University of Szeged
Faculty of Pharmacy
Department of Pharmaceutical Technology
Head: Prof. Dr. habil. Piroska Szabó-Révész D.Sc.

Summary of Ph.D. thesis

FORMULATION OPTIMIZATION OF SUSTAINED RELEASE AMMONIO METHACRYLATE COPOLYMER BASED MICROSPHERES

Péter Sipos

Supervisors:

Prof. Dr. habil. István Erős D.Sc.

University of Szeged, Department of Pharmaceutical Technology

Dr. habil. Ildikó Csóka Ph.D.

University of Szeged, Department of Drug Regulatory Affairs

Szeged

2008

1. INTRODUCTION

A sustained, constant drug level at the therapeutic optimum is needed in the blood in a number of pathological conditions. Therefore the preparation of controlled and targeted drug delivery systems is one of the most important tasks of pharmaceutical technology.

Colloidal drug delivery systems as micro- and nanoparticulate delivery systems are proper for the above-mentioned purposes. The value of these delivery systems as orally administered controlled-release dosage forms has been evident for years. The *micro*particulate delivery systems include mainly pellets, microparticles, lipospheres and macroemulsions. The *nano*particulate delivery systems include mainly lipid or polymeric nanospheres, microemulsions, liposomes, cochleates, and nonionic surfactant vesicles (niosomes). Drugs can be embedded within a polymeric/proteinic coat or matrix network in either a solid aggregated state or a molecular dispersion, resulting in the formulation of microcapsules or microspheres, respectively. The aqueous solubility, which becomes for many drugs the main drawback during formulation either in a liquid form or in a controlled release systems has been overcome by *microencapsulation techniques*.

Biodegradable and biocompatible polymer materials as drug carriers have been investigated in the recent 15 years in large number of studies in various drug delivery systems. In microparticles, the pharmacon diffusion can be easily controlled through the matrix structure, and also sensitive materials (drugs, peptides, hormones, vaccines, pDNA) can be protected against the external environment. The advantage is that the drug release can be controlled; microparticles have a long duration of action, and dosage frequency and adverse effects can therefore be reduced.

In this PhD work the aim was to prepare industrially applicable microsphere products. Since there was no preliminary experience in the Department of Pharmaceutical Technology, Szeged, in this field, the work was meanwhile completed with preformulation experiments which are prior to microsphere formulation in the logical order. This thesis follows the order of pharmaceutical technological formulation in the results and discussion part.

2. AIMS

The **main objectives** of the PhD work were to study the preparation and comparison of diclofenac sodium (DS, as model drug) containing stable microsphere novel compositions, with the application of multiple emulsion–solvent evaporation and spray-drying techniques. Furthermore to show the effect of compositional changes of the copolymer matrix on physicochemical characteristics, on the stability (*pharmaceutical technology aspect*) and on the drug release (*biopharmaceutical interest*).

The following **main groups** of the investigations were performed in this thesis:

- (**I**) preformulation study of the microspheres: (**i**) effect of the main processing parameters;

(**ii**) thermoanalytical examination of the components; (**iii**) assessment of the possible drug-copolymer interactions. Films with different ratios drug/copolymer were prepared by the solvent casting method and investigated by the thermal analysis (TA) and Raman spectroscopy (RS) methods.

- (**II**) Comparative study of the SE- and SD-microspheres: (**i**) to compare different preparation techniques, (**ii**) structural evaluations of the $W_1/O/W_2$ multiple emulsion and the microsphere products were carried out by the TA and RS methods together with physical and model mixtures.
- (**III**) Formulation optimization of the SE-microspheres: optimization of the characteristics is a challenging task, because there are no universal additives for all the active agents, and no universal preparation methodology. The (**i**) amount of W_1-phase; (**ii**) amount of W_2-phase; (**iii**) W_1/O emulsion stirring rate; (**iv**) drug/copolymer (DS/AMC) ratio; and (**v**) plasticizer/copolymer ratio were studied as main processing variables by qualitative factorial design study.
- (**IV**) Formulation optimization of the SD-microspheres: the (**i**) types and (**ii**) concentrations of different polar cosolvents, and the (**iii**) drug/copolymer ratio were studied as main processing parameters by quantitative factorial design study.

The following measurements were used to characterize the microsphere products:

(**i**) viscosity measurements of the organic phases and the W_1/O emulsions (rotational viscometer);

(**ii**) microscopic characterization of the emulsion droplets (Image analyser);

(**iii**) external morphology of microspheres (Scanning electron microscopy);

(**iv**) granulometric analysis (Laser diffractometry);

(**v**) determination of E and EE (Energy dispersive X-ray fluorescence analysis);

(**vi**) thermal behaviour and structural evaluation (Thermoanalysis – TG, DSC);

(**vii**) investigation of possible interactions between drug and polymer (Raman spectroscopy);

(**viii**) concentration of residual organic solvents (static head-space GC); and

(**ix**) *in vitro* drug release profiles of the microspheres compared by mathematical models.

3.A MATERIALS

Diclofenac sodium (DS) (Ph.Eur. 5) was used as hydrophilic model drug. *Ammonio Methacrylate Copolymer* (AMC) (Type B, MW = 150.000; USP/NF, Ph.Eur. 5./NF.) was selected as the biocompatible, but non-biodegradable hydrophobic frame-forming material of the microspheres, based on the low permeability and pH independent release properties.

