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Doctoral dissertation

Enhancement of the dissolution of meloxicam in binary systems

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Publications

I. **P. Reisi Nassab**, R. Rajkó, P. Szabó-Révész
Physicochemical characterization of meloxicam-mannitol binary system
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II. A. Szűts, O. Laczkovich, **P. Reisi Nassab**, Z. Aigner, P. Szabó–Révész:
Applicability of sugar esters in hot-melt technology
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III. **P. Reisi Nassab**, Zs. Tüske, P. Kàsa Jr., A. Bashiri-Shahroodi, P. Szabó-Révész
Influence of work of adhesion on dissolution rate in binary solid systems
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IV. A. Bashiri-Shahroodi, R. Rajkó, **P. Reisi Nassab**, P. Szabó-Révész
Preparation of a solid dispersion by a dropping method to improve the rate of dissolution of meloxicam
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V. ***P. Reisi Nassab****, G. Blazsó, T. Nyári, G. Falkay, P. Szabó-Révész*
In vitro and in vivo investigations on the binary meloxicam-mannitol system
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Abstracts

I. **P. Reisi Nassab**, P. Szabó-Révész, A. Bashiri-Shahroodi, I. Erös
Dissolution properties of meloxicam-mannitol binary system
6th Central European Symposium on Pharmaceutical Technology and Biotechnology. Siófok, Hungary. May 25-27, 2005
Eur. J. Pharm. Sci. (2005), 25/S1, S161-162

II. P. Szabó-Révész, **P. R. Nassab**, R. Rajkó
Dissolution properties of meloxicam-mannitol binary system
5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology. Geneva, Switzerland. 27-30 March, 2006

III. **P. Reisi Nassab**, P. Szabó-Révész
Effect of particle size and carrier on dissolution behaviour of meloxicam
European Federation for Pharmaceutical Sciences. Verona, Italy. April 26-27, 2006

IV. **P. Reisi Nassab**
Physicochemical characterization of meloxicam-mannitol binary systems
Ph.D Tudományos nap. Szeged, Hungary. May 3, 2006

V. **P. Reisi Nassab**, P. Kàsa Jr, P. Szabó-Révész
Effect of particle size and carrier amount on dissolution properties of meloxicam
Congressus Pharmaceuticus Hungaricus XIII. Budapest, Hungary. May 25-27, 2006

VI. **P. Reisi Nassab**, A. Szűts, P. Szabó-Révész
Dissolution improvement of meloxicam for development of solid dosage form
Gyógyszerkutatàsi Szimpózium. Debrecen, Hungary. November 24-25, 2006

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1. Introduction

The oral route is the one most frequently used for drug administration. Oral dosage forms are usually intended for systemic effects resulting from drug absorption through the various epithelia and mucosa of the GI tract. Compared with other routes, the oral route is the simplest, the most convenient and safest means of drug administration.

The physicochemical properties of a drug play an important role in drug absorption. Solubility is one of the physicochemical properties of the drug material. On the other hand, solid drugs need to dissolve before they can be absorbed.

The poor solubility of drug substances in water and their low dissolution rate in the aqueous gastro-intestinal fluids often lead to insufficient bioavailability and is one of the most difficult and unsolved problems in pharmaceutical technology. It is estimated that more than 35% of the known drugs and more than 25% of the newly discovered drugs face such a problem. Choosing a proper method to improve solubility remains one of the most challenging aspects in pharmaceutical technology. That is why different methods have been used to overcome this problem, e. g: increasing the surface area of a drug, complexation, salt formation, etc. but using a water-soluble material as a carrier is one of the easiest methods.

On top of that, fast dissolution is important in the case of some diseases in which the fast effect of the drug is necessary, such as analgetics, antipyretics, etc.

Drugs are absorbed in two general ways, by passive diffusion and by specialized transport mechanisms. Lipid solubility and the degree of the inonization of the drug at the absorbing site influence the rate of diffusion. Several specialized transport mechanisms are postulated, including active and facilitated transport.

Once the drug is absorbed, it can exert a therapeutic effect either locally or at a site of action remote form that of administration.

Oral dosage forms have to go through long processes to be absorbed by the body, pharmaceutical methods can be useful in this field to increase the bioavailability of the drug.

2. Aim

The main aim was to improve the dissolution rate of meloxicam (ME) (a new member of NSAID) in capsule form. Meloxicam is practically insoluble in water but its permeability is high so we can classify it in class II of the Biopharmaceutical Classification System (BCS).

- First of all the influence of the particle size of pure MEs was investigated on dissolution rate. According to Noyes-Whitney, decreasing of particle size (increasing of surface area) is one of the methods to increase dissolution rate.
- In the second step the water-soluble carrier was used to improve the dissolution of ME. Binary systems (physical mixture and melted products) were made by using different amounts of mannitol as a carrier.
- DSC, X-ray diffractometry, morphological investigation and chemometric methods were used to study the crystal's structure of ME and its products.
- Cohesion and adhesion, as well as surface free energy were investigated in order to possibly predict dissolution rate.
- The last step was the investigation of the efficacy of ideal interactive mixtures on animals (rats).

3. Literature

There are factors that affect oral drug absorption, these factors can be classified as

- External factors (physicochemical properties of the drug)
- Internal factors (intestinal conditions: anatomy and physiology of GI)

All the factors are important with respect to absorption.

3.1 Factors affecting oral drug absorption

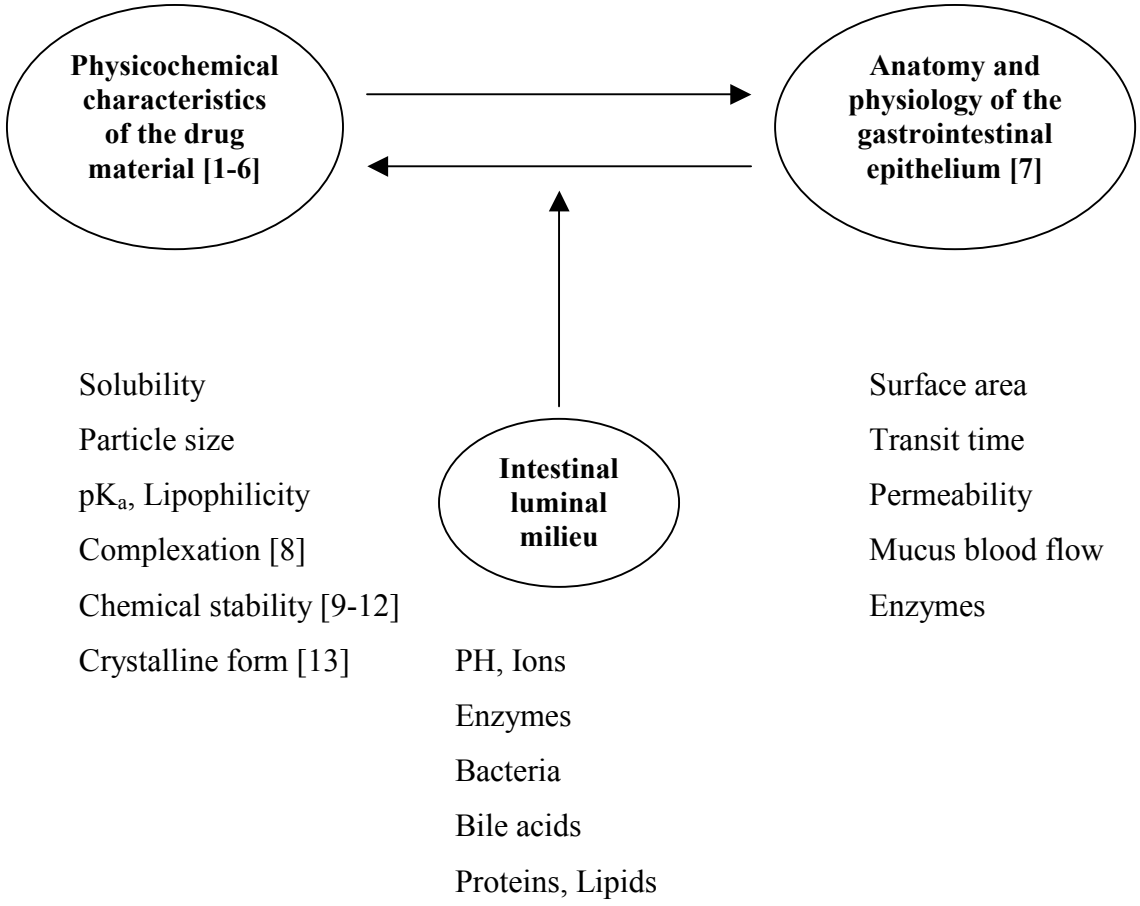

Fig. 1 Interrelationships between the factors affecting oral drug absorption

The physicochemical characterizations of a drug that may effect an increase in oral absorption, are as follow:

- **Solubility**

The extent to which the dissolution proceeds under a given set of experimental conditions is refferred to as the solubility of the solute in the solvent. All drugs, by whatever route they are administered, must exhibit at least limited aqueous solubility for therapeutic efficiency. Thus relatively insoluble compounds can exhibit erratic or incomplete absorption, and it might be appropriate to use more soluble salt or other chemical derivatives.

Alternatively, micronizing, complexation or solid dispersion techniques might be employed to increase the solubility or dissolution rate of insoluble materials [14,15].

- **Lipophilicity, partition coefficient and pK_a**

The lipid solubility or lipophilicity of drugs has long been recognized as a prerequisite for transcellular diffusion across the intestinal membrane.

Traditionally, the lipophilicity of drug substances is expressed as the partition coefficient or distribution coefficient (log *P*) between n-octanol and an aqueous buffer (pH 7.4), which is pH-dependent in the case of ionizable compounds [16]. In general, compounds with low log *P* are poorly absorbed, whereas compounds with log *P*>-1 offer satisfactory absorption. It is important, however, that the drug possess an optimum lipophilicity, as too low or too high lipophilicity may result in less than optimum oral bioavailability. For example, in the case of disodium we did not observe any significant improvement in oral absorption of disodium cromoglycate (log *P*< -3) despite the fact that lipophilicity was increased to a lipophilic (diethyl) ester prodrug (log *P*= 1.78) [17].

This observation was attributed to a marked decrease in water solubility simultaneously with increases in lipophilicity. Similarly, it was reported for the first time that high lipophilicity (log *P*>3.5) decreases drug transport across the intestinal epithelial cells and could be accounted for loss of *in vivo* biological activity [18]. The ”cut-off” point of *P* value, that is the *P* value corresponding to an optimal transepithelial passage of drug, was found to be around 3000 [19].

