drug, except for the 1:4 and 1:6 PMs K-25, where it was 15 and 21-fold.

The dissolution rates for the solid dispersions were significantly greater than those for the PMs and NIF. In SGM, there was a 2.3–5.26-fold improvement; in SIM, it was 26–51-fold. PVP may also have an enhancing effect on the wettability and dispersibility of the drug in the dissolution medium. This should retard any agglomeration or aggregation of the particles, which can slow the dissolution process. Differences were observed according to the preparation methods; spray drying gave better *K* values than solvent evaporation in both media.

Table XIV. Kinetic parameters in SGM and SIM calculated by Langerbucher equation					
NIF and Products		**Correl. coeff. r^2**	***K* SGM**	**Correl. coeff. r^2**	***K* SIM**
NIF		0.976	**0.0049**	0.997	**0.0069**
PMs C-15	1:1	0.998	0.0054	0.797	0.0038
	1:2	0.998	0.0053	0.819	0.0040
	1:4	0.997	0.0053	0.682	0.0032.
	1:6	0.997	0.0047	0.844	0.0036
PMs C-30	1:1	0.997	0.0039	0.708	0.0029
	1:2	0.992	0.0032	0.845	0.0051
	1:4	0.998	0.0042	0.854	0.0038
	1:6	0.999	0.0041	0.836	0.0050
PMs K-25	1:1	0.983	0.0034	0.931	0.0026
	1:2	0.992	0.0042	0.901	0.0032
	1:4	0.994	0.0056	0.992	**0.1092**
	1:6	0.999	0.0043	0.993	**0.1478**
SPs K-25	1:1	0.990	**0.0119**	0.991	**0.3254**
	1:2	0.876	**0.0161**	0.990	**0.3113**
	1:4	0.996	**0.0113**	0.973	**0.1776**
	1:6	0.992	**0.0153**	0.934	**0.2750**
SPDs C-15	1:5	0.974	**0.0258**	0.907	**0.3491**
	1:10	0.867	**0.0215**	0.909	**0.3441**

8.2.3. Investigation of in vitro *membrane diffusion*

7.88 mg of NIF was able to diffuse out during 150 min into SGM, and 9.35 mg into SIM. For all the products except the SPs, the diffusion was more advantageous than that of NIF into SGM. The diffusion of the products was also evaluable in the case of SGM.

As regards the PMs, the diffusion from the 1:4 compositions exhibited a 2-fold increase after 150 min. A 4.5-fold diffusion increase was measured for the SPs (Fig. 20) and a 2-fold improvement for the SPDs. The last part of the diffusion plots had a saturated character, as a consequence of the increased amount of diffused drug.

This is the explanation of the significant differences in the values of the K_d. Table XV lists these diffusion rate constants: NIF had a K_d value of 2.36 10^{-3} cm min^{-1}, while the K_d values for the samples were improved. The PM with a mass ratio of 1:4, and the SD and SPD with a mass ratio of 1:1 displayed the highest K_d values.