The solvent dichloromethane (CH_2Cl_2) and the cosolvents, acetone (Me_2CO), methyl ethyl ketone (MeCOEt), *n*-propanol (*n*PrOH) and *n*-butyl acetate (*n*BuOAc) were of reagent grade. The non-ionic surfactants sorbitan mono-oleate (HLB = 4.3) and polyoxyethylene 20 sorbitan mono-oleate (HLB = 14.9), the protective carrier poly(vinyl alcohol) (MW = 72 000) and the plasticizer polyethylene glycol stearate were of pharmacopoeial grade (Ph.Eur. 5).

3.B PREPARATION

Preformulation

In the preformulation study different **films** were prepared by the solvent casting method. **Physical** and **model mixtures** were prepared for thermal analysis and Raman spectroscopy investigations. In contrast with the physical mixture, the model mixture allows the preparation of a solid solution of drug in the copolymer matrix.

Conventional solvent evaporation technique

In the $W_1/O/W_2$ emulsion–solvent evaporation method, the aqueous solution of drug (W_1) in the lipophilic solvent (containing copolymer, plasticizer, and the W/O emulsifier) was emulsified. The W_1/O emulsion was then dispersed into the W_2-phase containing the O/W emulsifier and protective carrier. Solvent evaporation and solidification of the microspheres proceeded at RT and normal atmospheric pressure, under continuous stirring. Microspheres were collected by centrifugation, drying was performed by vacuum filtration or freeze-drying.

Spray-drying technique

Microspheres were prepared using a Büchi B-191 Laboratory Spray-dryer with a standard 0.7 mm nozzle. The microspheres were separated in the novel high-performance cyclone.

The $W_1/O/W_2$ emulsion was spray-dried. The process was performed at the same conditions (air flow: 11.6 $l \cdot min^{-1}$; pressure: 5 bars; pump rate: 2.1 $ml \cdot min^{-1}$). The inlet temperature was set above the boiling point of the solvents (140 °C).

3.C INVESTIGATION METHODS

Design of experiment (DOE). To evaluate the contribution of each factor with different levels on responses, qualitative and quantitative factorial based design was conducted.

Rheological measurements. The absolute viscosity (mPas) of the organic solvent mixtures, the dynamic viscosity (mPas) of the organic phase and that of the W_1/O emulsion were measured with a *rotational viscometer*.

Morphological study. Microscopic observations were made with a LEICA image analyser. Scanning electron microscopy was used to determine the surface characteristics and the external morphology of the microspheres.

Particle size analysis. The microspheres were sized by laser diffractometry (Malvern Mastersizer). Parameter D [4.3] was used to describe the average particle size.

Drug entrapment (%, w/w) and **encapsulation efficiency** (%) were determined with energy-dispersive X-ray fluorescence analysis (EDXRF).

Thermoanalytical measurements were performed using the same thermal program (25-400 °C heating range; 10 °C min^{-1} heating rate) under a dynamic flow of N_2 and Ar. TG (mass loss (%, w/w) vs. temperature), and DTG (derived mass loss vs. temperature) curves were plotted. The thermograms and the changes in enthalpy (ΔH, $J \cdot g^{-1}$) were recorded.

Raman spectroscopy measurements. The ingredients, the physical and model mixtures, the films and the microspheres were characterized (n = 3).

Analysis of residual organic solvent and cosolvent. The levels of residual organic solvent and cosolvents within the microspheres were determined by static head-space GC analysis.

Cumulative drug release and release profiles. A modified paddle apparatus (Apparatus II, Ph.Eur. 5) was used for the experiments. The dissolution parameters were: surfactant-free PBS; pH 7.42; 37 ± 0.5 °C; mixing rate of 100 $1 \cdot min^{-1}$. Six types of kinetic models were applied to process the *in vitro* data.

4. RESULTS AND DISCUSSION

4.1. PREFORMULATION STUDY OF THE MICROSPHERES

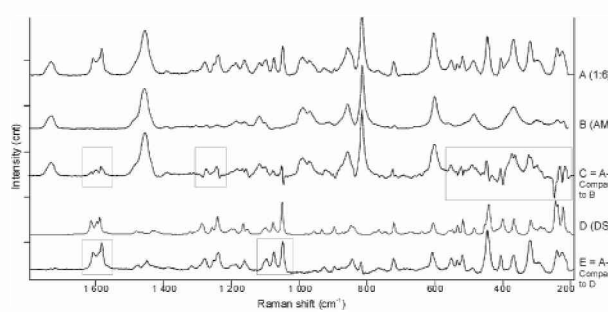
Prior to the preparation of microparticles, it is necessary to identify the state of the drug in the polymer matrix and the compatibility of the components. In addition, thermal investigations are important before high-temperature preparation methods (i.e. spray-drying) would be applied. The distribution of the drug inside the microparticles is an important factor, because the drug can crystallize during preparation, resulting a decreased solubility rate and a polymorphic form. The molecular dispersion of the drug ensures a higher dissolution rate in the gastrointestinal tract, but in the crystalline state, when the drug diffuses out of the matrix leaving channels, the drug dissolution rate can increase to such an extent that this rate could exceed the required sustained release rate. This preformulation study involved the characterization of the dispersed/dissolved state of the drug, the thermal stability and the properties of drug-containing AMC film (using TA), and determination of the possible interactions between the drug and the copolymer (using RS). A specific objective was to determine an appropriate drug/copolymer ratio for the drug entrapment.