- **Permeability**

Permeation rate is dependent upon the size, relative aqueous and lipid solubility and ionic charge of drug molecules, factors that can be altered through molecular modifications.

The absorbing membrane acts as a lipophilic barrier to the passage of the drug molecule [20]. The partition coefficient, for example between oil and water, is a measure of lipophilic character. The majority of drugs are weak acids or bases and, depending on their pH, exist in an ionized or unionized form.

Membranes of absorbing mucosa are more permeable to unionized forms of drugs than to ionized species because of the greater lipid solubility of the cell membrane, which results in the binding or repelling of the ionized drug, thereby decreasing penetration [21].

Therefore, the dominating factors that influence the absorption of weak acids and bases are the pH at the site of absorption and the lipid solubility of the unionized species [22]. These factors, together with the Henderson-Hasselbalch equations [23] for calculating the proportions of ionized and unionized species at a particular pH, constitute the pH-partition

theory for drug absorption. However, it has to be mentioned that other factors are clearly involved in absorption.

- **Polymorphism**

Practically all drug substances are handled in powder form at some stage during manufacture into dosage forms. However, for those substances composed of, or containing, powders or compressed powders in the finished product, the crystal properties and solid-state form of the drug must be carefully considered.

It is well recognized that drug substances can be amorphous (i.e. without regular molecular lattice arrangements), crystalline, anhydrous, at various degrees of hydration or solvated with other entrapped solvent molecules, as well as varying in crystal hardness, shape and size. In addition, many drug substances can exist in more than one form, with different molecular packing arrangements in the crystal lattice.

This property is termed plymorphism, and different polymorphs may be prepared by manipulating the conditions of particle formation during crystallization, such as solvent, temperature and rate of cooling. It is known that only one form of a drug substance is stable at a given temperature and pressure, with the other forms, termed metastable, converting at different rates of the stable crystalline form.

The different polymorphs vary in physical properties such as dissolution and solid-state stability, as well as processing behaviour in terms of powder flow and compaction during tableting in some cases. These different crystalline forms canbe of considerable importance in relation to the ease or difficulty of formulation and as regards stability and biological activity.

As might be expected, higher dissolution rates are obtained for metastable polymorphic forms; for example, the metastable form of chloratetracyline hydrochloride exhibits improved rate and extent of bioavailability. In some cases, amorphous forms are more active than crystalline forms [24-28].

3.1.2 Biopharmaceutical Classification System (BCS)

According to Amidon a biopharmaceutical classification system has been proposed which classifies drugs into four classes according to their solubility across the gastrointestinal pH range and their permeability across the gastrointestinal mucosa [29-31].

According to the solubility classification by WHO, high solubility is applied when: the highest single dose of the drug divided by the highest solubility of the compound over the pH range 1-7.5 at 37ºC is < 250 ml, high permeability is applied to a drug when: the absorbed amount is > 90% (measured in human, animal, suitable cell lines).

Class I: high solubility/high permeability
Class II: low solubility/high permeability
Class III: high solubility/low permeability
Class IV: low solubility/low permeability

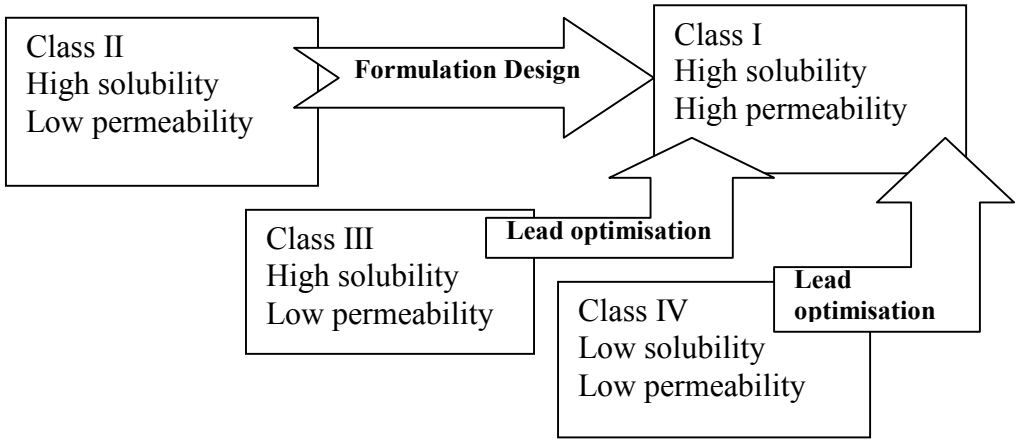

Fig. 2 Biopharmaceutical Classification System

As it can be seen, changing a class to the other one requires some techniques which can keep the efficacy of the active agent meanwhile improve its solubility and permeability to perform a sufficient effect or cause a faster action of it.

3.2 Improving of dissolution rate

The dissolution of a solid in a liquid may be regarded as being composed of two consecutive stages.

1. First is an interfacial reaction that results in the liberation of solute molecules from the solid phase. This involves a phase change, so that molecules of solid become molecules of solute in the solvent in which the crystal is dissolving. Because it is in direct contact with undissolved solid, its concentration will be C_S (a saturated solution).
2. The solute molecules must migrate through the boundary layers surrounding the crystal to the bulk of the solution, at which time its concentration will be C. This step involves the transport of these molecules away from the solid-liquid interface into the bulk of the liquid phase under the influence of diffusion of convection. Boundary layers are static or slow-moving layers of liquid that surround all wetted solid surfaces. Mass transfer takes place more slowly through these static or slow-moving layers, which inhibit the movement of solute molecules from the surface of the solid to the bulk of the solution. The concentration of the solution in the boundary layers changes therefore from being saturated (C_S) at the crystal surface to being equal to that of the bulk of the solution (C) at its outer most limit [8, 32-34].

Like any reaction that involves consecutive stages, the overall rate of dissolution will depend on whichever of these steps is the slowest (the rate-determining or rate limiting step).

In dissolution the interfacial step (1) is virtually instantaneous and so the rate of dissolution will be determined by the rate of the slower step (2), of the diffusion of the dissolved solute across the static boundary layer of liquid that exists at a solid-liquid interface.

The dissolution of a drug is described in a simplified manner by the Noyes-Whitney equation [35]:

$$\frac{dm}{dt} = \frac{DA(C_S - C)}{h}$$

where $\frac{dm}{dt}$ is the dissolution rate, D is the diffusion coefficient of the compound, A is the surface area of the dissolving solid, C_S is the solubility of the compound in the dissolution medium, C is the concentration of drug in the dissolution medium at time t and h is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound [8].

The main possibilities for improving dissolution according to this analysis are to increase the surface area available for dissolution by decreasing the particle size of the solid compound and/or by optimizing the wetting characteristics of the compound surface, to decrease the boundary layer thickness, to ensure sink conditions for dissolution and improve the apparent solubility of the drug under physiologically relevant conditions.

Many methods have been described in relation to the determination of the rate of release of drugs into solution from tablet and capsule formulations, because such release may have an important effect on the therapeutic efficiency of these dosage forms. Methods such as [36-38]: Beaker method, Flask-stirrer, Rotating basket method, Paddle method, Rotating and static disc methods.

The most attractive option for increasing the release rate is the improvement of solubility through formulation approaches. Here is a summary of the various formulation and chemical approaches that can be taken to improve solubility or to increase the available surface area for dissolution and as a result to increase absorption.

I. Physical modifications

- Particle size: Grinding and micronization [39-42]
- Modifications of the crystal habit: Polymorphs [43, 44], pseudopolymorphs (including solvates) and complexation/solubilization (use of surfactants, cyclodextrines, various solvents) [45-52]
- Drug dispersion in carriers: Eutectic mixtures, solid dispersions (non-molecular) and solid solutions [53-58]

II. Chemical modification

- Soluble prodrugs
- Salts [59, 60]

Particle size and carrier

As it was mentioned changing of particle size is the first choice of modifications. Following the administration of the medicine, the dosage form should release the drug into solution at the optimum rate, this depends on several factors, one of which will be the particle size of the drug. According to Noyes and Whitney, the rate of solution of a solid was related to the law of diffusion thus particles having small dimensions will tend to increase the rate of dissolution [32].

Decreasing the particle size of the compound by milling the drug powder theoretically results in an increase in the available area for dissolution, but in some cases the micronized powder tends to agglomerate [8].

Molecules at the surface of a material have a net inward force exerted on them from the molecules in the bulk: this is the basis of surface energy. Surface energy is important, as every interaction (except the mixing of two gases) starts with an initial contact between two surfaces. If this surface interaction is favoured then the process will probably proceed, whereas if it is not favoured then it will be limited. A good example of the role of surface energy is the wetting of a powder by a liquid, where the powder cannot dissolve until the liquid makes good contact with it [61].

Change in the wetting of powders can affect the processes of wet drug dissolution. A rough estimate of the wettability of a hydrophobic drug by a given medium can be obtained from the contact angle at the liquid/solid interface and the structure of the drug. When a compound is not very well wetted by water, that is to say its contact angle is high, native surfactants in the GI tract may assist the wetting of the drug by the luminal fluids, so that the ability of the fluid to penetrate between particles and into pores is increased [62, 63].

Water-soluble carriers (ex. polyol), have attracted attention as the most popular substances used in binary systems to increase the rate of dissolution of an active ingredient in a physical mixture or melted product (solid dispersion) [64-66].

β-D-mannitol was chosen to serve as a carrier in binary system with meloxicam. Mannitol is a sugar alcohol with a sweet taste but a low calorie content, it is stable to heat and melts without decomposition [67, 68]. It is widely used in the pharmaceutical and food industries.

3.3 Non-Steroidal Anti-Inflammatory Drugs (NSAID)

The anti-inflammatory, analgesic and antipyretic drugs are heterogeneous group of compounds, often chemically unrelated, which nevertheless share certain therapeutic actions and side effects. The protype is aspirin, hence these compounds are often referred to as aspirin-like drugs. They are also frequently called non-steroidal anti-inflammatory drugs or NSAID [69].

Meloxicam (**ME**) (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-benzothiazine-3-carboxamide-1,1-dioxide) is a highly potent non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class of oxicam derivatives.

ME is a potent inhibitor of cyclooxygenase (COX), and in several models exhibits selectivity for the inducible isoenzyme COX2. It is used to treat rheumatoid arthritis, osteoarthritis and other joint diseases [70-72]. Besides its main function as an anti-inflammatory drug, it is also emerging as a useful agent in Alzheimer's disease and cancer (mainly colorectal and adenocarcinoma) treatment [73-76]. Other advantages of meloxicam are:

- less irritation of gastrointestinal and local tissue (dermal, rectal and ocular), although meloxicam is mainly absorbed in the duodenum (intestinal absorption) [77].
- fewer renal side-effects as compared with other NSAIDs [78-80].

