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Ph.D. thesis

FORMULATION OPTIMIZATION OF AMMONIO METHACRYLATE COPOLYMER BASED SUSTAINED RELEASE MICROSPHERES

presented by
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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**

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III. ABBREVIATIONS

AcN	Acetonitrile;
AMC	Ammonio Methacrylate Copolymer;
API	Active pharmaceutical ingredient;
β-CD	β-cyclodextrine;
BCS	Biopharmaceutics drug classification system;
BSA	Bovine serum albumine;
CDR	Cumulative drug release;
CH_2Cl_2	Dichloromethane or Methylene chloride;
$CHCl_3$	Chloroform;
CHT	Chitosan;
D [4.3]	Weighted average of the volume distribution;
DH	Diltiazem hydrochloride;
DH/CHT	Diltiazem HCl/ chitosan ratio;
DMA	Dynamic mechanic analysis;
DOE	Design of experiment;
DS	Diclofenac sodium (model drug);
DS/AMC	Diclofenac sodium/copolymer ratio;
DSC	Differential scanning calorimetry;
DTA	Differential Thermal Analysis;
E	Drug entrapment (%, w/w);
EDXRF	Energy-dispersive X-ray fluorescence analysis;
EE	Encapsulation efficiency (%);
η	W_1/O emulsion viscosity (mPas);
FDA	US Food and Drug Administration;
FTIR	Fourier-transformed infrared spectroscopy;
GC	Gas chromatography;
GI	Gastrointestinal (tract);
HLB	Hydrophilic-lipophilic balance;
HPMC	Hydroxypropyl methylcellulose;
ICH	International Conference on Harmonisation;
IR	Infrared spectroscopy;
MeCOEt	Methyl ethyl ketone;
Me_2CO	Acetone;
MeOH	Metanol;
*n*BuOAc	*n*-butyl-acetate;
*n*PrOH	*n*-propanol;
NSAID	Non-steroidal anti-inflammatory (and analgesic) drug;
PBS	Phosphate-buffered saline;
PDE	Permitted daily exposure ($mg \cdot day^{-1}$);
PEG	Poly(ethylene glycol);
PEGS	Poly(ethylene glycol stearate);
PEGS/AMC	Plasticizer/Copolymer ratio;
Ph.Eur.	European Pharmacopoea (as Editio No. 5.);
PLGA	Poly(lactic-*co*-glycolic acid);
PMMA	Poly(methyl methacrylate);
PVA	Poly(vinyl alcohol);
PVP	Poly(vinyl pyrrolidon);
R^2	Correlation coefficient;
RS	Raman spectroscopy;
RT	Room temperature;
S.D.	Standard deviation ($p < 0.05$);
SD-microspheres	Microspheres prepared by the spray-drying technique;
SDS	Sodium dodecyl sulphate;
SE	(The conventional) Solvent-evaporation technique;
SE-microspheres	Microspheres prepared by solvent-evaporation technique;
SEM	Scanning electron microscopy;
SPAN	Width of the particle size distribution;
SSA	Specific surface area (powder surface/powder volume) ($m^2 \cdot g^{-1}$);
T_D	Degradation / decomposition temperature;
T_g	Glass transition temperature;
T_m	Melting temperature;
TA	Thermal analysis;
TEM	Transmission electron microscopy;
TG	Thermogravimetric analysis;
TNF	Tumor necrosis factor;
TRIS	Trishydroxymethylaminomethane buffer;
USP	United States Pharmacopoea (as Editio No. XXIII);
W_1 phase	Inner aqueous phase of the $\mathbf{W_1}/O/W_2$ multiple emulsion;
W_1/O	Water-in-Oil (primary) emulsion;
$W_1/O/W_2$	Water-in-Oil-in-Water multiple emulsion;
W_2 phase	Outer aqueous phase of the $W_1/O/\mathbf{W_2}$ multiple emulsion;
XRD	X-ray diffractometry;
Φ_1	Volume fraction of water in the W_1/O emulsion;
Φ_2	Volume fraction of W_1/O emulsion in the $W_1/O/W_2$ emulsion

1. INTRODUCTION AND AIMS

Research, development and sales of drug-delivery systems are increasing at a rapid pace throughout the world. This worldwide trend will intensify in the next decade as cuts in public health expanses demand lower costs and higher efficacy. To meet this demand, many efficient drugs currently in use will be reformulated within delivery systems that can be value-added for optimal molecular activity. 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[1].

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 nanoparticles, microemulsions, liposomes, cochleates, and nonionic surfactant vesicles (niosomes). APIs 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, the related papers are numbered in chronological order.