Problem statement: at high drug/polymer ratios, the quantity of the polymer may be insufficient to englobe the drug. At low ratios, drug dissolution is prevented, while it takes more time for the drug to get to the gastrointestinal juice.

The Raman spectrum of the DS (Fig. 1D) was subtracted from the spectrum of the film with a drug/copolymer ratio of 1:6 (Fig. 1A), and the result (Fig. 1C) was compared with the spectrum of AMC (Fig. 1B). For determination of the changes in drug, the difference spectrum of the model mixture (A) and AMC (B) was calculated. The result (E) was compared with the drug spectrum (D); the differences could be well observed in the regions overlapping with AMC bands (1500-1400 cm^{-1} and 850-800 cm^{-1}).

Figure 1.

Raman spectra of
(A) film
(drug/copolymer = 1:6;
(B) AMC;
(C) film minus drug;
(D) drug;
(E) film minus AMC.



Conclusions of the preformulation study

The following conclusions and implications can be drawn:

Thermoanalytical studies confirmed that DS can behave as a plasticizer in drug-copolymer films, which was indicated by decreasing glass transition temperature (T_g) of the AMC, depending on its dispersity level in the copolymer matrix. A partial *solid solution* of drug was formed at drug/copolymer ratios of 1:12 and 1:8. No significant difference was revealed by any major compositional changes, except for the effects of the different drug contents of the measured films.

Raman spectroscopy confirmed that DS and AMC were compatible with each other. There were only small changes, such as broadening and shifting of the peaks corresponding to the $O^1C^8O^2$ ions of DS (1581 cm^{-1}) and the quaternary ammonio groups of AMC (900-800 cm^{-1}), indicating the decrease in the vibrational relaxation time. The dichlorophenyl ring stretching of DS (1590 cm^{-1}) was missing, which could otherwise indicate an ionic interaction. The strength of the other possible interactions between the drug and the copolymer chains seemed to be too weak to have an additional retaining effect of drug from dissolution. These investigations facilitated the selection of the appropriate drug/copolymer ratios (1:6, 1:8, 1:12) in the preformulation study of the microsphere preparation.

4.2. COMPARATIVE STUDY OF SE- AND SD-MICROSPHERES

In this comparative study the effects on the thermal behaviour of microspheres of the type and amount of four polar cosolvents and the preparation methods were investigated. The formulations were designed by varying the independent variables, the batches were evaluated on the basis of SEM, DSC and RS measurements.

Problem statement: the preliminary study suggested that the type and increased amounts of polar cosolvents could increase the risk of confluence of the W_1 and W_2 phases, which could cause marked changes in physical structure and thermal behaviour, with significant relationships between the independent variables and the main thermal events.

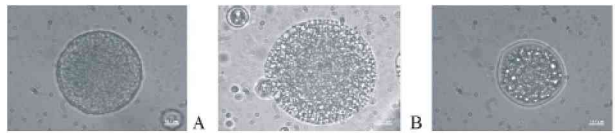
Formulation design (qualitative) was performed to determine the significance of differences in the main DSC events of the microspheres. The factors selected as *independent variables* were: the log P of the cosolvents ($\mathbf{X_1}$), the preparation method ($\mathbf{X_2}$), and the cosolvent concentration (%, w/w) ($\mathbf{X_3}$). Table 1 shows the levels and actual values of the independent variables. Thus, Me_2CO, *n*PrOH, MeCOEt, or *n*BuOAc were mixed individually with CH_2Cl_2 as organic solvent. The batches were evaluated on the basis of SEM, DSC and RS measurements. Thermal events 1-3 (°C) ($\mathbf{Y_1}$), ΔH values ($J \cdot g^{-1}$) ($\mathbf{Y_2}$), and encapsulation efficiency (EE) (%)($\mathbf{Y_3}$), as *dependent variables* were examined.

Table 1. Levels and values of the independent variables (non-randomized)

Levels	Values		
	$\mathbf{X_1}$ (log P)	$\mathbf{X_2}$ (prep. method)	$\mathbf{X_3}$ (cosolvent conc.) (%, w/w)
-1	0.234 (Me2CO)	Spray-dried (SD)	0
-0.3	0.559 (*n*PrOH)	----------	25
+0.3	0.736 (MeCOEt)	----------	50
+1	1.822 (*n*BuOAc)	Solvent evaporation (SE)	75

The state of the W_1/O emulsion droplets determines the morphology of the final microparticles. The W_1/O emulsion droplet structure was changed dramatically by increasing the drug/copolymer ratio (Figs 2A-C). Increase of the drug/copolymer ratio (X_3: -1→+1) at a fixed volume of the cosolvent (X_2: 0) resulted in an increase in the W_1 droplet size due to the influx of water and merging. The emulsion droplets exhibited rupture of the interfacial layers; the physical stability therefore became critical. This alteration in the W_1 droplet structure drastically decreased the EE value of the microspheres, in accordance with the literature. When the drug/copolymer ratio was fixed at 1:16 (X_3: +1), increase of the cosolvent concentration (X_2: 0→+1) resulted in an increased W_1 droplet size. Despite of the large W_1 droplet size, the copolymer precipitation rate increased due to the higher amount of cosolvent, increasing the EE.