Like many other NSAIDs, ME is practically insoluble in water. ME can be graded in Class II of the Biopharmaceutical Classification System, which means low aqueous solubility and rapid absorption (high permeability) through the gastrointestinal tract [77].

Some methods are used to improve the dissolution rate of meloxicam, such as: complexation by cyclodextrin, salt formation, solid dispersion with PEG 6000 [42, 49, 60, 81].

3.4 In vitro/in vivo correlation (IVIVC)

In general, the prediction of oral drug absorption in humans is either an indirect result from bioavailability measurements obtained from evaluation of *in vivo* animal data, usually from the rat or dog, or can be predicted from *in vitro* permeability methods, such as partition coefficients, Caco-2, Ussing chamber, intestinal perfusions, etc [82-85].

Conceptually, IVIVC describes a relationship between the *in vitro* dissolution/release versus the *in vivo* absorption. This relationship is an important item of research in the development of drug delivery systems. *In vitro* dissolution testing serves as a guidance tool to the formulator regarding product design and in quality control [86], it is of specific importance especially for modified-release dosage forms, which are intended for the purpose of prolonging, sustaining, or extending the release of drugs. By applying mathematical principles, such as linear system analysis or moment analysis, data describing *in vitro* as well as *in vivo* processes can be obtained [87-89].

Developing a predictable IVIVC depends upon the complexity of the delivery system, its formulation composition, method of manufacture, the physicochemical properties of the drug and the dissolution method.

IVIVC models can be developed by defining the correlation(s) between *in vitro* dissolution as *in vivo* input rate. A successful IVIVC model can be developed if *in vitro*

dissolution in the rate limiting step in the sequence of events leads to the appearance of the drug in the systemic circulation following oral or other routes of administration. Thus the dissolution test can be utilized as a surrogate performance of the product.

For orally administered dugs, IVIVC is expected for highly permeable drugs, or drugs under dissolution rate-limiting conditions, which is supported by the BCS [90-91]. For extended-release formulations following oral administration, modified BCS containing the three classes (high aqueous solubility, low aqueous solubility, and variable solubility) is proposed [92].

Based on the type of data used to establish the relationship, three main levels are defined by the FDA [93-97]:

- **Level A**

A correlation of this type is generally linear and represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate (e.g., the *in vivo* dissolution of the drug from the dosage form). In a linear correlation, the *in vitro* dissolution and *in vivo* input curves may be directly superimposable or may be made to be superimposable by the use of a scaling factor. Nonlinear correlation, while uncommon, may also be appropriate, alternative approaches to developing a Level A, IVIVC are possible. Whatever the method used to establish a Level A IVIVC, the model should predict the entire *in vivo* time course from the in vitro data.

- **Level B**

A Level B IVIVC uses the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared either to the mean residence time or to the mean *in vivo* dissolution time. A Level B correlation does not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time values.

- **Level C**

A Level C IVIVC establishes a single point relationship between a dissolution parameter, for example, $t_{50\%}$ percent dissolved in 4 hours and a pharmacokinetic parameter (e.g., AUC, C_{max}, $T_{max)}$. A Level C correlation does not reflect the complete shape of the plasma concentration-time curve, which is the critical factor that defines the performance of ER (extend release) products. In addition to these levels, a combination of various level C is also described:

A multiple Level C correlation relates one or several pharmacokinetic parameters of interest to the amount of dug dissolved at several time points of the dissolution profile. For the

establishment of a correlation as described in the FDA guidance, various parameters can be used as presented in Table 1.

Table 1 Various parameters used in IVIVC depending on the level

Level	In vitro	In vivo
A	Dissolution	Input (absorption curves)
B	Statistical moments: MDT	Statistical moments: MRT,MAT,etc
C	Disintegration time, time to have 10, 50, 90% dissolved, dissolution rate, dissolution efficiency	C_{max}, T_{max}, K_a, time to have 10, 50, 90 % absorbed, AUC (total or cumulative)

Level A correlation uses all the information of the dissolution and absorption curves, in contrast to levels B or C. The establishment of a relationship implies the use of many formulations, each of them giving one pair of data (vitro and vivo). It is obvious that level B or C needs more data and, as they do not use all the information related to the vitro and vivo behaviour of the formulation, they are less powerful.

The FDA ranked the levels as follows:

A Level A IVIVC is considered the most informative and is recommended, if possible. Multiple Level C correlation can be as useful as Level A correlations. However, if a multiple Level C correlation is possible, then a Level A correlation is also likely and is preferred.

Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected.

Level B correlations are the least useful for regulatory purposes.

In accordance with the above-mentioned, there were some *in vivo* and clinical experiments in the case of meloxicam, either as an analgesic or an anti-inflammatory agent [98-102].

4. Materials and Methods

4.1 Materials

Meloxicam (Ph. Br. 2001) is a pale yellow powder. It is practically insoluble in water; very slightly soluble in alcohol and in methyl alcohol, slightly soluble in acetone; soluble in dimethyl formamide. The molecular weight of meloxicam is 351.4 and it melts at 253-255ºC. Mobic (generic name of meloxicam) is available as a tablet for oral administration containing 7.5 mg of meloxicam, mostly used in the case of rheumatoid pain.

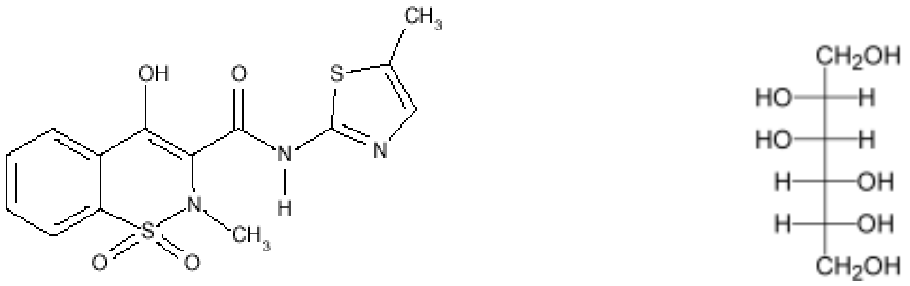

Fig. 3 Structures of meloxicam and mannitol

Mannitol (USP. 25th) is a white odourless crystalline powder or free-flowing granules with a sweet taste. 1g is soluble in 5.5ml of water, it is very slightly soluble in alcohol; practically insoluble in ether. The molecular weight of mannitol ($C_6H_{14}O_6$) is 182.17 and it melts at 166-168ºC. It is used in pharmacy as an excipient and diluent for solids and liquids, in pharmaceutical and food industries as a sweetener [67].

The particle sizes and specific surface areas of MEs and mannitol were measured by laser diffraction (Malvern Mastersizer 2000, Malvern Ltd., Worcestershire, UK). For the measurements, the materials were dispersed with air and deagglomerated at an air pressure of 0.5 bar. The particle size was determined in the range 0.02-2000 μm. The specific surfaces of the samples were calculated from the particle size data. The measurements were repeated three times.

Table 2 Particle sizes and specific surface areas of the materials

Material	d(10%) µm	d(50%) µm	d(90%) µm	Specific surface area (m^2/g)
ME1	50.50	106.66	206.20	0.071
ME400	1.62	25.74	131.15	1.370
ME2	0.72	2.49	5.97	2.514
Mannitol	17.61	86.74	239.45	0.226

4.2 Methods

4.2.1 Preparation of physical mixtures (PMs)

PMs of ME1, ME2, and ME400 with mannitol (PM1, PM2 and PM400**)** in (drug:carrier) ratios of 3:7 and 1:10 were obtained by mixing the individual components for 10 min in a Turbula mixer (Turbula WAB, Systems Schatz, Basel, Switzerland) at 50 rpm.

4.2.2 Preparation of melted products (MPs)

MPs of ME1 and ME2 and mannitol (ME1-MP and ME2-MP) in ratios of 3:7 (w/w) and 1:10 (w/w) were made as follows: The MEs were added to melted mannitol (170 °C) and the melts solidified at room temperature (20±1 °C). The products were triturated in a mortar and were sieved. The particle size range of the products was between 100 and 250µm.

- **Capsule filling**

The products of MP and PM binary systems were measured by balance and filled into hard gelatin capsules (No. 2) by hand. Each one of the capsules contained 15 mg of ME. (The therapeutic dose of ME is 7.5–15 mg.)

4.2.3 Dissolution studies

Dissolution tests were performed by using Pharmatest equipment (Hainburg, Germany) at a paddle speed of 100 rpm. 900 ml of artificial enteric juice pH 7.5 (± 0.1) (Ph.Eur.4) at 37 °C (± 0.5 °C) was used. The ME contents of the samples were measured spectrophotometrically at 362 nm (Helios α Spectronic, Unicam, Cambridge, UK). The dissolution experiments were conducted in triplicate.

Kinetic equations (e.g. First order, Zero order) were used to describe the rate of the dissolution [103, 104].

4.2.4 Contact angle measurements

Compacts of the powders (150 mg) were made with a highly polished stainless steel punch (13 mm in diameter) in a Specac hydraulic press (Specac, England) with a 20 s dwell time at a pressure of 4×10^8 Pa.

The contact angle (θ) of the solids was determined by means of the sessile drop technique (OCA 20 Dataphysics Instruments GmbH, Fielderstadt, Germany), using a charging pipette (Hamilton Microliter Syringe). Photos were taken with a video camera every second up to 30 s from the coming into contact of the drop with the compact. The contact angles were calculated from the contours of the drop. γ_s results were calculated from the contact angles at 1 s to avoid the error arising from drop penetration.

The liquids used for contact angle measurement were bidistilled water (γ^p = 50.2 mN/m, γ^d = 21.8 mN/m) and diidomethane (γ^p = 0 mN/m, γ^d = 50.8 mN/m).

4.2.5 Calculation of surface free energy (γ_s)

In the method of Wu [105], γ_s is taken as the sum of depressive (d) and polar (p) components. The γ_s data of solid materials can be determined by means of contact angle measurements on two liquids with known polarities. They can be assessed by solving two equations with two unknowns:

$$(1+\cos\theta)\gamma_1 = \frac{4(\gamma_s^d \gamma_l^d)}{\gamma_s^d + \gamma_l^d} + \frac{4(\gamma_s^p \gamma_l^p)}{\gamma_s^p + \gamma_l^p}$$

where θ is the contact angle, γ_s is the solid surface free energy and γ_l is the liquid surface tension.