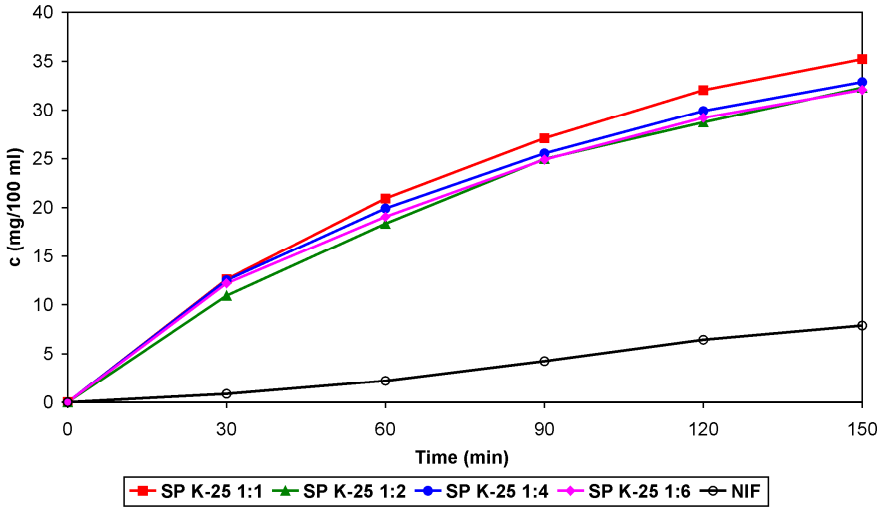

Fig. 20. Diffusion curves of NIF and SPs in SGM

Table XV. Diffusion constants (K_d) of NIF and products from SGM			
NIF and Products		$\boldsymbol{K_d}$ (10^{-3}) [cm/min]	**SD** (10^{-3}) ±
NIF		**2.36**	0.77
PMs C-15	1:1	3.49	2.24
	1:2	3.48	2.52
	1:4	3.63	2.47
	1:6	2.83	1.78
PMs C-30	1:1	3.75	2.78
	1:2	3.82	1.85
	1:4	**4.80**	2.83
	1:6	4.52	2.74
PMs K-25	1:1	3.74	2.76
	1:2	3.91	2.72
	1:4	4.60	3.10
	1:6	3.82	2.33
SPs K-25	1:1	**10.56**	6.81
	1:2	9.69	5.73
	1:4	9.86	6.87
	1:6	9.62	6.63
SPDs C-15	1:5	**4.21**	2.90
	1:10	3.33	2.41

*8.2.4. **Results of the thermal analysis** (DSC and HSM)*

DSC thermograms of NIF and its products are shown in Fig. 21. NIF gave a melting endotherm at 203.81 ^{o}C. NIF melting was not observed for any of the X-ray-amorphous solid

dispersions, as expected. The DSC curves did not reveal a melting peak for NIF in any of the solid dispersions (Fig. 22). This may be due to the interaction between NIF and PVP in these systems. In contrast with the XRPD patterns of the PMs K-25 (without any amorphous character of NIF), the melting point of the drug was not observed. This indicated a NIF:PVP solid-state interaction induced by heating.