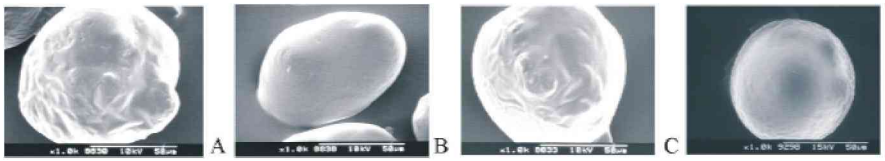
Figure 2. Representative image analysis of multiple emulsion droplets (magnification: 100x) (X_1; X_2; X_3): (A) -1; 0; -1; (B) -1; 0; 0; (C) -1; 0; +1.



The basic composition SE-microspheres, prepared with CH_2Cl_2 alone, were all nonporous and spherical in shape as expected (Fig. 3A), indicating a constant evaporation of CH_2Cl_2 and also uniform solidification. No signs of deformation were observed in the SEM pictures, which means that evaporation proceeded in conjunction with the solidification process.

There were no drug particles on the surface of the microspheres, and no signs of recrystallization or aggregation were observed. The surface of the drug-free basic composition SE-microspheres was smooth (Fig. 3B). The basic composition SD-microspheres displayed spherical particles with a smooth surface, without agglomeration, an uneven shape, or drug crystals on the surface (Fig. 3C). The drug-free basic composition SD-microspheres exhibited an intact and smooth surface (Fig. 3D).

Figure 3. Basic composition microspheres: (A) drug-containing SE-microspheres; (B) drug-free SE-microspheres; (C) drug-containing SD-microspheres; (D) drug-free SD-microspheres.



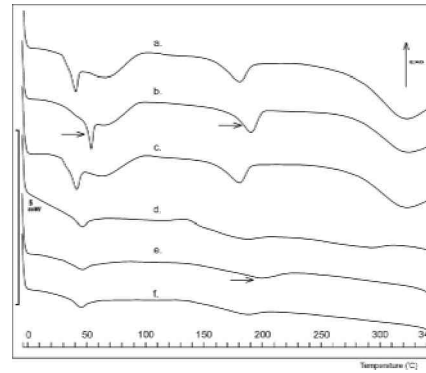
4.2.1. Conclusions of the comparative study

(1) In thermoanalytical studies it was found that neither the concentrations nor the types of the cosolvents changed the temperatures of the thermal events or the enthalpies significantly; coherence of the independent variables (log P and concentration of cosolvents, and preparation method) and the EE values could be observed. The noteworthy differences between the physical mixtures and the microspheres furnished evidence on the formation of a DS solid solution in the matrix (Fig. 4). The usage of polar cosolvents had less effect on the thermal behaviour of the microspheres; only the presence of the drug was of decisive importance.

Figure 4.

Typical DSC profiles of the microspheres:

(A) (100% CH_2Cl_2, spray-dried);
(B) (50% CH_2Cl_2, spray-dried, drug-free);
(C) (50% CH_2Cl_2, spray-dried);
(D) (100% CH_2Cl_2, solv. evap.);
(E) (50% CH_2Cl_2, SE, drug-free);
(F) (50% CH_2Cl_2, solv. evap.).



(2) Raman spectroscopy demonstrated that only the nature of the preparation method caused significant variations in the structure of the microspheres. Raman spectroscopy revealed weak

interactions between AMC and DS in the microspheres, without sufficient strength to exert a retaining effect on drug from dissolution.

The results confirmed that both SE- and SD-techniques can be used for microsphere production, in spite of the thermal treatment nature of the spray-drying.

4.3. FORMULATION OPTIMIZATION OF SE-MICROSPHERES

The formulation optimization of drug-containing sustained-release SE-microspheres was investigated in paper **[I]**. The investigations focused on the determination and understanding of the influence of preparation parameters on the W_1/O emulsion, and on the structure and characteristics of the SE-microspheres. The optimization was carried out on the basis of the *qualitative* design study. The factors selected as *independent variables* were: the ratio of the primary emulsion (W_1/O) and the external aqueous phase (W_2) ($\mathbf{X_1}$), emulsion stirring rate (rpm) ($\mathbf{X_2}$), the drug/copolymer ratio ($\mathbf{X_3}$), and the plasticizer/copolymer ratio ($\mathbf{X_4}$). Table 2 shows the levels and actual values of the independent variables. Several parameters were examined as *dependent variables*: η – as W_1/O emulsion viscosity (mPas) ($\mathbf{Y_1}$), D [4,3] – as particle size ($\propto$m) ($\mathbf{Y_2}$), specific surface area (m^2/g) ($\mathbf{Y_3}$), E – as drug entrapment (%, w/w) ($\mathbf{Y_4}$) and EE – as encapsulation efficiency (%) ($\mathbf{Y_5}$).

Table 2. Levels and values of the independent variables (non-randomized)

Levels	Values			
	X_1 ($W_1/O:W_2$)[a]	X_2 (stirring rate)[b]	X_3 (DS/AMC)	X_4 (PEGS/AMC)
-1	1:5	14400	1:50	1:10
-0.3	1:10	17600	1:25	1:5
+0.3	1:15	20800	1:16	1:3.3
+1	1:20	24000	1:12	1:2.5

[a]: the ratio of the primary emulsion (W_1/O) and the external aqueous phase (W_2);
[b]: the stirring rate in the first step of emulsification;

Conclusion of the characterization of SE-microspheres

- **W_1/O : W_2 phase ratio (X_1)**: A fourfold increase of the amount of the W_2 phase resulted in significant decreases in particle size and EE. With decreasing particle size, the specific surface area/particle volume ratio increased, in conjunction with a decrease in the cumulative amount of drug released.