The **main objectives** of the PhD work were to study the preparation and comparison of novel stable microsphere compositions containing DS as model drug, using AMC 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 (*pharm. 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 DS-AMC interactions. Films with different ratios DS/AMC were prepared by the solvent casting method and investigated by the TA and 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**) ratio DS/AMC; and (**v**) ratio PEGS/AMC 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**) ratio DS/AMC 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;

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

(**iii**) external morphology of microspheres (SEM);

(**iv**) granulometric analysis (PSA); (**v**) determination of E and EE (EDXRF);

(**vi**) thermal behaviour and structural evaluation (TA);

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

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

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

2. LITERATURE REVIEW

2.1. THEORY OF MICROENCAPSULATION

Microencapsulation techniques are widely used in the development and production of improved drug- and food-delivery systems; and to enhance material stability, reduce adverse or toxic effects, or extend material release for different applications in various fields of manufacturing[1]. To this time, the use of some interesting and promising therapeutic materials has been limited clinically because of their restrictive physicochemical properties, which have required frequent administration. These substances may become more widely used in a clinical setting if appropriate microencapsulation techniques can be designed to overcome their intrinsic inconveniences. During the past two decades, pharmaceutical technologists have succeeded in controlling the drug-absorption process to sustain adequate and effective plasma drug levels over a prolonged period of time by designing oral or parenteral *microparticulate delivery systems*. The ultimate objective is to control and extend the release of API from the microparticles without attempting to modify the normal biofate of the API in the body after administration and absorption. In the past decade, ongoing efforts have been made to develop drug carriers specifically to the intended target organ, while reducing the total amount of drug administered and increasing the therapeutic efficacy. The site-specific *microparticulate delivery systems* allow an effective API concentration to be maintained for a longer interval in the target tissue and result in decreased side effects associated with lower plasma concentrations in the peripheral blood circulation. The use of microparticles for drug delivery is not limited to any specific illness, rather they can be widely applied in many situations where continuous/controlled/targeted drug administration is essential.

Microparticles are usually formed by the controlled precipitation of polymers and can be divided to the groups of: (**i**) microcapsule (spherical geometry with a continuous core region surrounded by a continuous shell; reservoir systems); (**ii**) microsphere (spherical matrix with dispersed or dissolved entrapped drug; matrix systems); and (**iii**) irregular geometry with a number of small droplets or particles of core material. Microparticles have many advantages: (**i**) delayed or sustained release; (**ii**) prevention of side effects related to the presence of the drug in the stomach; (**iii**) protection of the drug from degradation in the acidic environment of the stomach; (**iv**) reduction in frequency of administration and avoidance of peak and valley effects in blood level; (**v**) biocompatibility; (**vi**) easy preparation; (**vii**) relative stability; and in special cases (**viii**) to obtain controlled or targeted release.[2] Microparticles are widely discussed in the

literature, this is why the literature review part of this thesis mainly focuses on microspheres prepared by the W/O/W emulsion-solvent evaporation method. Some other preparation methods are also mentioned. A number of microparticle preparation methods are listed in Table 1.[3-9]

Table 1 Frequently used encapsulation processes

Physico-chemical encapsulation processes			**Mechanical encapsulation processes**	
1	complex coacervation[10-13]		8	spray drying[19]
2	polymer-polymer incompatibility		9	spray chilling[20]
3	interfacial polymerization in liquid media		10	fluidized bed coating
4	in situ polymerization[14]		11	electrostatic deposition
5a	in-liquid drying[15]	solvent evaporation[16]	12	centrifugal extrusion
5b		solvent extraction[17], quenching[18]	13	spinning disk or rotational suspension separation
6	thermal and ionic gelation in liquid media		14	polymerization at liquid-gas or solid-gas interface
7	desolvation in liquid media		15	pressure extrusion or spraying into solvent extraction bath
			16	matrix grinding[21]

2.1.1. Emulsion methods to the encapsulation process

Emulsification techniques have been developed in order to achieve successful encapsulation, and prevention of degradation of API. This preparation method for s consists of two, three or more phase systems (O – oil; W – water; S – solid; G – glycerol). The system of different emulsion methods towards microparticle formation are assessed in Fig. 1. Water-containing systems are often used as:

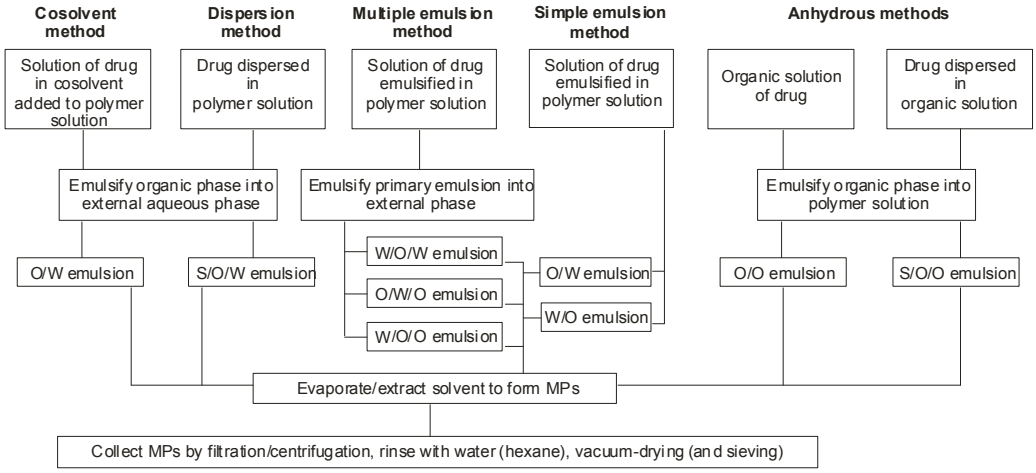
- **O/W**: dispersion of the organic polymer and lipophilic drug solution into an aqueous phase;[22,23]
- **S/O/W**: the technique is based on suspension of the drug in organic solvents;[24,25]
- **W/O, G/O** and **W/O/O**: the hydrophilic drugs (e.g. insulin[26] and TNF-α;[27]) are unlikely to migrate out of the medium, resulting high EE;[28,29]
- **W/O/O/O**: it ensures microparticles of the class of reservoir type drug delivery devices.[30] The oil in the W/O emulsion prevents both the contact between the internalized drug and the polymer/solvent systems, and possible denaturization of i.e. protein.
- **O/W/O**: formulating microspheres loaded with a hydrophobic drug;[31]
- **W/O/W**: in case of O/W emulsions, poor EE was observed with hydrophilic drugs.[32] This emulsion technique is more complex,[33,[II]] with more processing variables to be controlled.

Anhydrous emulsion systems have also been developed as:

- **O/O**: high protein loading could be achieved; it is comprised of an organic polymer phase emulsified in an immiscible oil,[8] i.e. AcN+ CH_2Cl_2 and corn oil,[34] where the drug is insoluble in

the external oil;[35] the **S/O/O** technique is allowed the micronized drug substance and a polymer solvent (nonpolar)-cosolvent (polar) system; the **S/O/O/O** system could be i.e. oil suspension of drug-AcN-mineral oil system.[27]

Figure 1. Emulsion methods



The evaporation of the organic solvent can be accomplished in three different ways:

- (**i**) evaporation (RT) stopped before complete elimination, the partially solid microparticles are transferred into an emulsifier solution, where the evaporation is pursued;[32]
- (**ii**) the emulsion is continuously stirred (RT) until evaporation is completed[36] and;
- (**iii**) emulsion is placed into a rotary evaporator under vacuum and warmed.[37]

The $W_1/O/W_2$ multiple emulsions

Increased interest in sustained and controlled drug delivery systems and higher bioavailability has led to intensive research on $W_1/O/W_2$ multiple emulsions.[38,39] The advantages offered by multiple emulsions as drug delivery carriers for oral administration include protection against enzymatic hydrolysis and degradation, and enhanced absorption through the intestinal wall.[40] These advantages were already shown for controlled release products in vitro[41] and in vivo[42] after oral administration, as well as after parenteral administration.[43]

Formation and stability of $W_1/O/W_2$ emulsions are mainly influenced by two factors: (**i**) the structure of W_1-phase/oil interface and its saturation by the emulsifier; and (**ii**) the number and size of the multiple droplets and the possible interactions between them.[44,45,[II]] $W_1/O/W_2$ emulsions are thermodynamically unstable, which results in various problems such as leakage of the API from the W_1-phase, flocculation of W_1- and $W_1/O/W_2$ emulsion droplets, and phase

separation. To ensure stable $W_1/O/W_2$ emulsions, (**i**) interfacial complexation of macromolecules in the W_1-phase with a lipophilic surfactant in the oil phase;[46] and (**ii**) formation of polymeric gels in the oil or aqueous phases[47-50] can be applied.