The work of cohesion (W_c) is twice γ_s, since two identical surfaces interact:

$$W_c = 2\gamma_s$$

The work of adhesion (W_a) is the energy that arises when two surfaces come into contact:

$$W_a = 4\left[\frac{\gamma_1^d \gamma_2^d}{\gamma_1^d + \gamma_2^d} + \frac{\gamma_1^p \gamma_2^p}{\gamma_1^p + \gamma_2^p}\right]$$

4.2.6 Differential scanning calorimetry (DSC)

Thermal analysis was carried out with a DSC821° instrument (Mettler-Toledo GmbH, Switzerland). Sixteen milligrams of sample was weighed into a non-hermetically sealed aluminium pan.

The samples were heated from 25 to 300 °C at a heating rate of 5 °C/min. The instrument was calibrated by using indium. All the DSC measurements were made in argon atmosphere and the flow rate was 100 ml/min. From the DSC curves, the calorimetric enthalpy, ΔH (integral normalized value) and the peak temperature (T) were calculated by software (Stare version 6).

4.2.7 Powder X-ray diffractometry (XRPD)

XRPD was performed with a Philips X-ray diffractometer (PW 1050/70 PW 1710), where the tube anode was Cu with Kα = 1.54242 Å. The pattern was collected with 50 kV of tube voltage and 40 mA of tube current in step scan mode (step size 0.035, counting time 1 s/step). The instrument was calibrated by using silicium produced by Philips. The setting error to silicium ethanol was not more than $0.01/2\Theta$.

4.2.8 Chemometric method

The method of multivariate curve resolution with alternative least squares (MCR-ALS) [106-108], as a chemometric method, can decompose the data matrix to profiles (composition profiles and pure diffractogram profiles) with the use of certain constraints [109-111].

The usual assumption in multivariate resolution methods is that the experimental data follow a bilinear model similar to the Lambert–Beer law in absorption spectroscopy. In matrix form, this model can be described as

$$\underset{I\times K}{\mathbf{R}} = \underset{I\times N}{\mathbf{D}} \cdot \underset{N\times K}{\mathbf{C}^{\mathrm{T}}}$$

where **R** is the response matrix (i.e. the counts in a diffractometry measurement against 2 theta from sample to sample), **D** is the diffractogram profile matrix of the components, and **C** is the composition profile matrix for the samples. The matrix dimensions are indicated below the symbols of the matrices in the equations, where index I denotes the number of 2Θ values, K is the number of samples, and N means the number of crystalline

components of the samples (mixtures) to be analysed. Suitably chosen initial estimations of **D** or **C** are optimized

by solving Eq. (1) iteratively by alternating least squares optimization:

$$\underset{N\times I}{\mathbf{D}^{+}} \cdot \underset{I\times K}{\mathbf{R}^{*}} = \underset{N\times K}{\mathbf{C}^{\mathrm{T}}}$$

$$\underset{I\times K}{\mathbf{R}^{*}} \cdot \underset{K\times N}{(\mathbf{C}^{\mathrm{T}})^{+}} = \underset{I\times N}{\mathbf{D}}$$

where the matrix **R*** is the reproduced data matrix obtained by principal component analysis for the selected number of components, and $^{+}$ means the pseudoinverse [112].

Unfortunately, this decomposition is very often not unique because of the rotational and intensity (scaling) ambiguities [113,114]. The rotational ambiguities can be moderated or even eliminated if convenient constraints can be used [109-111]. Tauler and coworkers developed a Matlab code for MCR-ALS with some constraints [115].

4.2.9 Morphological study

The binary systems were investigated with a scanning electron microscope (Hitachi 2004 S, Hitachi Scientific Instruments Ltd, Tokyo, Japan). A polaron sputter coating apparatus (Bio-Rad SC502, VG Microtech Uckfield, UK) was applied to create electric conductivity on the surface of the samples. The air pressure was 1.3-13.0 mPa

4.2.10 *In vivo* study

48 rats (male, SPRD, 200-220 g) were divided into 8 groups. Appropriate amounts, (determined in pilot experiments) of pure ME1, ME400 and PMs were filled into special capsules. These capsules were then administered into the stomach of the rats by using a Capsules-kit (CapsuGel, Greenwood. USA), which is specific for preclinical studies.

A local inflammatory response was elicited by the injection of 0.1 ml of Carrageenan (Viscarin, Marine Colloids Inc, Springfield, USA) into the left hind paw. The contralateral foot was injected subcutaneously with isotonic saline (control). Edema (caused by Viscarin) was measured with a plethysmometer (7140, Hugo Sachs Electronic GmbH, Germany) 5 h after the injection of Carrageenan.

The differences in the extent of edema formation due to the various pretreatments were expressed as percentages of the control value. Bonferroni and Dunnett tests were applied in the *Post-hoc* analysis.

5. Results and discussion

5.1 Influence of particle size on dissolution rate

The drug profiles of pure ME1 with *d*(90%)=206.20µm and ME2 with *d*(90%)=5.97µm were first studied. The wettability of the particles was very low because ME is a poorly water soluble drug. Difference in release between the two drugs could be observed after 60 min. The effect of the higher specific surface area of ME2 was not manifested because of the tendency of the small (~6 µm) crystals to agglomerate. After the dissolving of the capsule, the particles that were in a very hydrophobic form clustered in the artificial enteric juice (Fig. 4).

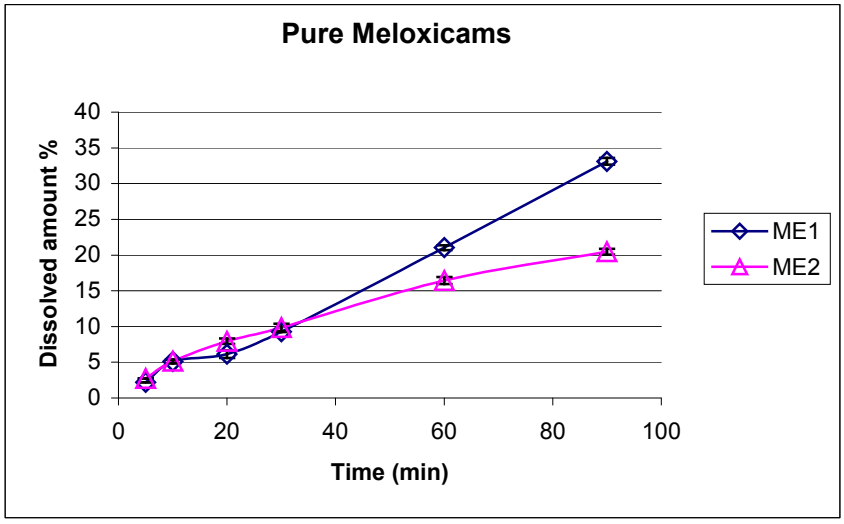

Fig. 4 Rate of dissolution of ME1 and ME2

The kinetic parameters (for ME1 and ME2) of dissolution process show that the dissolution rate (slope) is very slow (Table 3).

Table 3 Kinetic parameters of pure meloxicams

Samples	Zero Order	
	Slope	**R^2**
ME1	0.360	0.991
ME2	0.204	0.978

None of the pure meloxicams could reach 100% dissolution and it was proved that: particle size is not the only factor, which influences the dissolution rate, that is why mannitol was used for the next step.

5.1.1 ME-mannitol binary systems

The effects of the particle size of the MEs and the role of mannitol were studied in the binary systems by using PMs.

- ***Physical mixture (PM)***

The dissolution rate results showed that the amount of mannitol (ratio) and its specific surface area did not influence the rate of dissolution of ME1. This is evidence that the ME1 and mannitol particles with the same particle size only mixed together (Fig. 5 and Fig. 6).

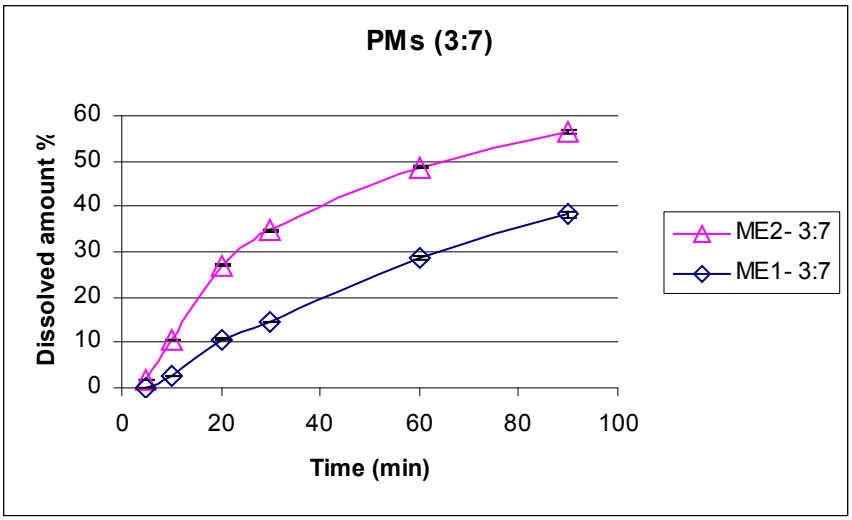

Fig. 5 Rate of dissolution of MEs from PMs in 3:7 ratio

Even in this case none of the MEs could achieve 100% dissolution.

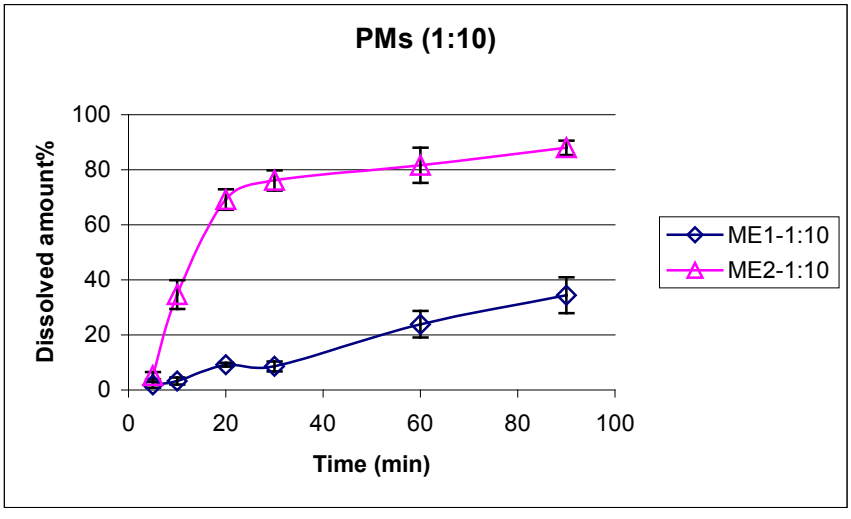

Fig. 6 Rate of dissolution of MEs from PMs in 1:10 ratio

In the case of ME2, on increasing of the amount of mannitol (1:10), dissolution was faster. This is connected with the specific surfaces of ME2 and mannitol. ME2 with small particles presumably adheres to the surface of mannitol. Nevertheless, it should be noted that the total amount of ME2 did not dissolve.