- **Stirring rate (X_2)**: In the preparation of the W_1/O emulsion, a stirring rate of 24,000 rpm was inappropriate, because the mechanical stress damaged the composition, leading to unsuitable drug release characteristics.

- **Ratio DS/AMC (X_3)**: The increase of drug concentration resulted in an increase in particle size and more viscous and more stable W_1/O emulsion (thicker oil layer) yielded an enhanced EE.

- **Ratio PEGS/AMC** (X_4): Increase of plasticizer concentration led to a significant decrease in particle size, and the more hydrophilic structure significantly increased the drug release.

4.4. FORMULATION OPTIMIZATION OF SD-MICROSPHERES

The objective of this part of the work was to optimize and simulate the alterations of the process parameters and to ensure microsphere product quality according to the *PAT* (Process Analytical Technology) system. Appropriate preparation techniques should be designed and complex investigations of the effects of the main physicochemical factors should be performed to overcome the drawbacks of the microparticles.

The optimization was carried out on the basis of the average effects of the dependent variables and a 3^3 factorial design study. The factors selected as *independent variables* were: the log P ($\mathbf{X_1}$), and the concentrations of the Class 3 polar cosolvents ($\mathbf{X_2}$), and the drug/copolymer ratio ($\mathbf{X_3}$). Several parameters were examined as *dependent variables*: η (mPas) ($\mathbf{Y_1}$), production yield (%) ($\mathbf{Y_2}$), particle size (∝m) ($\mathbf{Y_3}$), EE (%) ($\mathbf{Y_4}$), and Q_6 – as released drug amount in 6 h (%) ($\mathbf{Y_5}$). Me_2CO, MeCOEt or *n*BuOAc were mixed individually with CH_2Cl_2 as organic solvent. To verify the robustness of the optimization, Me_2CO was replaced with the similarly water-soluble *n*PrOH and the factorial design was also accomplished for *n*PrOH. Although the release profile is a useful feedback for the evaluation and recognition of coherences in matrix systems, it is complicated to draw conclusions regarding the structure of the microspheres from the release profiles without an adequate amount of supporting evidence. *The required parameters* were low values of W_1/O emulsion viscosity (η) and particle size; relatively high values of production yield and EE; and Q_6 values in the ranges of 20-80% in 1-6 h. Table 3 shows the levels and actual values of the independent variables.

Table 3. Levels and values of the independent variables (non-randomized)

Levels	**Values**		
	X_1 (log P)	**X_2 (cosolvent conc.) (%, w/w)**	**X_3 (DS/AMC)**
-1	0.234 (Me_2CO)	25	1:32
-1A	0.559 (nPrOH)		
0	0.736 (MeCOEt)	50	1:24
+1	1.822 (*n*BuOAc)	75	1:16

The individual and joint effects of independent variables on the properties of AMC-based SD-microspheres were investigated. Table 4 summarizes the optimization process between the required microsphere product parameters and the levels of the independent variables, furnishing a basis for predictions of further quantitative data. Low and medium (-1 / 0) levels of X_1, high (+1) X_2 and low (-1) X_3, as independent variables, were used to obtain microspheres with a relatively high production yield (Y_2: 69-71%) and EE (Y_5: 42-53%), and low particle size (Y_3: 141-145 ∝m). It was difficult to identify

the optimum levels of the variables to attain Q_6 in the range of 20-80% in 1-6 h, because the high rate of drug release of particular batches increased the average effects to such an extent that they exceeded the purpose of this work, in spite of their statistical significance. For sustained and relatively low drug release, MeCOEt as cosolvent was appropriate at low and medium (-1 and 0) levels of X_2 and X_3, as were *n*PrOH and *n*BuOAC at low and medium (-1 and 0) X_2. The robustness of the optimization process was confirmed by the replacement of Me_2CO with *n*PrOH, the effects of the independent variables were significant, except of Y_5 response.

The following results were obtained as concerns the independent variables:

- **Log P of cosolvent (X_1):** The CH_2Cl_2+cosolvent composition was the key factor controlling the properties of the microspheres according to the demand of the formulator. Me_2CO and MeCOEt were clearly the best cosolvents in this work, these cosolvents best increased the precipitation of AMC during the spray-drying process, and ensured low η. The cosolvents *n*BuOAc and especially *n*PrOH gave less reasonable results, despite the similar microsphere surface structures, different EE and Q_6 values were obtained. The final sequence of the cosolvents was *n*BuOAc < *n*PrOH < MeCOEt < Me_2CO as concerns their utility for sustained release microspheres.
- **Cosolvent concentration (X_2):** A high level of X_2 had a much higher positive effect; the optimum parameters could be reached with X_2 in the sequence of 50<25<75% w/w.
- **The drug/copolymer ratio (X_3):** For optimization of the microsphere characteristics, the ratio of 1:32 (X_3: -1) proved effective. Conversely, at the ratio of 1:16 (X_3: +1), in spite of the rapid preparation process, the less stable W_1/O emulsion droplets could not retain the drug inside during preparation and EE decreased due to the osmotic effect of the W_1 phase.