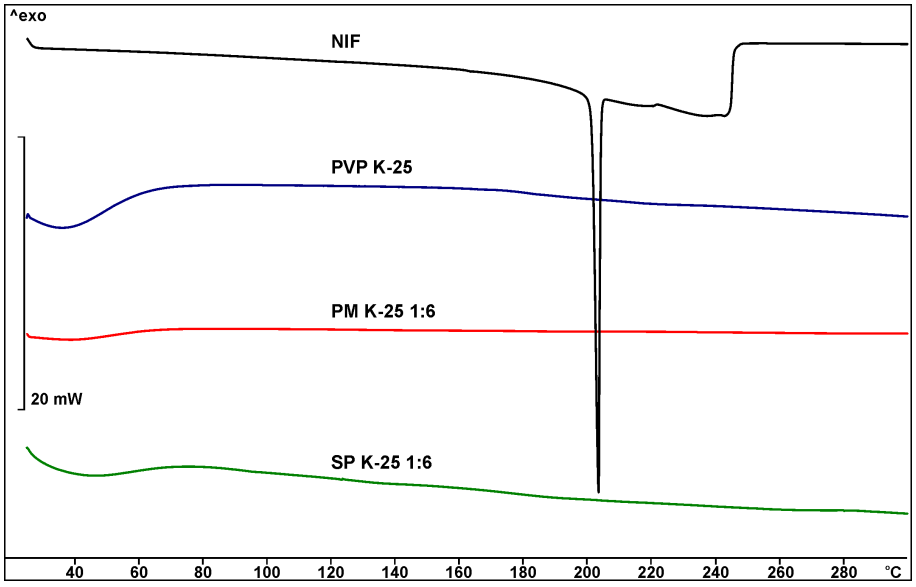

Fig. 21. DSC curves of NIF, PVP K-25 and samples

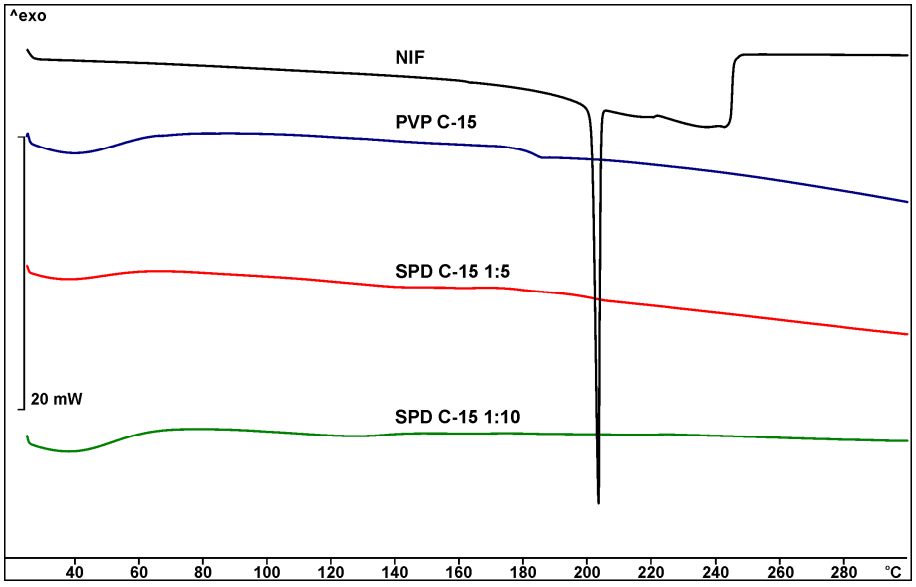

Fig. 22. DSC curves of NIF, PVP C-15 and samples

To visualize the changes in the PM K-25 samples during heating, HSM was used. This technique is complementary to DSC and may facilitate the interpretation of the DSC results. The different thermomicroscopic investigations indicated that, for the PMs K-25, NIF was

dissolved in the melted PVP. Changes in the PMs K-25 during heating were detected by HSM. The photographs in Fig. 23 demonstrate the morphology of PM K-25 in 1:1 ratio from the beginning of heating up to 157 ^{o}C. With increase of temperature, the PVP began to melt, and above 110 ^{o}C its melting was complete. It may be seen that the NIF particles were dispersed in the melt and subsequently dissolved with rising temperature. During heating, the drug particle size was steadily reduced, showing its dissolution in the PVP melt. The NIF was completely dissolved in the melt of PVP at close to 157 ^{o}C, a temperature about 50 ^{o}C lower than the melting point of the pure drug. This finding can explain the absence of any sign of melting in the DSC thermograms of the PMs K-25.

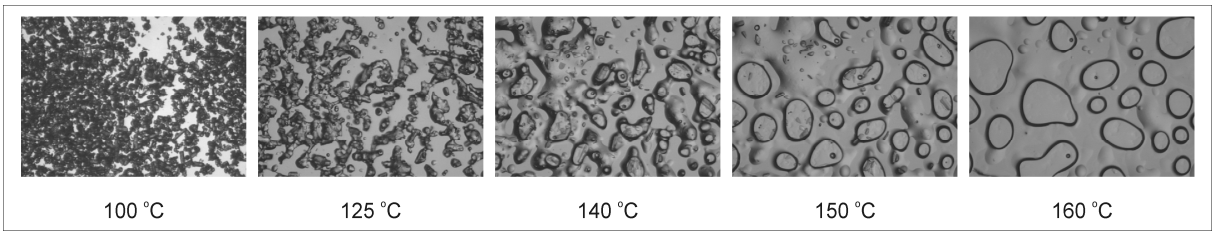

Fig. 23. HSM photographs of PM K-25 1:1

8.2.5. Fourier transform infrared spectroscopy

The interactions between the polymers and NIF were also studied by IR spectroscopy. Evaluation of spectral changes was performed by subtraction of the polymer spectrum from the spectra of the samples prepared by various methods. Two relatively broad and weak bands, at 1495 cm^{-1} and 846 cm^{-1}, marked with arrows in Fig. 24 [A], were used to determine the proper subtraction parameter.

All difference spectra deduced from the samples prepared by physical mixing of the ingredients, were practically identical to the spectrum of the pure NIF, indicating negligible interaction between the polymer and it. There was no considerable effect due to the various mass ratios between them (Fig. 24 [B]).

On the other hand, the solvent evaporation method resulted in essential changes in the molecular state of the NIF. Two strong bands disappeared from the original spectra, at 1615 and 1428 cm^{-1}, and a new one developed at 1683 cm^{-1} (Fig. 24 [C, D]). The most feasible explanation for these changes can be given as follows. The compounds containing carboxylate and amino groups are always in zwitterionic state in solid crystalline form, like amino acids. The dissolution of the samples in solvents such as alcohol or acetone usually reverses the proton transfer. As a result of the preparation procedure, the proton transfer was prevented in the highly dispersed NIF, so the antisymmetric and symmetric stretching bands of the

carboxylate group disappeared and the C=O stretching mode of the carboxylate group developed in the spectra. Fast evaporation of the solvent and the formation of an amorphous, highly dispersed, solid phase prevents the transfer of the proton. This is a higher energy state since the enthalpy difference between the carboxyl O-H and the ammonium N^+-H bond and the lattice energy originating from the coulombic interactions of the crystalline state are also missing. A higher energy state results in a higher equilibrium concentration and higher solubility.