Table 4 shows the slopes of PMs (according to the first kinetic order). The dissolution rate of PMs is faster than that of pure ME1 and ME2 but 10 parts of mannitol is not enough for perfect dissolution of micronized ME2.

Table 4 Kinetic parameters of MEs in PMs

Samples	First Order	
ME1 PM	**Slope**	**R^2**
3:7	-0.002	0.995
1:10	-0.002	0.991
ME2 PM		
3:7	-0.004	0.948
1:10	-0.057	0.953

- ***Melted product (MPs)***

Another possibility for the processing of ME–mannitol systems is melt technology, based on the melted mannitol as carrier containing the MEs in dispersed form.

This form may be a eutectic mixture, a solid dispersion with a non-molecular distribution of the drug material or a solid solution with a molecular distribution of the drug material. Of course, the type of drug distribution influences the dissolution profile.

In general, for crystalline binary systems, a molecular distribution of the drug ensures fast release. The components and their ratios in the samples prepared by melt technology were identical to those of the PMs. Fig. 7 demonstrates the influence of melt technology on drug release. For MP-ME1, the amount dissolved of ME1 was almost twice as much as in the case of the PMs. It seems in the case of MP-ME2 with ratio of 3:7, the amount of mannitol was not enough to reach 100% dissolution.

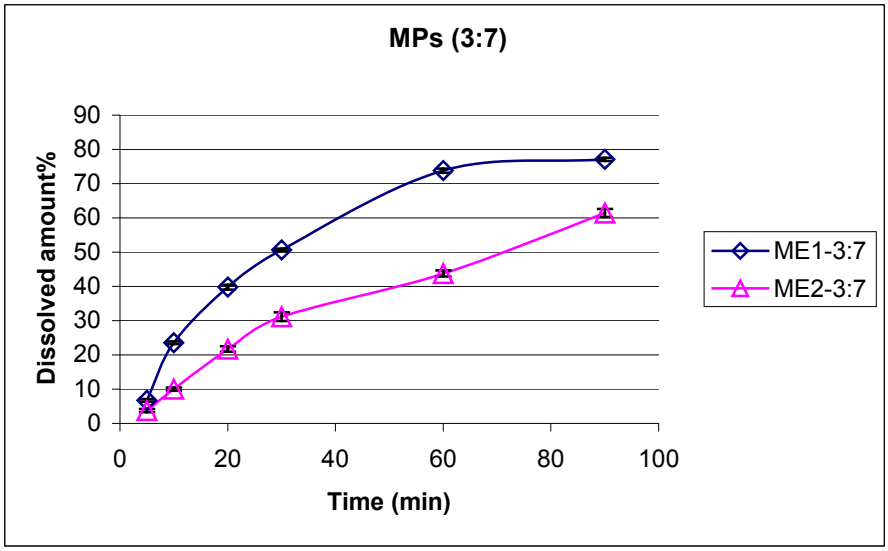

Fig. 7 Rate of dissolution of MEs from MPs in 3:7 ratio

For the MP-ME2 samples, we achieved perfect dissolution with a higher concentration of mannitol (10 parts). The results revealed the different effects of melt technology on the rate of dissolution of the MEs (Fig. 8). Although the particle size of ME2 is smaller than ME1, the cohesion between ME2 particles is strong enough to slow down the wetting of particles by artificial enteric juice.

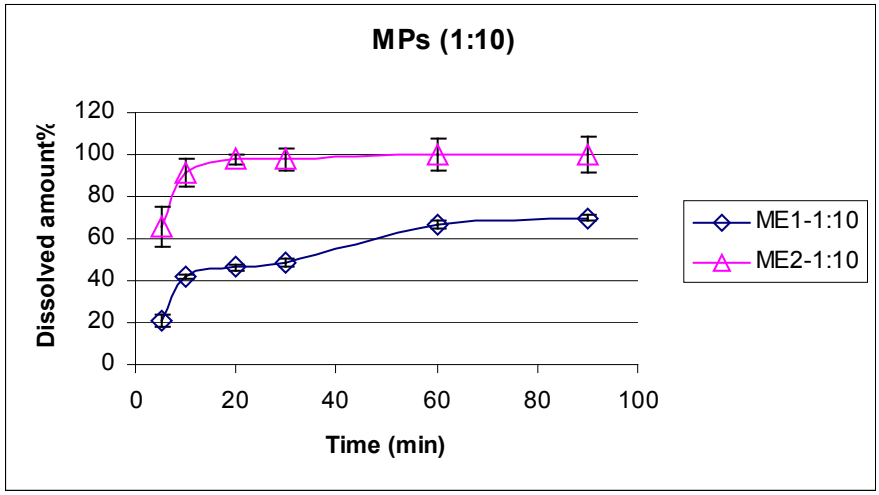

Fig. 8 Rate of dissolution of MEs from MPs in 1:10 ratio

90% of MP2-1:10 is dissolved within 10 min. It means that the rate of dissolution is very fast in the beginning, the curve has a plateau in 100% dissolved amount within 20 min. It is shown by kinetic parameters of MPs (Table 5).

Table 5 Kinetic parameters of MEs in MPs

Samples	First Order	
ME1 MP	**Slope**	**R^2**
3:7	-0.007	0.948
1:10	-0.006	0.907
ME2 MP		
3:7	-0.004	0.989
1:10	-0.012	0.632

5.2 Investigations on ME-mannitol binary systems

5.2.1 Differential scanning calorimetry

The DSC curves of the starting compounds exhibited a sharp endothermic peak at 165 °C, corresponding to the melting point of mannitol, and at 260 °C, the melting point of MEs (Fig. 9).

The melting point of MEs is followed by an exothermic peak, which means the transformation or recrystallization of the drug material.

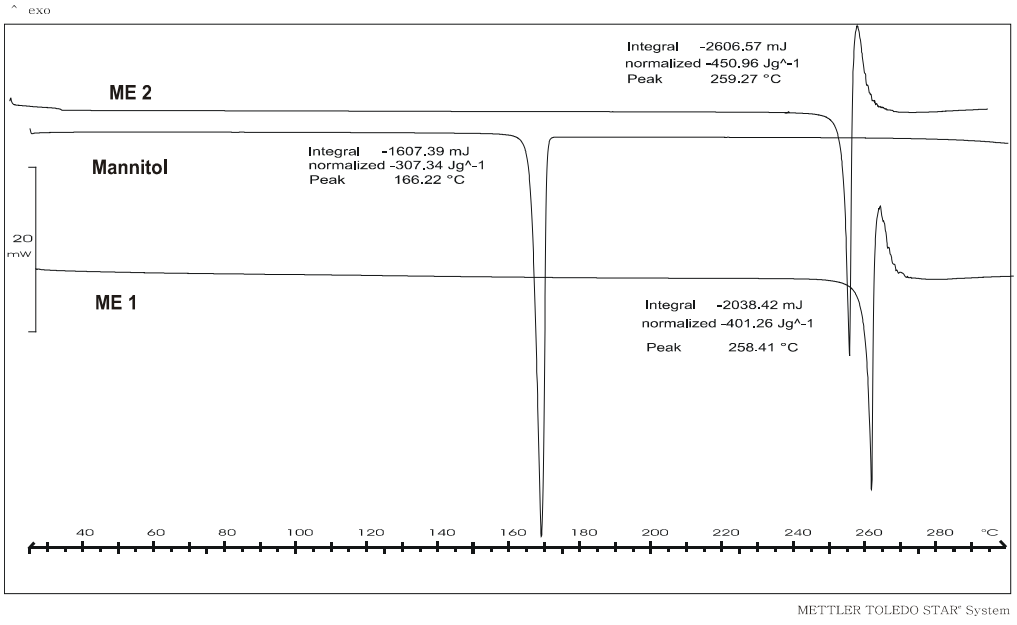

Fig. 9 DSC curves of ME1, ME2 and Mannitol

The DSC scans of the PMs and the MPs of ME–mannitol always included two endotherms, attributed to the separate melting processes of the two components (Fig. 10). It can be seen in the DSC curve of the PM that a proportion of the ME was dissolved in the melted mannitol (a broad, double endothermic peak), but the samples prepared by melting

technology did not display the double peak because this dissolution might occur during the preparation of the samples.

The peaks observed for the ME1 cases were analogous to those observed for ME2 at the corresponding ratios. A small additional peak is seen at a ratio of 1:10, but not at 3:7.

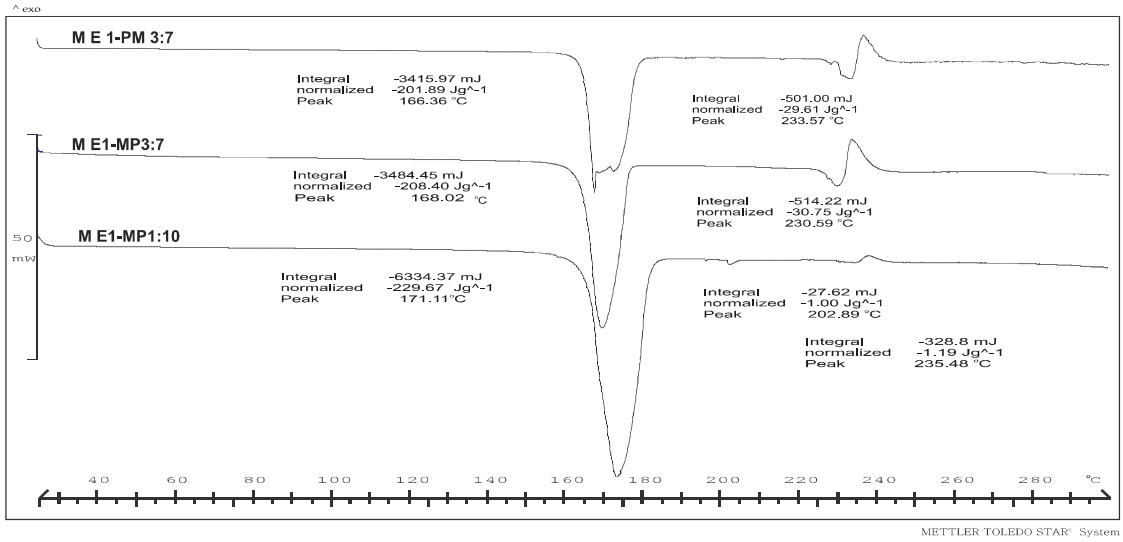

Fig. 10 DSC curves of PMs and MPs

We wanted to confirm the findings by powder X-ray investigation of the appearance of the new endothermic peak between the melting points of mannitol and ME.