Table 4. Optimization of levels of independent variables according to required effects

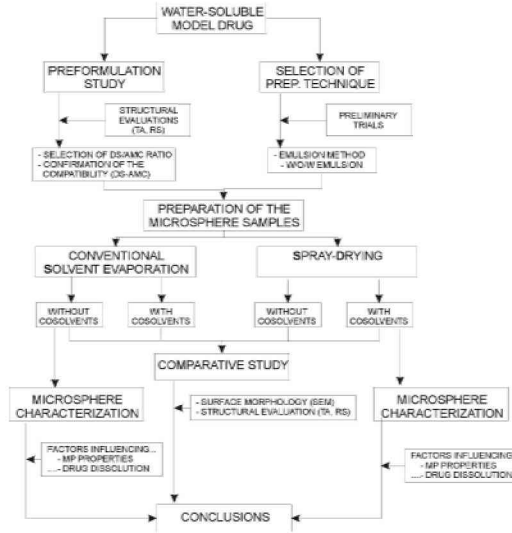
Responses	Required effects (Relative values)	Required levels		
		X_1	X_2	X_3
Y_1	Low W_1/O viscosity	-1	+1	+1
Y_2	High production yield	-1 / 0	-1 / +1	-1
Y_3	Low particle size	-1	-1 / +1	-1
Y_5	High EE	-1 / 0	+1	-1
Y_6	$Q_6 \rightarrow$ 20-80% in 1-6 h	0 / +1	+1	-1 / +1

5. SUMMARY

The preformulation study towards microspheres was aimed in this thesis, followed by the formulation optimization and evaluation of the prepared SE- and SD-microspheres. Figure 5 shows the summary of the optimization steps of this PhD work.

Figure 5.

Summary – a recommended protocol of microsphere development followed in this thesis



Preformulation study

Physical mixtures of AMC with DS, protective carrier and plasticizer separately and in combinations and a drug-copolymer model mixture were prepared for preformulation mesurements. None of the major compositional changes revealed any significant difference, which could indicate a strong ionic interaction between the drug and the copolymer, according to RS evaluation. TA and RS investigations showed that the drug/copolymer ratio can be selected from a wide range in the formulation optimization of SE- and SD-microsphere preparation, in conformity with the therapeutic aim.

DH-containing microspheres: TA suggested that the presence of the crystalline form of DH was not observed in the chitosan based microspheres, as an indication of the molecular dispersion of DH in the chitosan matrix. It was established that the preparation conditions influenced the particle size; furthermore, the microspheres were spherical. Based on the investigations, the ratio DH/chitosan = 1:1 was suggested as the best ratio.

Formulation optimization of the SE- and SD-microspheres:

The formulation optimization section of this thesis focused on the determination and understanding of the influence of preparation parameters (stirring rate, phase ratios, drug/copolymer and plasticizer/copolymer ratios) on the W_1/O emulsion, and on the structure and characteristics of the microspheres. The morphology, the physicochemical properties and in vitro dissolution behaviour of the microspheres obtained were discussed.

Evaluations of the potential of EDXRF apparatus in EE determination have been performed, its application for our purpose can be considered a novelty. The emulsification process generated microspheres in high yield with a particle size range of 100-300 ∞m.

The following contributions can be assessed to the preparation of microspheres:

(**1**) In the preparation of the W_1/O emulsion at elevated stirring rate led to microspheres with unfavorable characteristics. The viscosity of the W_1/O emulsion up to 90 mPas ensured acceptable microsphere product.

(**2**) Increase of the drug concentration resulted in an increase in particle size, and a more viscous and more stable W_1/O emulsion (thicker oil layer) yielded an enhanced EE. Increase of drug content and the plasticizer concentration had opposite effects on particle size. A covalently not bound plasticizer was applied, which lead to more hydrophilic microsphere structure and a consequent significant increase of drug release. The plasticizer concentration did not influence the viscosity of the W_1/O emulsion (η).

(**3**) The results obtained in the quantitative factorial design study of SD-microspheres showed that the use of Class 3 cosolvents and alteration of the drug/copolymer ratio proved effective in the optimization process. Linear relationships were observed between the independent (log P and concentration of the cosolvents, and the drug/copolymer ratio) and the dependent (η, preparation yield, particle size, EE and Q_6) variables. It was found that the *polar cosolvents* used can serve as effective ingredients, replacing CH_2Cl_2 in 25-75% w/w concentration to prepare AMC-based microspheres. Irrespective of their type, even at high concentration (75% w/w) the cosolvents caused only minor structural changes and differences in DSC events, while the microspheres varied in their physicochemical properties. The analysis results confirmed the dispersed state of the drug in the microspheres. The DSC measurements confirmed the parameter stability of the microspheres.

In the comparative study major differences in DSC events were observed only between the SE- and SD-microspheres and the drug-free and drug-containing microspheres.