Microparticles formed from a relatively stable W/O/W emulsion typically have four representative internal structures: (**i**) microcapsular; (**ii**) multivesicular; (**iii**) porous capsular and (**iv**) matrix type.[51]

2.1.2. Spray-drying as evaporation technique

During spray-drying, the solvent evaporates quickly due to the thermal energy provided, which leads to quick polymer solidification, resulting in a higher EE[52]. The larger SSA of the spray-dried particles and the elevated temperature of the drying reduce considerably the amount of residual solvent inside the microspheres.[53] The spray-drying preferentially applied when biodegradable polymers[54] were used (PLA,[55,56] albumin,[57] CHT[58]), and it was concluded that PS, morphology, API loading and release are not affected by the cyclone type.[19] The main advantage over other methods is that it is a continuous *one-step process*, offering good reproducibility, potential for scale-up, and preparation yields in the range of 90-100%.[58-59] High processing temperatures may cause organic solvent or water to dry quickly, so that the polymer chains lack time to settle homogeneously, forming an *amorphous* structure. The solvent uptake by the microparticles is prevented but effective subsequent drying is guaranteed (vacuum, lyophilizer), to eliminate residual solvent.[60]

2.2. INGREDIENTS

The main ingredients of microspheres prepared by the emulsification-solvent evaporation method applied in this thesis are (**i**) the API, (**ii**) the copolymer, (**iii**) the organic phase and (**iv**) distilled water.

Active pharmaceutical ingredients (APIs)

A great number of pharmacons have been considered for incorporation. Toxic drugs, which can cause severe side effects, or lipophylic drugs (BCS Class IV), which may require large doses to promote absorption, can be administered only with a lower frequency and smaller quantity. Table 2 shows examples of the variety of specific medications.

Table 2. Generally used active pharmaceutical ingredients

1	antibiotics	Amoxicillin;[61] gentamicin;[54] vancomycin[62]	2	antispastic (intrathecal)	baclofen[63]
3	anaesthetic	bupivacaine[64]	4	antivirals	desferrioxamine[65]
5	bisphosphonate (parenteral)	pamidronate disodium salt[66]	6	radioembolization therapy	holmium-acetylacetone[67]
7	chemotherapy	tegafur (albumin microparticle)[68]	8	anti-HIV drugs[69]; anti-cancer drugs[70]	
9	antimicrobial	metronidazole[71]; tetracycline (therapy of the periodontal pocket)[72]			
10	NSAIDs	paracetamol (porous thermoplastic cellulose pellets)[73]; DS[74,75]; piroxicam[76]; acetaminophen[77]; ketoprofen[78]			
11	steroid hormones	levonorgestrel[79] (bioerodible contraceptive implantable device); progesterone[18]			
12	proteins	Vibrio Cholera antigen (as outer membrane protein)[80]; insulin[26]			
13	peptides	- octreotide acetate[81]; leuprolein[54]; β-lactoglobulin[82] ($W_1/O/W_2$); - somatostatin[8] and vapreotide (somatostatin analogue)[83] (O/W and O/O); - amino acid peptides as controlled release oral vaccination[84]; -recombinant human growth hormone[85]; human chorionic gonadotropin hormon[86]			
14	albumin (as water-soluble antigen)	- bovine serum albumin (BSA)[87] as nasal platforms across nasal mucosa[88], and oral vaccine delivery[89]; - human serum albumin (HSA) W/O[90]- and $W_1/O/W_2$[91]-techniques; spray-drying[19]			

Peptides, vaccines, immunmodulators. Microparticles are able to protect the *peptides and proteins* against the degradation by enzymes, and in some particular cases to improve their passage through biological barriers. Peptide-based microparticle-vaccines offer several advantages over live, attenuated or inactivated vaccines[92] for the following advantages: (**i**) enhanced stability; (**ii**) no infectious agent is present; (**iii**) less expensive large scale production; and (**iv**) chemically defined product. Microparticles can be used as *potent vaccine adjuvant* for the induction of cytotoxic T lymphocytes against recombinant p55 gag from HIV-1.[93] The encapsulation of a recombinant form of the surface glycoprotein of HIV-1 MN strain (MN rgp120) into microsphere-vaccine could ensure a single administration by providing a sustained release of antigen over time to achieve high neutralizing antibody titers.[94] *Immunomodulator* (monophosphoryl lipid A) was incorporated into microspheres to bias and enhance the immune response towards a type 1 T-helper response.[92] Microparticles loaded with Cyclosporin A as an *immunosuppressive agent*[95] and with *influenza virus vaccine*[96] were investigated to the comparative immune response. Investigations were performed to encapsulate TNF-α;[79] interferon-α;[97] nerve growth factor;[98] and recombinant human erythropoietin[52] into biodegradable microparticles and nanoparticles. Microparticles loading lysozyme as model enzyme were also prepared.[99]