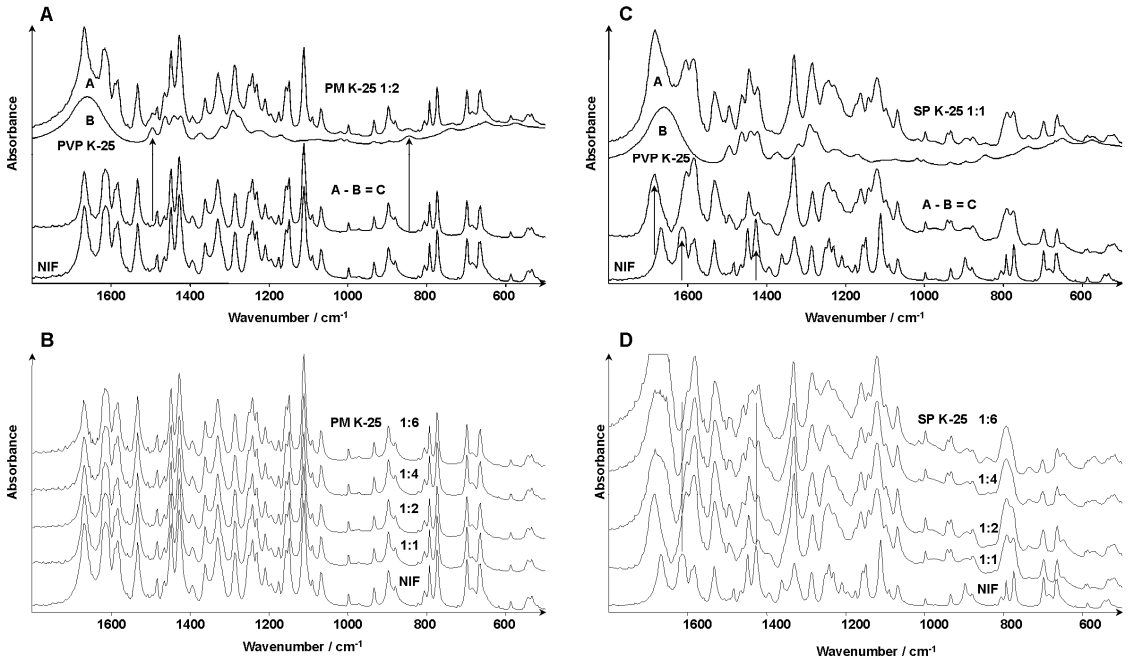

Fig. 24. FT-IR spectra of (A) NIF and PVP K-25, (B) NIF and PMs K-25, (C) NIF and SD K-25 1:1, (D) NIF and SDs K-25

The spray-drying method resulted in similar changes in the molecular state of NIF to the SPs. Two strong bands disappeared from the original, spectra at 1615 and 1428 cm^{-1}, the antisymmetric and symmetric carboxylate stretching modes of the zwitterion form, which is characteristic for amino acids. A new one developed at 1683 cm^{-1} (see Fig. 25), the C=O stretching mode of the protonated carboxyl group. Dissolution in alcohols reverses the proton transfer and NIF returns into the neutral, amine-carboxylic form. The preparation of the samples, the interaction between the polymer and the highly dispersed, amorphous, solid phase prevents the proton transfer. This is a higher energy state since the enthalpy difference between the carboxyl O-H and the ammonium N^+-H bond and the lattice energy originating from the Coulombic interactions of the crystalline state are also missing. The higher energy state for the solid phase results in a higher equilibrium concentration and a higher solubility.