(**4**) The optimum level of variables was attempted to choose, keeping Q_6 in the range of 20-80% in 1-6 h in SD-microsphere preparation by quantitative factorial design study. The robustness of the optimization process was investigated and confirmed by the replacement of Me_2CO with *n*PrOH. Me_2CO and MeCOEt were found to be the best cosolvents, which facilitated the precipitation of AMC best during spray-drying, and ensured low W_1/O emulsion viscosity. The final sequence of cosolvents was *n*BuOAc > *n*PrOH > MeCOEt > Me_2CO as concerned their utility in the preparation process of sustained release SD-microspheres. The cosolvent concentration favourably used showed a sequance of 50 < 25 < 75% w/w, and drug/copolymer ratio = 1:32 proved to be optimal in SD-microsphere formulation.

(**5**) The drug release rate was controlled mainly by drug diffusion, whereas the models of Higuchi and Baker–Lonsdale proved to conform to each dissolution profile ($R^2 > 0.95$). The kinetic study allowed the conclusion that the Higuchi square root of time model was the best-fitting model with which to describe

the release kinetics of the examined batches. It was found that, when deviations occurred either in the microsphere structure or in the matrix homogeneity, the release profiles of the microspheres conformed to the Baker-Lonsdale matrix dissolution model.

(**6**) At 75% w/w, Class 3 cosolvents gave < 1000 ppm residuals which meets the requirements of the ICH guideline at single dosing per day, while 100% w/w CH_2Cl_2 in SD-microspheres gave residual exceeding the limits (808.5 ppm).

The potential use of drug-containing SE- and SD-microspheres for sustained release is supported by these studies. The spray-drying and the use of polar cosolvents proved to be promising alternatives for the rapid and successful microparticle formulation. The reduction of the particle size can be an important objective of the development, as AMC-based *colloidal sized particles* have already been successfully prepared with average size of 200-300 nm (unpublished result).Control of the drug release rate and the increase of the EE value are also proposed subjects for further investigations. In addition, the replacement of CH_2Cl_2 to polar cosolvents can be considered as one of the following steps towards green technologies.

6. ANNEX

Publications related to the subject of this thesis

[I.] ***P. Sipos****, I. Csóka, S. Srčič, K. Pintye-Hódi, I. Erős*

Influence of preparation conditions on the properties of Eudragit microspheres produced by a double emulsion method *(Drug Dev. Res., 64, 41-54. 2005)* IF: **0.891**

[II.] *A. Kovács, I. Csóka,* ***P. Sipos****, I. Erős*

Preparation, properties, stability and applicability scopes of complex emulsions in the cosmetics *(J. Oil Soap Cosm., 54, 100-109. 2005)* IF: ---

[III.] *T. Hekmatara, G. Regdon Jr.,* ***P. Sipos****, I. Erős, K. Pintye-Hódi*

Thermoanalytical study of microspheres containing diltiazem hydrochloride *(J. Therm. Anal. Cal. 86. 287-290., 2006)* IF: **1.438**

[IV.] ***P. Sipos****, M. Szűcs, A. Szabó, I. Erős, P. Szabó-Révész*

An assessment of the interactions between diclofenac sodium and ammonio methacrylate copolymer using thermal analysis and Raman spectroscopy *(J. Pharm. Biomed. Anal., Accepted, 2007)* IF: **2.121**

[V.] ***P. Sipos****, A. Szabó, I. Erős, P. Szabó-Révész*

Thermal behaviour of ammonio methacrylate copolymer - based microspheres prepared with polar cosolvents by different preparation techniques. A DSC and Raman spectroscopic study *(J. Therm. Anal. Cal. Accepted, 2008)* IF: **1.438**

[VI.] ***P. Sipos****, K. Pintye-Hódi, I. Erős, P. Szabó-Révész*

Formulation optimization of sustained-release ammonio methacrylate copolymer microspheres. Effects of concentration and log P of polar cosolvents, and role of the drug/polymer ratio ***(Under revision)***

Other publications related tothe subject of the PhD work

[VII.] ***P. Sipos****, I. Csóka, I. Erős*

Preparation and investigation of Eudragit microparticles, I. Microspheres – drugs with different water solubility *(Eur. J. Pharm. Sci., 25, S187-189, 2005)* IF: 1.949

[VIII.] *G. Regdon Jr., T. Hekmatara,* ***P. Sipos****, I. Erős, K. Pintye-Hódi*

Diltiazem hydrochloride and tolperisone hydrochloride containing microspheres and their thermoanalytical testing *(Eur. J. Pharm. Sci., 25, S176-178, 2005)* IF: 1.949

[IX.] *I. Erős, Zs. Makai, Z. Aigner, J. Bajdik,* ***P. Sipos***

Preparation and investigation of alginate based pharmaceutical dosage forms *(Eur. J. Pharm. Sci., 25, S94-96, 2005)* IF: 1.949

Other publications

[X.] *M. Shourbaji,* ***P. Sipos****, S. Maher, P. Szabó-Révész, I. Erős*

Liberation of active substance from pharmaceutical suspensions I., Effect of surfactants on the active substance dissolving *(Acta Pharm. Hung., 2007)* IF: ---

[XI.] *M. Mohnicke,* ***P. Sipos****, Y. Zhou, J. Ulrich, P. Szabó-Révész*

Spray drying techniques in the crystallization of riboflavin *(BIWIC, 2005,* ISBN: 3-86010-797-6*)* IF: ---

Abstracts and conference lectures related to the subject of this thesis

PP = Poster presentation, OP = Oral presentation

[1.] *K. Sütő, I. Csóka,* ***P. Sipos****, I. Erős,* Multiple emulsion as pharmaceutical dosage form, *Symposium on Lipid and Surfactant Dispersed Systems, Moscow, 167-168, 1999* PP