NSAIDs. Increased need for patient compliance and demand for improved therapeutic efficacy of NSAIDs suggest also the need for a sustained release oral drug delivery system.[58] In the case

of multiple dosing regimen of immediate release formulations, it has got the propensity of systemic accumulation, leading to side effects (i.e. indigestion, hemorrage, mucosal erosion and ulceration) or in some cases severe systemic toxicity. The mechanism is complex, and has been partly attributed to both systemic and local irritations due to local GI exposure, the physicochemical action on the gastric mucous,[100] and also to biliary excretion into the GI tract,[101] following all routes of administration, even non-oral routes, e.g. intravenous routes and rectal suppositories.[102] Microparticles formulation will decrease the dosing frequency, alleviate pain and other symptoms and at the same time avoid systemic accumulation. To minimize the side effects, NSAIDs are marketed as enteric coated and sustained-release preparations. Even these formulations have shown GI toxicity in clinical studies,[103] resulting increased exposure of the lower GI tract to the drug.[104] The formulator therefore has the choice of keeping a constant drug dissolution rate or minimizing the dissolved drug concentration.

Other applications. MCs containing alkannin and shikonin with a wide spectrum of biological activity were prepared to control its release rate.[105] For the formation of the synthetic seed, apical buds of apple rootstock can be encapsulated into alginate MCs.[106] Hollow microspheres were composed of discrete nickel nanoparticles, and coated with oriented carbon nanotubes.[107]

Polymers

The available polymers are classified based on their biodegradability (Table 3). *Biodegradable polymeric carriers* are widely used for various advantages,[108,109] like (**i**) good biocompatibility; (**ii**) easy administration (e.g. stereotaxic injection into the brain tissue), (**iii**) controlled release over prolonged periods of time, and (**iv**) complete erosion. They are approved by the FDA for human use.[110,111] Numerous synthetic but often *non-biodegradable polymers* are also available for use in controlled release systems.

Poly(methyl methacrylate) (PMMA) has been used as a sustained release coating in the pharmaceutical field.[122,124] PMMA-PEG blend polymer membranes are used as thermo-sensitive drug delivery systems, showing the T_g around the body temperature (32-42 °C);[123] they clearly open the tight junction, but with serious epithelial cell disruption.[125,126]

AMC, as the used form of PMMA, has been used as enteric coating[122] and sustained release coating material[127] in view of its biological safety,[124,128] and it has been used as a retardant in the formulation of sustained-release pellets,[129] thermosensitive membranes,[123] and matrix tablets.[124] AMC is the less hygroscopic PMMA copolymer and it is insoluble in digestive juices, but swells and becomes permeable, releasing the drug by diffusion.[130] AMC has a chemical purity and

stability,[131] it generally starts to degrade at the side-chain above 150 ºC, while depolymerization and other reactions of the main chain start above 180 ºC.[131] A weak ionic interaction can be observed with AMC and NSAIDs, which is related to its functional ammonio groups.[132] The thermal characterization was reported earlier,[129,131,134] and complementary FTIR spectrocopsic examinations were performed.[123,135] TA investigations were utilized to study AMC-based microspheres;[136,[I,IV,V,VI]] PMMA-grafted silica nanocomposites;[137] and PMMA-plasticizer interactions.[186] The drug-polymer possible interactions also were investigated with XRD and DSC devices.[139]

Table 3. Generally used polymers: biodegradable (A) and non-biodegradable (B)

A	poly(OH-butiric acid)[108]	poly(α-OH-acid) (PAHA)[112] and PAHA/PVA[112]		poly(ethylene oxide)[100]
	poly(δ-valerolactone)[113]	poly(isobutylcyanoacrylate)[96]	poly(ε-caprolactone)[11,16,25]	poly(sebacic anhydride)[76]
	poly(lactic acid) (PLA)[67]	PLA polymer with glycolic acid (PLGA)[92] and ethylene glycol (PELA)[80,91]		poly(ortho esters)[18,79]
B	poly(ethylene terephthalate) (PET):	polyethylene and poly(tetrafluoroethylene)	water-soluble polymers: sodium alginate,[74,114]	
	ethyl cellulose (EC),[12,115] microcrystalline cellulose (MCC),[116] hydroxypropyl (HPC) and hydroxypropyl methylcellulose (HPMC),[78,100] carboxymethyl (CMC) and sodium carboxymethylcellulose (NaCMC),[114] cellulose acetate phthalate (CAP) and butyrate/butanoate (CAB),[115] and cellulose gum[77]			
	PVA, PVA/NaAlg[117]	CHT[118-121]	PMMA[122], PMMA-PEG[123]	