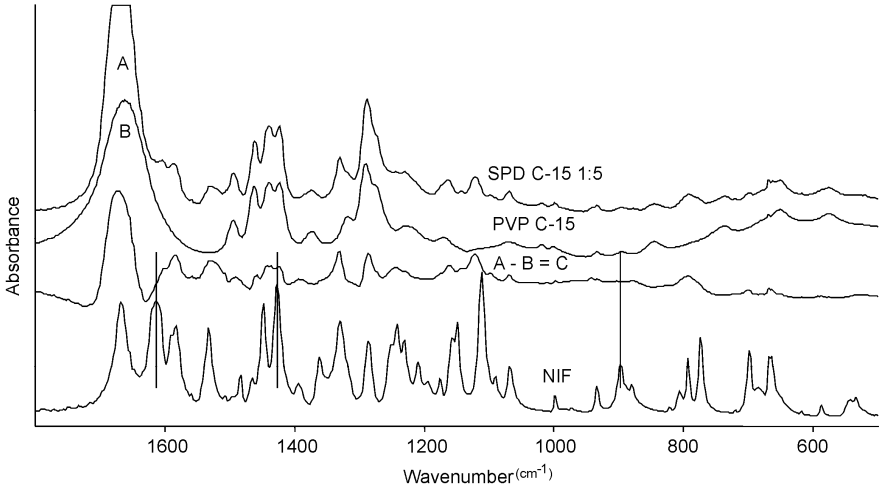

Fig. 25. FT-IR spectra of PVP C-15 and SPD C-15 1:5

8.2.6. X-ray powder diffraction analysis

The XRPD patterns of NIF, PVP K-25, PM K-25 1:6 and SP K-25 1:6 are shown in Fig. 26. PVP is an amorphous powder without crystalline structure. The presence of numerous distinct peaks in the XRPD spectrum indicates that NIF is a crystalline material; its characteristic peaks appear at diffraction angles 2θ at 8.18, 12.92, 16.33, 21.30 and 23.21^{o}. The XRPD peaks of NIF in the PMs K-25 were similar to those for the pure drug, indicating that the crystallinity of NIF was not changed in these products. However, the crystalline structures of NIF in all the solid dispersions were different from that of the pure drug, as revealed by the differences in their XRPD patterns. These patterns were similar to those for the PVPs. The absence of diffraction peaks indicated the presence of NIF in amorphous form.

The XRPD patterns of NIF, PVP C-15, and SPD C-15 1:5 are shown in Fig. 27. PVP is an amorphous powder with no crystalline structure. The absence of diffraction peaks indicated the presence of NIF in amorphous form, similarly to the SPs. *Sekikawa et al.* **[124]** pointed out that PVP might inhibit the association of the drug molecule to form the crystal nucleus and thereby inhibit crystal growth; the interaction between the drug and the PVP should be the inhibitory and/or retarding factor in the crystallization.