5.2.2 Powder X-ray diffractometry

The results of the structural investigation of the starting materials (ME1, ME2, and mannitol), the PMs and the MPs were as follows (Fig. 11).

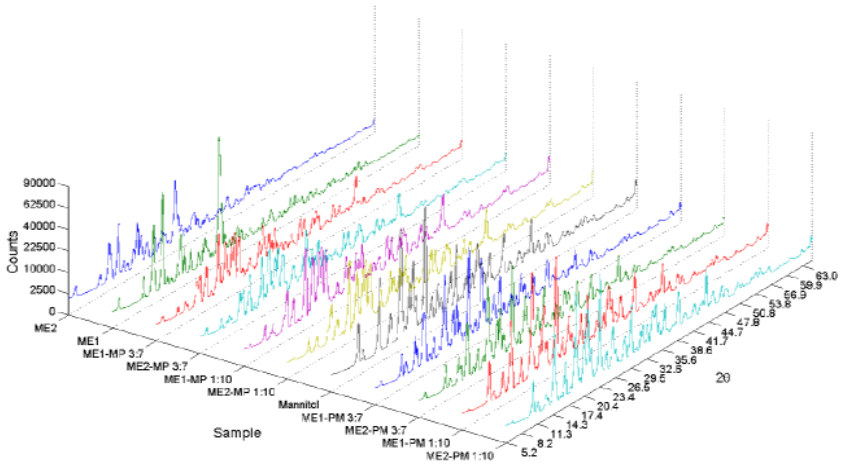

Fig. 11 X-ray diffractograms of ME1, ME2, mannitol, and ME–mannitol binary systems

The diffractograms of the PMs showed the characteristic values of the starting materials. Those of the MPs (3:7 and 1:10) had the same characteristic values as those of the PMs. The drug was distributed in the carrier in fine crystal (suspended) form.

A proportion of ME dissolved in the melted mannitol, but it was recrystallized during cooling. The remainders of the crystals were in a suspended form in the binary system during the melting process.

This method of drug distribution promoted drug release, but it was not perfect. The diffractometry on the MPs containing ME1 and ME2 (1:10) showed a new peak between those of ME and mannitol, referring to the mixed crystals. Presumably these mixed crystals could be seen at 202.89 °C in the DSC curves, and this is connected with the rapid dissolution in the case of the higher amount of mannitol (10 parts).

The X-ray evaluation of the binary systems raised the problem of the covered peaks (Fig. 11). To determine the differences between the X-ray diffractograms, and primarily to justify the presence of the mixed crystals, we used chemometric evaluation.

5.2.3 Chemometric method

This method is a new possibility for the evaluation of the results of X-ray investigations. After principal component analysis, it could be concluded that 3 latent variables (principal components) were sufficient to explain 98.01% of the variations in the total data according to the scree-plot (Fig. 12).

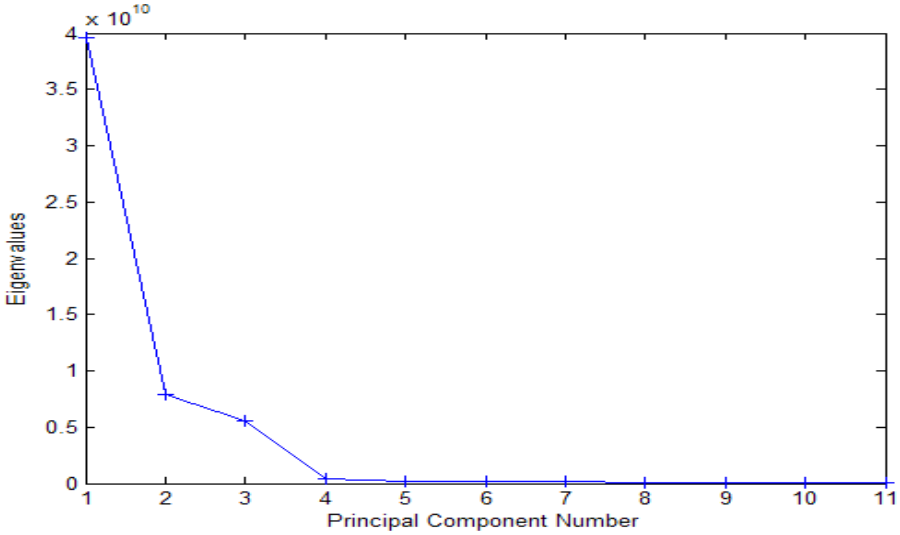

Fig. 12 Scree-plot demonstrating that three latent variables (principal components) are sufficient.

If we know the number of components, the multivariate curve resolution method can resolve the raw data to physically interpretable factors, i.e. the composition factors of the pure components (composition profiles) and the diffractogram factors of the pure components (diffractogram profiles), using constraints of non-negativity for both profile matrices.

The composition profiles show (see Fig. 13) that the solid line can be assigned to mannitol, and the dashed line to ME1, but the dotted line cannot be clearly assigned to ME2. The dotted line may represent a 'blend' of the pure components ME2, mannitol, and ME1, i.e. a new crystalline form. The 'blend' appeared in predominant amounts in the MPs, but only as a minor component in the PMs.

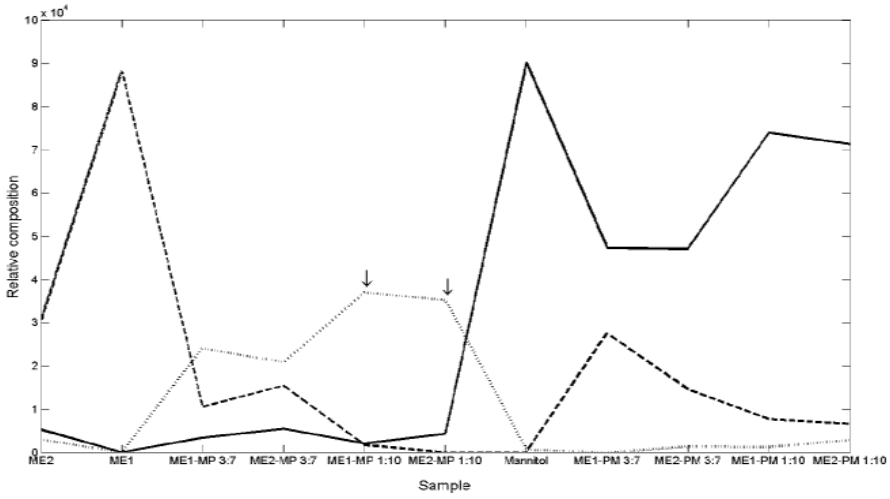

Fig. 13 Composition profiles of the three crystalline components given by MCR. Solid line: mannitol; dashed line: ME1; dotted line: 'blend'.

The evaluation of the X-ray measurements with MCR confirmed the conclusions drawn from the DSC measurements: ME-MP 1:10 gave the highest amount of the new crystal form; the new endothermic peak (Fig. 10) appeared only at the 1:10 ratio. The PMs predominantly display the characteristics of mannitol.

Conclusion:

This study of the dissolution of meloxicam–mannitol binary systems in artificial enteric juice has revealed that both the particle size and the amount of mannitol are important factors influencing the dissolution of ME. It was shown that preparation of a PM is more useful when the ME is in micronized form (μm). In the case of a MP, a high amount of mannitol (1:10) is used but even in this case the ME particle size plays an important role.

Thus, the amount of mannitol and the ME particle size act in conjunction to increase the dissolution of ME.

5.3 Optimization of particle size of meloxicam in physical mixtures (PMs)

Because the 100% dissolution could not be achieved by a simple physical mixture (even by increasing the amount of mannitol), we decided to find a proper particle size. That is why ME1 was milled to make a ME400 (it has to be mentioned that ME400 showed the best result among 2 other particle sizes ME100, ME200).

Influence of work of adhesion on dissolution rate in binary solid systems

The aim of this part is to investigate the surface properties of different physical mixtures of meloxicam (ME) and to find a possible relation between the surface properties and the dissolution of physical mixtures.

5.3.1 Contact angle measurement and surface free energy

The data of contact angles in Table 2 reveal that the ME samples have medium wettability (θ_{water} = 54.4–69.4°).

Mannitol as a water soluble carrier possesses very good wettability (θ_{water} = 24°). The wettability of the MEs is in every case lower than that of mannitol.

The total surface free energies of the pure MEs are lower than that of mannitol. The polarity of surfaces, which is connected with surface free energy, decreases with the reduction of the particle sizes of MEs, consequently, with increases in the hydrophobic surface area.

The work of cohesion and the work of adhesion yield appreciable information on the interactions between the particles (Table 6). The dissolution of the pure MEs was slow because of the low polarity values.

Table 6 Surface properties data of the pure components

Samples	**Contact angle (°)**		**Surface free energy (mN/m)**	**Polarity (%)**	**Work of cohesion (mN/m)**	**Work of adhesion (mN/m)**
	Water	**SD (±)**				
ME1	54.4	2.4	67.9	27.8	135.8	144.7
ME400	59.9	1.2	65.9	24.4	131.8	140.7
ME2	69.4	3.7	59.2	19.4	118.3	129.5
Mannitol	24.0	4.5	80.6	40.7	161.2	-

The work of cohesion of ME samples is in every case lower than that of mannitol. This is necessary for the spreading of the ME over mannitol. The work of adhesion between mannitol and ME samples is in every case higher than the work of cohesion of the

corresponding ME samples. This is also a necessary condition for the spreading of the active agent on the surface of the excipient (Table 7).

Table 7 Spreading coefficients of the MEs over carrier (mannitol) (S_{12}) and of mannitol over the MEs (S_{21})

1	2	$S_{1/2}$	$S_{2/1}$
		mN/m	
ME1	**Mannitol**	7.9	-20.4
ME400	**Mannitol**	9.6	-26.7
ME2	**Mannitol**	8.8	-20.6

It is interesting that the works of cohesion and adhesion are lower for ME2 than that for ME1 and ME400 and the spreading coefficient of the ME2 over mannitol (S1/2) is between the spreading coefficients of ME1 and ME400. These conditions are necessary for the spreading of one component over the surface of the other, though the particle size and the proportions of the components can modify the arrangement of the particles under different conditions.