[2.] ***P. Sipos****, I. Csóka, I. Erős,* V/O/V típusú emulzióból előállított mikrorészecskék hatóanyag-felszabadulásának jellemzése, *Országos PhD-hallgatói konferencia, Gödöllő, 2000* PP

[3.] *I. Csóka, K. Sütő,* ***P. Sipos****, I. Erős,* Characterization of multiple emulsions, *6th European Congress of Pharmaceutical Sciences, EUFEPS 2000, Budapest, Eur. J. Pharm. Sci., S15, 2000* OP

[4.] ***P. Sipos****, I. Csóka, I. Erős,* Mikroszféra – új lehetőség a hatóanyagok biohasznosíthatóságának fokozására, *Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium, Visegrád, 2001* OP

[5.] ***P. Sipos****, I. Csóka, I. Erős,* Preparation of Eudragit microspheres with different internal matrix structures by means of different solvent evaporation techniques, *3rd International Conference of PhD Students, Miskolc, 177-179, 2001)* OP

[6.] ***P. Sipos****, I. Csóka, I. Erős,* Preparation of Eudragit microspheres with different internal matrix structures by means of different solvent evaporation techniques, *4. Zentraleuropäischen Symposium für Pharmazeutische Technologie, Wien, S262-263, 2001* PP

[7.] *I. Csóka, I. Erős,* ***P. Sipos****, K. Sütő, E. Bodnár, E. Soós-Csányi, M. Makai,* Drug liberation from emulsion drug delivery systems. 3. Drug liberation from multiple emulsions, *8th Conference on Colloid Chemistry, Keszthely, 68.p., 2002* PP

[8.] *I. Csóka, K. Sütő, T. Pénzes,* ***P. Sipos****, I. Erős,* Összetett emulziók stabilitása, *V. Nemzetközi Kozmetikai és Háztartásvegyipari Kongresszus, 87.p., 2002* PP

[9.] ***P. Sipos****, I. Csóka, I. Erős,* Eudragit mikroszférák előállítása módosított V/O/V összetett emulziók segítségével, *XIV. Országos Gyógyszertechnológiai Konferencia, Hévíz, 79.p., 2002* PP

[10.] ***P. Sipos****, I. Csóka, I. Erős,* Porlasztva szárítással előállított Eudragit mikroszférák vizsgálata, *Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium, Visegrád, 2002* OP

[11.] ***P. Sipos****,* Összetett emulziós technikával előállított Eudragit mikroszférák vizsgálata, *VI. Clauder Ottó Emlékverseny, Budapest, 17.p., 2002* OP

[12.] ***P. Sipos****, I. Csóka, I. Erős,* Összetett emulziós technikával előállított Eudragit mikroszférák vizsgálata, *XII. Congressus Pharmaceuticus Hungaricus, Budapest, 2003* PP

[13.] *T. Hekmatara, G. Regdon Jr.,* ***P. Sipos****, I. Erős, K. Pintye-Hódi,* Thermoanalytical testing of microspheres containing diltiazem hydrochloride and tolperisone hydrochloride, *8th International Conference on Pharmacy and Applied Physical Chemistry, PhandTA 8., Monte Verita, 22.p., 2004* PP

[14.] ***P. Sipos****, Zs. Makai, I. Erős,* Gyógyszertartalmú mikrorészecskék szemcseméret-analitikája, *IX. Szemcseméret-analitikai, Környezetvédelmi és Portechn. Szimpózium, Balatonfüred, 96-97., 2004* PP

[15.] ***P. Sipos****, I. Csóka, S. Srčič, K. Pintye-Hódi, I. Erős,* Influence of preparation conditions on the properties of Eudragit microspheres produced by a double emulsion method, *PharmaBioTec Europe, Symposium of Pharmaceutical Biotechnology, Trieste, 2004* PP

[16.] ***P. Sipos****, I. Erős, P. Szabó-Révész,* Mikrorészecskék – Új lehetőségek a hatóanyagok biológiai használhatóságának fokozására, *A Magyar Tudomány Ünnepe, Szeged, 2004* OP

[17.] *E. Bányai,* ***P. Sipos****, I. Erős,* Paracetamol tartalmú mikrorészecskék a gyógyszerészetben, *XXXIX. Rozsnyay Mátyás Emlékverseny, (national placed 2nd), Lillafüred, 2004* OP

[18.] ***P. Sipos****, I. Csóka, I. Erős,* Preparation and investigation of microparticles, Microparticles with different coating material. Drugs with different water solubility, *6th Central European Symposium on Pharmaceutical Technology and Biotechnology, Siófok, 2005* PP

[19.] ***P. Sipos****, P. Szabó-Révész, I. Erős,* Mikroszférák előállítása és vizsgálata különböző vízoldékonyságú hatóanyagokkal, *XIII. Congressus Pharm. Hungaricus, Bp., 105.p., 2006* PP

[20.] ***P. Sipos****, I. Erős, P. Szabó-Révész,* Nyújtott gyógyszer-felszabadulást biztosító mikroszférák előállításának optimálása kísérlettervezéssel, *7. KeMoMo – QSAR Miniszimpózium, Szeged, 2007* OP

Other abstracts and conference lectures related to the subject of this thesis

[21-40.]