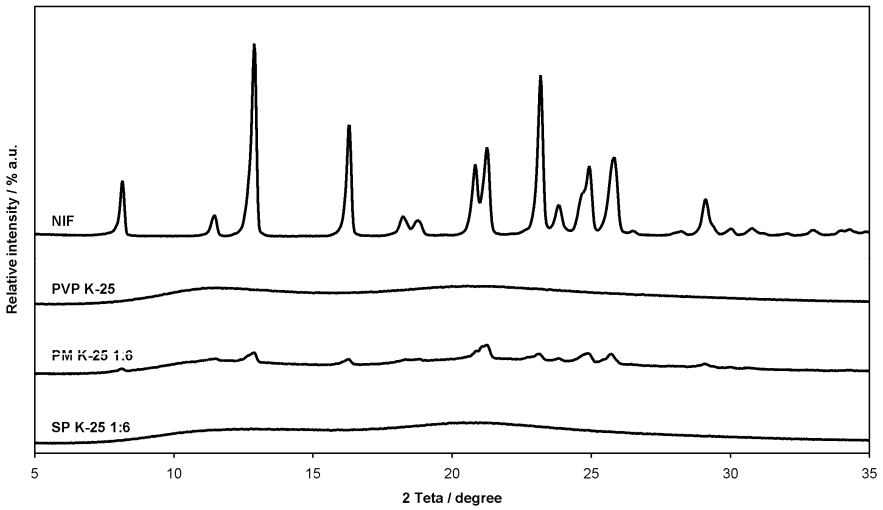

Fig. 26. XRPD patterns of NIF, PVP K-25, PM K-25 1:6 and SP K-25 1:6

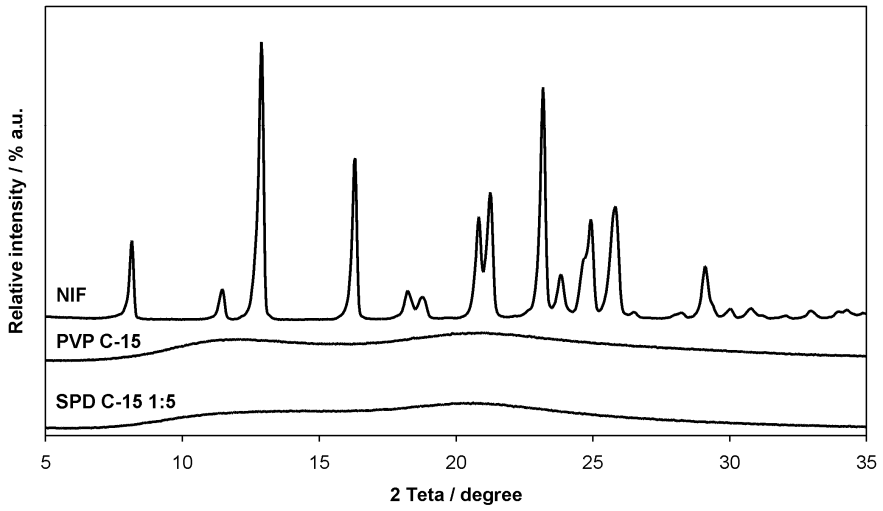

Fig. 27. XRPD patterns of NIF, PVP C-15 and SPD C-15 1:5

9. SUMMARY

In consequence of the poor water-solubility of the pharmaceutical ingredient, NIF, my aim was to increase its solubility and dissolution rate by applying several methods. This work involved a preformulation study to introduce the technological possibilities of a generic formulation.

The research work can be summarized as follows:

1) The solubility-increasing effects of the available CD derivatives were determined under uniform conditions. It was found, that the solubility of NIF was always increased by the CDs, and especially for NIF with HP-β-CD to 2.5-fold.
2) Different preparative mole ratios (2:1, 1:1, 1:2 and 1:3) and three methods (PMs-physical mixtures, KPs-kneaded products and USs-ultrasonicated products) were applied to form complexes, and 15 m/m% PVP K-90 was used to prepare ternary systems (PMs+PVP and KPs+PVP) to improve the efficacy of complexation.
3) To observe the good effect of a water-soluble polymer, PVP, on the complexation, solid dispersions were prepared in various mass ratios (1:1, 1:2, 1:4, 1:5, 1:6 and 1:10), using different types of PVP and applying three methods (PMs-physical mixtures, SPs-solvented products and SPDs-spray-dried products).
4) The wettability study indicated that the products had a hydrophilic character as compared with NIF. Significantly lower wetting angles were measured for all samples, the decrease ranging from 71º to 26º. There was a parallel result for the wettability relative to the saturation concentration in water. A significant difference was observed for the SPDs, where the concentraration was 413 μg/ml compared with the pure NIF (26 μg/ml).
5) A concerns the morphology of the solid dispersions, determined via particle size analysis, the average lenght of the crystalline drug was changed during the solvent evaporation method, the size decreasing from 45 μm to 13 μm. These products have small particles, which is important in the formulation of solid dosage forms.
6) 38 different samples were examined as regards their dissolution with the rotating basket tester in SGM and SIM. The dissolution was better in SIM for all samples, except the binary kneaded products. The same dissolution phenomena were observed for the KPs, KPs+PVP, SPs and SPDs in SGM, where a 4-fold solubility increase was detected. In SIM, depending the preparation methods, 3.5 (KPs), 7.5 (KPs+PVP) and 14 (SPs and SPDs)-fold increases were demonstrated.