Table 8 Surface properties data of ME-mannitol PMs in a ratio 3:7

Samples	Contact angle (°)		Surface free energy (mN/m)	Polarity (%)
	Water	SD (±)		
PM1-3:7	38.2	2.4	75.3	35.6
PM400-3:7	57.9	1.8	66.9	25.9
PM2-3:7	60.3	1.6	63.5	24.7

The contact angles of the PMs are between those of the ME and mannitol (Table 8). Interestingly, a difference may be observed between the PM1 group and the PM400 and PM2 groups. The θ_{water} values for the latter (PM400 and PM2) are closer to the contact angle of the ME than to that of mannitol. This phenomenon is more marked at a ratio of 3:7. This presumably means that at this ratio the ME is spread over the surface of mannitol, whereas at a ratio of 1:10 the ME does not totally cover mannitol (Table 9).

In the PM1 group, the two different particles are side by side, so the good wettability of mannitol can be observed. The same tendency can be seen in the surface free energies of the PMs as in the contact angles in accord with the presumed positions of the two different particles.

Consequently, the surface free energy and polarity data on the PM1s were closer to those on mannitol than to those on ME1.

For the PM400s and PM2s, the coverage of mannitol by the ME results in the surface free energies approximating to those for the pure MEs.

Table 9 Surface properties data of ME-mannitol PMs in a ratio 1:10

Samples	Contact angle (°)		Surface free energy (mN/m)	Polarity (%)
	Water	SD (±)		
PM1-1:10	31.4	2.1	77.9	38.4
PM400-1:10	42.8	4.9	73.9	32.9
PM2-1:10	54.4	1.2	66.4	27.8

5.3.2 Dissolution study

Dissolution studies were done on ME400 and its binary systems, the results of this investigation were compared to the other 2 MEs and their binary systems. he dissolution rates of the pure MEs (without mannitol) with different particle sizes were studied (Fig. 14). On decreasing the particle size, the dissolution rate for ME400 increased, but a small particle size was not sufficient to achieve a dissolution rate of 40% in 90 min.

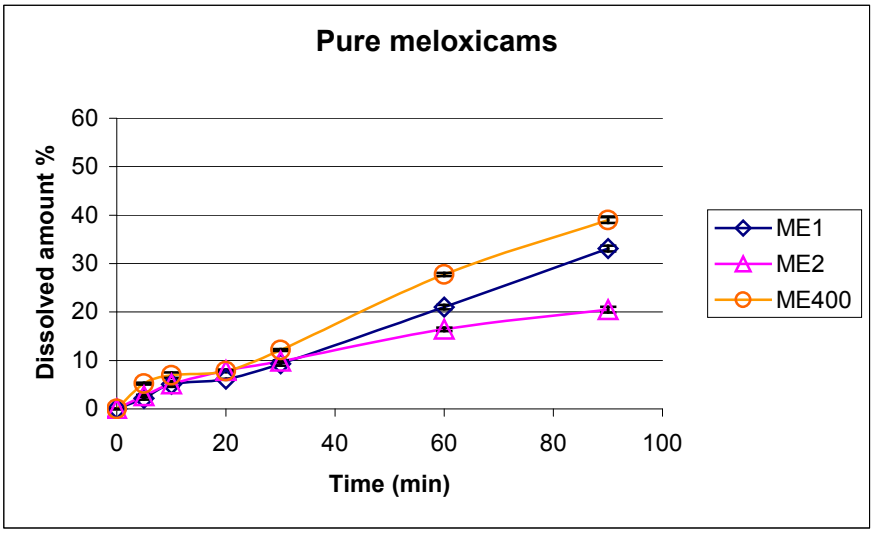

Fig. 14 Rate of dissolution of pure meloxicams (ME1, ME2, ME400)

The values of slope of the pure MEs in which the dissolution rate is very slow, the dissolution rate constants show the differentiations. The slope of ME2 is smaller than the other two because of the agglomeration of the micronized ME2 (Table 10).

Table 10 Kinetic parameters of pure meloxicams

Samples	Zero order	
	Slope	**R^2**
ME1	0.360	0.996
ME2	0.204	0.978
ME400	0.415	0.986

The dissolution rate of ME400 was increased to 80% just by adding mannitol (7 parts) and the difference between ME1 and ME400 was more than 40%, but unfortunately the whole PM400 did not dissolve (Fig. 15).

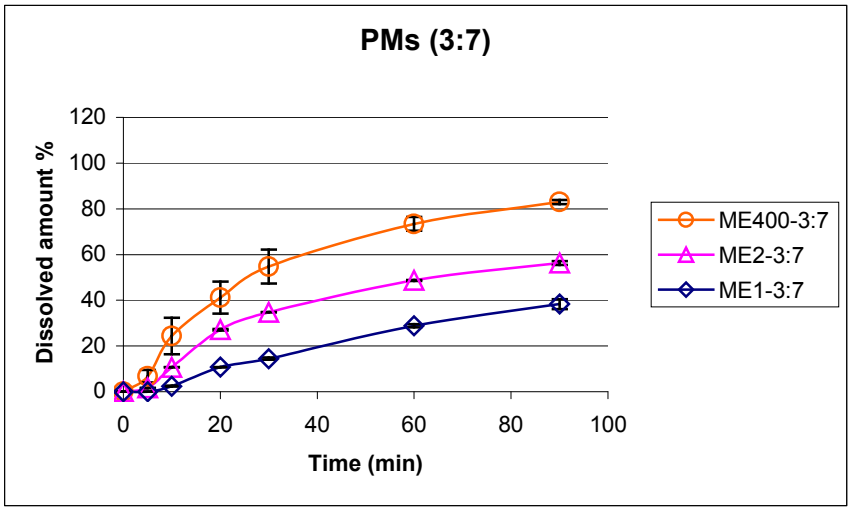

Fig. 15 Rate of dissolution of ME1 and ME400 from PMs in 3:7 ratio.

Through the use of PMs in a ratio of 1:10 the dissolution was improved for PM400 and PM2 as compared with the dissolution of the pure MEs. For PM1, the amount of drug dissolved did not differ from that of the pure ME1. The dissolution profile of PM1 with a ratio of 1:10 differed from those of PM2 and PM400 (Fig. 16).

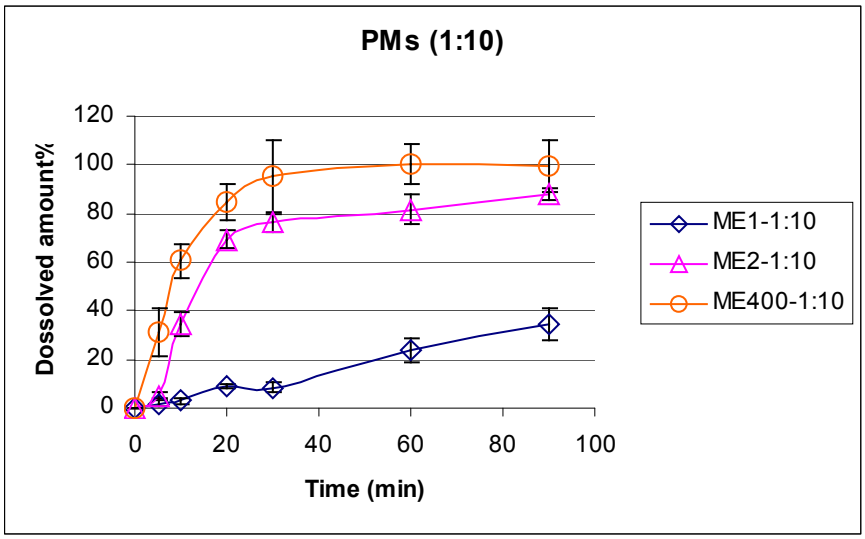

Fig. 16 Rate of dissolution of ME1 and ME400 from PMs in 1:10 ratio

Even though the particles of the milled ME1 (ME400) were not as small as those of ME2, it was able to attain a higher surface area in contact with the dissolution medium and the dissolved amount was improved to 100% (Table 11).

Table 11 Kinetic parameters of ME in PMs400

Samples	First order	
	Slope	R^2
PM400 3:7	-0.019	0.981
PM400 1:10	-0.105	0.998

Establishment of the ideal ratio meant not only that the maximal dissolution was approached, but also the rate of dissolution of the ME (with appropriate particle size) was improved so that almost the whole PM400 was dissolved by 40 min.

Such an improvement occurred only in the case of MEs with small particle sizes. Small ME particles spread over the surface of mannitol. Through the improvement of the content of smaller particles in the binary mixtures, the cohesion between the ME particles was increasingly pronounced. From this aspect, the dissolution of PMs with a ratio of 3:7 was unfavourable.

Some investigations were done to find the reason for better results in the case of ME400. The surface energy of MEs was also studied to find a possibility to predict a dissolution rate from it.

Conclusion

The preparation of a PM is one of the easiest ways to increase the dissolution of insoluble drugs, but it is sometimes not effective enough to be used by researchers. Through the establishment of an ideal ratio for the binary system and an appropriate particle size for the ME, the use of a PM can be as effective as any other method, involving, for example, a eutectic mixture or complexation.

PMs with different particle sizes and different amounts of mannitol showed different dissolution rate results. PM400- 1:10 reached a complete dissolution.

5.3.3 Morphological study

The scanning electron micrographs support the conclusions drawn from the wettability measurements and the investigations of the interparticle interactions. As it can be seen meloxicam and mannitol have big particles, but the particles of mannitol are rougher than in meloxicams (Fig.17 and Fig. 18).

- ***Pure meloxicam and mannitol***

Fig. 17 SEM micrograph of ME1

Fig. 18 SEM micrograph of Mannitol

The mannitol and ME particles are in close proximity because of the large particle size of ME1, which is close to that of mannitol; only a small particle fraction of ME1 can adhere to the surface of mannitol (Fig. 19, Fig. 20 and Fig. 21).

- ***PMs1***

Fig. 19 SEM micrograph of PM1-3:7

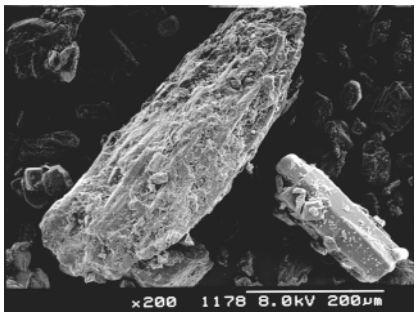

Fig. 20 SEM micrograph of PM1-3:7

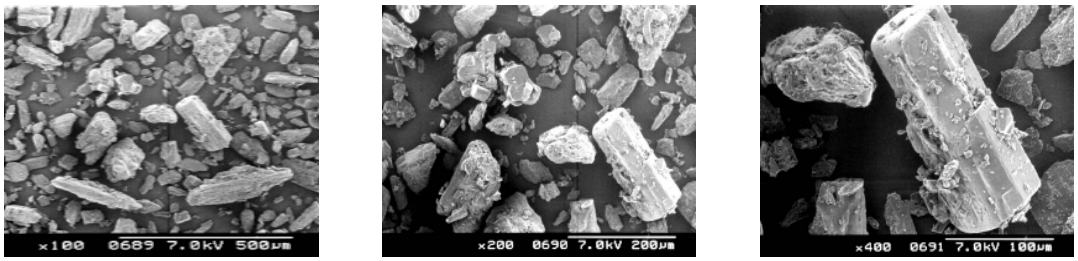

Fig. 21 SEM micrographs of PM1-1:10

- ***PM2 and PM400***

ME400 and ME2 have smaller particle sizes than those of mannitol. After mixing, therefore, each particle of mannitol will be a core surrounded by small particles of MEs.