Chitosan (CHT)[[III]] is a hydrophilic, biocompatible and biodegradable natural polysaccharide of low toxicity, and is used to include controlled release delivery systems either for implantation or for oral delivery.[59] As a week base, it is sparingly soluble in water and practically insoluble in all common organic solvents and solutions at pH < 6.5 but dissolves in solutions of most organic acids.[140,141] The cationic nature enables it to establish a strong attractive force with the negatively charged lipid bilayers. Because of its easy availability as a second abundant polysaccharide next to cellulose, CHT has a great potential for pharmaceutical applications.[142]

Organic solvents and cosolvents

Organic solvents and cosolvents are commonly used in the case of emulsification-solvent evaporation method because of their (**i**) limited water solubility; (**ii**) good solubility towards a range of encapsulating polymers; (**iii**) low boiling point; and (**iv**) high evaporation rate. The selection of organic solvents for obtaining a good production yield and advantageous characteristics of the product is mainly restricted by their residual toxicity. Attempts have been

made to use less toxic solvents or to select an efficient preparation technique in the future for environmental and health reasons.[143-146] Cosolvents are generally used as a 'poor' or driving solvent for the polymer.[147] At the interface, the cosolvents, that have less or no affinity to the polymer diffuse out first from the polymeric 'quasi-emulsion' droplets and, at the same time the polymer starts to precipitate at the interface,[146] leading to higher EE.[80] The 'good' solvent, with high affinity to the polymer, have a delayed diffusion from the diminshed droplets. These diffusion steps, which are greatly affected by the properties of the solvents, like water-miscibility, boiling point, viscosity, amount, and particularly the interactions between polymer and solvent, play a crucial role in the successful formulation. It was shown that the polarity does not play a role in the formulation[146]. The use of organic solvents in order to prepare microspheres has been investigated previously, e.g. (**i) Class 2** solvents: $CHCl_3$,[53,90,148] 1,2-dichloroethane,[149] cyclohexane,[12,115] CH_2Cl_2,[33,84,112] THF,[80] and MeOH;[8,150] and (**ii**) **Class 3** solvents: Me_2CO,[16,86,146] EtOH,[45,151,152] AcN,[34] isopropanol,[16] MeOAc,[17] ethyl formate,[18,19] and EtOAc.[13,63,91,99,153] The concentration of Class 2 solvents in the product should be limited according to USP and ICH guidelines[154,155] at every levels of formulation. In addition, it is an industrial requirement to test the amount of residual organic solvents for stability reasons. The enclosed residual solvent migrates over time and it can act as a plasticizer, modifying the T_g of the polymer (generally lowering).

Vegetable oils[156] such as arachis, cottonseed, sunflower, soybean,[27] corn,[113] olive, castor, sesame oil[35] are best preferred as they are hydrophobic and biocompatible. In addition, liquid paraffin,[17,157] and molten wax[158] are also used in the O/W emulsion systems.

Additives

A variety of additives are incorporated in the emulsion phases as surfactants, plasticizers, pigments, antiadherents (fumed silica), preservatives, protective coating colloids and stabilizers.

Plasticizer. Plasticization results in a decrease in the intermolecular forces between polymer chains, promoting flexibility, generally causing a decrease in the T_g and T_m values.[159,160] Plasticizer affects film-forming temperature from colloidal polymer dispersions, the mechanical properties of the resulting films,[161] and the drug release.[162] Plasticizer acts as a pore-forming agent;[91] and it can promote mucosal adhesion,[125] and decrease the biomolecule adsorption and consequently inhibit the API uptake by the cells from the reticuloendothelial system.[159]

Protective colloid. It must have the following properties to ensure the stability of emulsions during encapsulation through (**i**) high surface activity (interfacial tension < 10 dyn/cm); (**ii**) high viscosity in the used phase; (**iii**) adequate electrical charge; (**iv**) film adsorbed on the surface of droplets; and (**v**) low concentration.[163] Among protective colloids used as β-CD and SDS form monomolecular interfacial films, while polysaccharides (pectin, sodium alginate,[5]), proteins (gelatin, serum albumin); synthetic cellulose derivatives (MEC, HPMC, CMCNa[5]), synthetic nonionic polymers (PVA, PVP), gelatine,[80] and the tensioactive BSA protein.[112] form multimolecular films. PVA is frequently used either in a single[85] or a multiple emulsion.[164] The concentration range was found to be optimal at 0.5-2% w/w,[165] higher PVA concentrations leading to an increased viscosity of the W_2 phase, which limited the mechanical breaking of the W_1/O emulsion into small droplets,[166] resulting in a significant increase in the PS,[101] and the absence of pores.[14]