7) The intensity of the dissolution depended on the preparation method. The PMs and the USs always displayed prolonged dissolution profiles. The addition of PVP and the use of an organic solvent, such as ethanol, methanol or acetone containing amorphous material led to rapid dissolution. It was also typical that the saturation concentration was reached in 5–10 min for solid dispersions, while the samples containing CDs needed 15–20 min to reach the same state. The dissolution rate increase was 1.5–2.5-fold for binary and ternary systems with CD, and 5–51-fold for the solid dispersions.
8) According to the BCS, NIF has good permeability and very bad solubility, which is reflected by the *n*-octanol distribution, which showed the high *n*-octanol solubility and low water solubility of NIF. The products resulted in a 2–4-fold better diffusion as compared with the original NIF in SGM. NIF had a K_d value of 2.36 10^{-3} cm min^{-1}, while the K_d values for the samples were improved. The SD and SPD with a mass ratio of 1:1 displayed the highest K_d values.
9) The DSC curves demonstrated crystalline NIF for samples containing CD. When PVP was used, the melting point of the drug was not detected. During heating, the drug particle size was steadily reduced, showing its dissolution in the PVP melt. The NIF was completely dissolved in the melt of PVP at close to 157 ^{o}C, a temperature about 50 ^{o}C lower than the melting point of the pure drug. This finding can explain the absence of any sign of melting in the DSC thermograms of the PMs K-25.
10) The structural characterization, like the FT-IR spectra, did not show new bonds in the case of samples with CDs. Thus, no inclusion complexation was presumable. However, for the solid dispersion systems, where two strong bands disappeared from the original spectra, at 1615 and 1428 cm^{-1}, a new one developed at 1683 cm^{-1}.
11) The crystalline structures of NIF in all the solid dispersions were different from that of the pure drug, as revealed by the differences in their XRPD patterns. These patterns were similar to those for the PVPs. The absence of diffraction peaks indicated the presence of NIF in amorphous form. This is a higher energy state for the solid phase, resulting in a higher equilibrium concentration and a higher solubility.

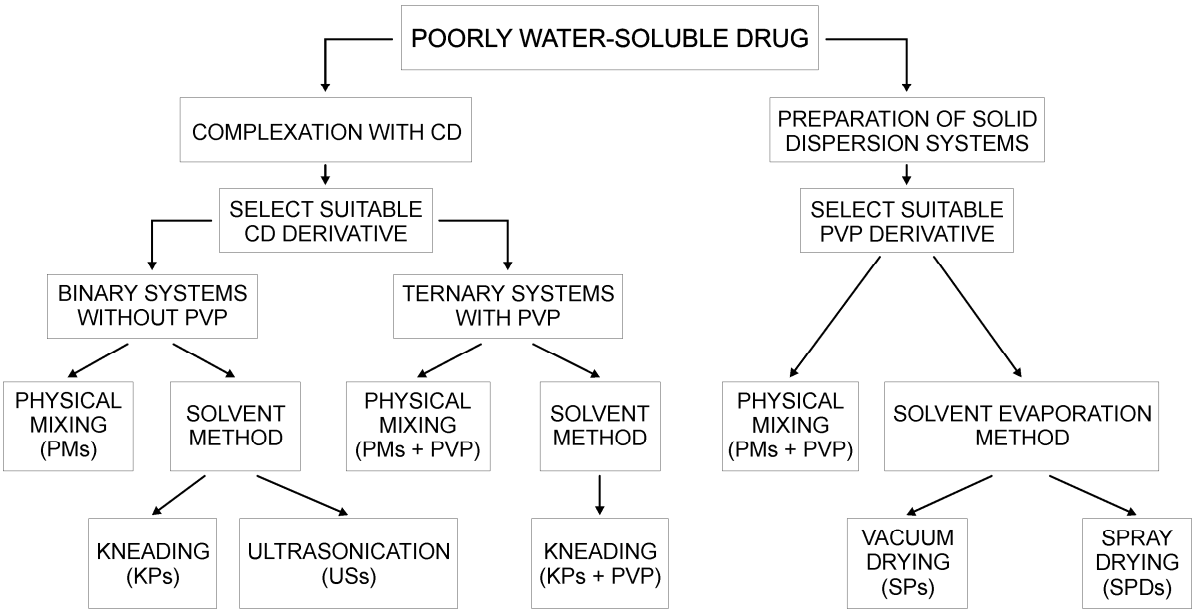

Fig. 28. Summarized technological protocol of the Thesis

To summarize the results, the goal of this study that the solid dispersion systems are more favourable than using CD to improve the solubility and dissolution rate of nifluminic acid. Using CD, I suggest the ultrasonicated binary and the kneaded ternary 1:3 products to prepare semisolid dosage forms. In these cases the permeability and wettability properties of the drug are very useful. Spray-drying, like the solvent evaporation method using 30 v/v% of ethanol, resulted in rapid drug release, it is suitable for solid dosage form formulation. After this substantial preformulation study, further biopharmaceutical investigations should be performed, like gastric toxicity, blood level concentration and oedema inhibiting effect of the products. Fig. 28 present the steps of the technological protocol in the case of nifluminic acid. These methods may help by the generic formulation of other poorly water-soluble pharmacons, so the applied drug quantity and the unwanted side-effects can therefore be decreased.

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