For PM2, the micronized ME2 is spread over the surface of the carrier, but tends to agglomerate because of the small particle size and the higher specific surface area (Fig. 22 and Fig. 23).

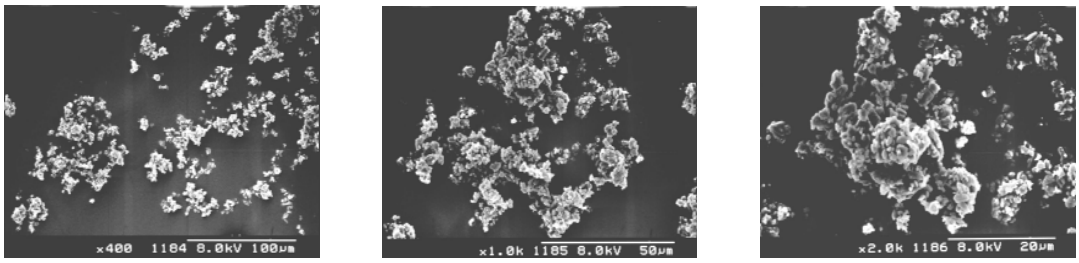

Fig. 22 SEM micrographs of PM2-3:7

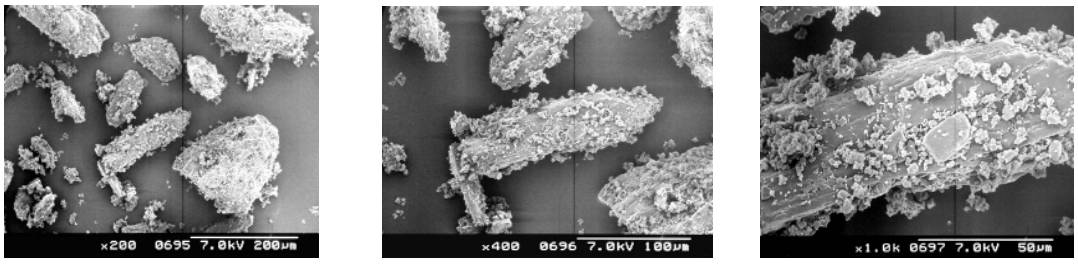

Fig. 23 SEM micrographs of PM2-1:10

For PM400, the situation is the same, except for the tendency to agglomerate. In the case of PM2 the proportion of smaller ME particles is higher, and the coverage of the mannitol crystals is more significant (see θ and γ for the mixtures) (Fig. 24).

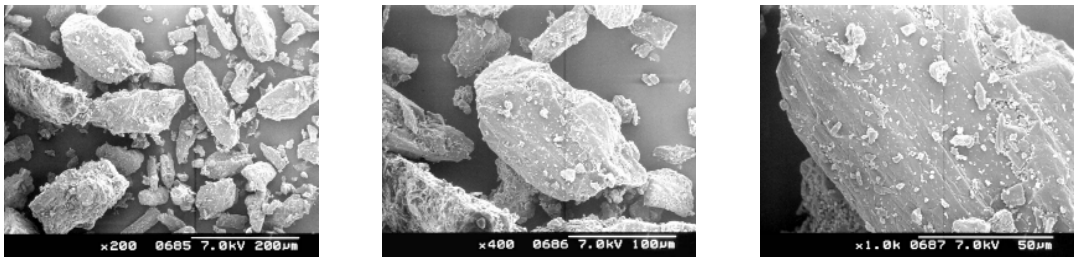

Fig. 24 SEM micrographs of PM400-1:10

Conclusion

As demonstrated earlier, the interparticle interactions in two component mixtures are influenced extensively by the particle size and the proportions of the components. The present results show that this phenomenon may be applied for dissolution improvement. With the ideal particle size and ideal proportions of the carrier and drug, the interparticle interactions are optimally influenced by the surface properties and particle size. Of the binary mixtures applied here, PM400 with a ratio of 1:10 proved ideal for 100% dissolution of the drug to be attained within 90 min. The dissolution of the drug from binary mixtures could be predicted from the surface properties and morphological study of the PMs.

5.4 In vitro and in vivo investigations on the binary meloxicam-mannitol system

After the successful *in vitro* experiments, the anti-inflammatory effects of the pure MEs and PMs were investigated on rats. This study was approved by the Committee on Animal Research, University of Szeged, Hungary (IV/4316-/2002).

PMs of ME1 and ME400 were chosen as the biggest particle size and the particle size which is proper for dissolution. After the administration of pure meloxicams and PMs to the inflamed rat, the differences in the extent of edema formation due to the various pretreatments were expressed as percentages of the control value. The results are shown in Table 12 in comparison with the *in vitro* results and in Fig. 25 for the *in vivo* investigations.

Table 12 *In vivo* and *in vitro* results of MEs, mannitol and PMs

Samples	*In vitro* (90 min) dissolved amount (%)	Anti-inflammatory effect (%)
ME1	33.11	45.94
ME400	39.07	47.432
PM1-3:7	38.30	58.21
PM400-3:7	83.00	58.71
PM1-1:10	34.40	53.57
PM400-1:10	99.60	71.48
Mannitol	100	3.82
Empty capsule	0	0

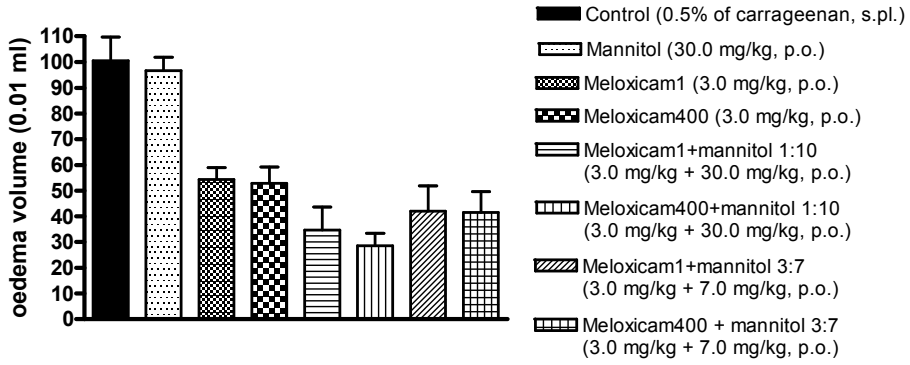

Fig. 25 Anti-inflammatory effects of ME1, ME400, mannitol and different PMs of meloxicams and mannitol on rats

The results were compared with each other "point to point" as it was mentioned in the case of Level A. As it can be seen, the particle size of the pure MEs did not influence either the dissolved drug amount (*in vitro*) or the anti-inflammatory effect.

Conclusion

The particle size of the pure MEs (ME1 and ME400) did not influence either the dissolved drug amount (*in vitro*) or the anti-inflammatory effect. On decreasing the particle size, the dissolution rate for ME400 increased, but a smaller particle size was not sufficient to achieve a dissolution rate of 40 % in 90 min and the anti-inflammatory effect of 50 %. A further decrease of the particle size of the ME resulted in the agglomeration of the small particles.

It was demonstrated earlier that 3 factors affected the dissolution of the drug material in binary ME-mannitol system:

1. The ME particle size with a given specific surface
2. The amount of carrier (mannitol) with enough specific surface for ME particles
3. The interaction (adhesion) between the ME and mannitol particles

Each factor is important in increasing the rate of dissolution of ME and all three must act together. However, with the ideal particle size of the drug (ME400) and the ideal ratio of ME400 and mannitol (1:10), the total dissolution of the drug was achieved (99.60 % within 90 min). In this interactive mixture, mannitol functioned as a core covered with a monolayer of ME particles. This was confirmed in *in vivo* experiments. PM400 1:10 exhibited the best (71.48%) anti-inflammatory effect. Statistically, a positive correlation was found between the *in vitro* and *in vivo* data ($P < 0.05$, $R^2 = 0.937$).

The results confirmed the applicability of the interactive binary physical mixture for the increase of dissolution and better bioavailability of water-insoluble drugs.

6. Summary

The anti-inflammatory drug, meloxicam (ME) has poor water solubility. The object of this project was to improve the rate of dissolution of meloxicam in capsule form.

- ME samples with different particle sizes were investigated without a carrier. A smaller (micronized) particle size did not improve the dissolution of the drug.
- Mannitol, a sugar alcohol, is a cheap and readily available excipient which was used as a carrier in different ratios, in physical mixtures and melted forms (Binary systems).
- Differential scanning calorimetry (DSC) and X-ray diffractometry were used to investigate the characteristics of meloxicam–mannitol binary systems. Multivariate curve resolution (MCR) as a chemometric method was applied to interpret the X-ray diffractograms.
- According to the results, the amount of mannitol and the particle size of ME were important factors in the rate of dissolution. For the perfect dissolution of ME, the melt technology (MP) was used which resulted in mixed crystals. This technology was made by 10 parts of mannitol and 1 part of ME2 with about 6 μm in average particle size.
- In physical mixtures (PMs), the interactions between the particles are determined by the forces of adhesion and cohesion. The size and the specific surface area of the particles are also determinative factors in the interparticle interactions. However, with the ideal particle size of (d 90% = 131.15 μm) the drug (ME400) and the ideal ratio of ME and mannitol (1:10), the total dissolution of the drug was achieved. In this case, mannitol functioned as a core covered with a monolayer of ME particles.
- Contact angle, surface free energy, polarity, work of adhesion, and work of cohesion of the drug, the carrier, and their physical mixtures were calculated.
- According to the results, contact angle, surface free energy, polarity and work of adhesion can be used as critical parameters to characterize powder interactive mixtures and to determine the optimum dissolution profile.
- A study was made of the anti-inflammatory effects on rats of pure meloxicam (ME) with different particle sizes and of physical mixtures of the binary ME-mannitol system.

- The level of local inflammation was significantly decreased when the amount of mannitol was the highest and the particle size of meloxicam was d(90%) 131.15 μm. These results are the same as the ones achieved in *in vitro* experiments.

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