Surfactant. Studies indicated that the emulsifier film strength is more important than the initial droplet size in improving $W_1/O/W_2$ emulsion stability.[167] Frequently used *nonionic* surfactants are the sorbitan fatty acid esters (Spans[29,145] – 80, 83, 85); ethoxylated sorbitan fatty acid esters (Tweens[167] – 20[82], 80[168]); and lecithin.[113] Pore-formation can be prevented by the emulsifier, thus the release profile and the extent of the burst release can be reduced.[84] Polymers typically interact with *anionic* surfactants, and their propensity is related to the length of its alkyl chains.[169]

2.3. FACTORS THAT DETERMINE THE PROPERTIES OF MICROPARTICLES

A range of production parameters influence the physicochemical parameters of the resulting microspheres.[170] Critical formulation parameters for the $W_1/O/W_2$ *preparation process* are:

Mechanical stirring. When W_1/O emulsion is prepared by vortex-mixing, the obtained microspheres are large,[171] however, when by sonication is applied, a microfine and homogeneous emulsion is formed.[172] The EE was reported to increase with increasing mixing rate,[173] whereas other authors found no relationship between these parameters.[174]

Viscosity. The more viscous the polymer solution is, the more difficult it is to break it down into smaller droplets, which leads to larger microparticles. A highly viscous phase and low mixing intensity can be useful in the preparation of microparticles containing sensitive drugs. Increase in the W_1/O viscosity is related to an increase in the EE,[144] but W_1-phase with higher

viscosity will permit the water pass into this phase resulting in swelling and releasing their content into the W_2-phase.[175]

Osmotic gradient. The W_1 phase usually contains stabilizers (protein, surfactant). The semi-permeable surfactant membrane allows some concentration difference, but once the maximum limit is reached (around 10% w/w), transfer of the water droplets through the oil phase will occur. When the W_2-concentration is nil, water can penetrate into the W_1-droplets, resulting increased PS and viscosity of W_2 phase. When the W_2-concentration is twice the W_1-concentration, internal water will migrate ($W_1 \rightarrow W_2$) resulting smaller droplets.[117]

Volume of the phases. The volume of the *W_1-phase* affects the solidification time, as it decreases, an increase in E[176] and a small decrease in PS[29] can be observed. Low *oil phase* volume yields a viscous and concentrated polymer solution, so it is more difficult for the oil phase to be broken into smaller droplets, which results in increased PS[163] and porous matrix. The increase in the *W_2-phase* volume leads to an increase in both the PS and E,[146] which is related to the reduced mixing or dispersion efficiency during the 2nd emulsification step due to the larger volume. Generally there is a practical limit of increasing the W_1- (Φ_1) and W_2-phase (Φ_2) fractions ($0.60<\Phi_1<0.75$ and $0.60<\Phi_2<0.80$), because either the W_1/O emulsion will become far too viscous to be dispersed, or it might invert.

Type of organic solvent-cosolvent. Ever since microparticles have been formulated, the problem of the organic solvent as an important parameter has been present. The integrity of the forming microsphere wall is controlled by the rate of extraction of the organic solvent to the W_2 phase and also by the rate of its evaporation from the W_2 phase. The rate of solvent extraction is limited by the water-solubility of the organic solvent used, while the evaporation rate depends on its boiling point.

When polar *co*solvent is used in the organic polymer solution and is emulsified into the aqueous medium, at the water-organic interface, cosolvents with low affinity for the polymer are the first to diffuse out from the W_1/O emulsion droplet (depending on their physicochemical properties) until it attains equilibrium with the W_2-phase.[145] Addition of a polar cosolvent and therefore fast partitioning and extraction can decrease the interfacial tension between the organic and aqueous phases, and form a dense wall, which can prevent the confluence of the aqueous phases, and ensure a low PS and a dense microsphere structure with high EE.[175,177] Addition of a cosolvent can increase the porosity, leading to drug loss and therefore a lower EE.[175,178]

Polar cosolvents may act in two opposite ways: (**i**) increasing the polymer precipitation rate and (**ii**) at the same time decreasing E, due to the confluence of the aqueous phases; thus, there can be a sensitive balance between these effects.

Temperature. *Below RT* the diffusion and evaporation rate of solvents become slow.[87] *Above 30 °C*, it is easier for the droplets to collide with each other and they may coalesce together at the same time with solidification, since the viscosity of the oil medium is lower at higher temperature.[29] When the solidifying microspheres are exposed to $T > T_g$ of polymer, it will change to its rubbery state which is more flexible and fluent, so the polymer can move through the matrix and fill gaps and coat the existing drug crystals, as in situ micro-coating.[179]

Stabilizers. Addition of buffers (TRIS or PBS[82]) *to the W_1-phase* could promote an influx of water from the W_2-phase due to a difference in osmotic pressure. The addition of salts *to the W_2-phase* results in formation of a dense and homogenous polymer matrix, although they could reduce the solubility of organic solvents in water, resulting the precipitation of polymer.[45]

2.4. MICROSPHERE CHARACTERIZATION METHODS

New applications of microparticles necessitate successful technology transfer, industrial scale-up, and reliable investigation methods also in preformulation and in formulation steps.

Design of experiment (DOE). Optimization with factorial based designs and analysis of the response surfaces is a powerful, efficient and systematic tool that shortens the time required for the development of dosage forms and improves research and development work.[180,181] DOE aids the evaluation of the results of the measurements mentioned below.

Rheological measurements. It can be carried out to investigate the viscosity of the: (**i**) solvent mixture; (**ii**) aqueous and oil phases; and (**iii**) simple/multiple emulsions.

Morphological study. The microparticles can be studied for appearance and the emulsions for droplet type using SEM and optical microscopy, respectively.

Particle size analysis. Microparticles could be sieved with a combined sieving system. One of the commonly used techniques for assessing the PS distribution, SSA and SPAN[58] appeared to be laser diffractometry. Photon correlation spectroscopy (PCS), the Coulter® Multisizer II equipment[31] and light or electron microscopy can also be used.[97]

Drug entrapment (E) and encapsulation efficiency (EE). Very common method to measure the drug entrapment, when microparticles are dissolved with applicable solvent, then filtered and analysed with UV-spectrofotometry.[25,182] Protein and peptide content could measure with protein

assay: HPLC-method,[8,83] and Bio-Rad microassay.[82] IgG and IgA levels can be monitored by ELISA method.[96] DSC and XRD[183] and EDXRF[184,185] also were used to measure the actual E value.[184] Evaluations of the potential of EDXRF apparatus in microparticles have been performed,[I] its application for our purpose can be considered a novelty.

Thermoanalytical measurements (TA). TA is a useful tool in investigating e.g. the solubility of the drug in the polymer.[186] However it should be emphasized that such a solubility is determined at the melting point of the drug and not at ambient temperature. The most common techniques are TG, DSC and DMA, in which structure-dependent physical properties of polymers and drug-loaded polymeric delivery systems are measured when subjected to a controlled temperature program.[187] Interesting types are the modulated temperature DSC (MTDSC)[188], and the 'Heat-cool-reheat' technique when after the 1st heating step the sample is cooled and reheated to delete the disturbing effect of the adsorbed water, so the T_g characteristic to the polymer can be measure clearly.[67]

Raman spectroscopy (RS). Based on the measurement of Raman-scattering by a molecule, RS, FT-Raman, and surface-enhanced Raman (SERS) are used for the structural analysis of molecules, the vibrational characterization of drugs,[189,190,191] the characterization of drug stability, the quantification of complex mixtures, furthermore to confirm the possible interactions,[192] and to differentiate crystalline forms of the materials.[193]

FTIR measurement. It can also be used to characterize the parameters mentioned in connection with RS, often together with other techniques (FTIR + TGA + DSC).[194,195]

Analysis of residual organic solvents and cosolvents. Manufacturers are required to remove residual solvents completely or keep them below acceptable limits, as complete removal is often not possible. Few reports of residual solvent effects are available, such as the effect of residual CH_2Cl_2 on the crystallinity of the drug.[13,196]

Cumulative drug release and release profile studies. The knowledge of the *BCS* characteristics of a drug can also be utilized by the formulator to develop a more optimized dosage form based on fundamental mechanistic, rather than empirical information.[197]

The *in vitro* dissolution rates of the microparticles can be measured at defined rpm in 37±1 °C buffer solution/deionized water mixture of defined pH according to the USP Drug Release Test 2 criteria. Dissolution in the GI tract takes place under heterogeneous conditions, this is one of the reasons why different *buffer solutions* (citrate, acetate, phosphate or other) are used, although most of them do not correspond to the physiological situation in the human GI-tract. The use of

surfactants in the dissolution systems has physiological significance also as natural surfactants like bile salts (wetting, micellar solubilization, and/or deflocculation). Gastric juice has a relatively low surface tension, (42.7 dyn·cm^{-1}) compared with water (70 dyn·cm^{-1}) which aids in the wetting of both hydrophobic and hydrophilic particles. As *in vivo* animal studies, generally male New Zealand white rabbits, rhesus monkeys, wild type and transgenic mice can be used,[85] and the correlation of the in vitro/in vivo evaluations should be clearly established.[83,96]

The types of oral *biodegradable polymeric sustained release systems* according to the drug release are:[198]

(**i**) diffusion-controlled systems (reservoir device-microcapsules; and matrix device-microspheres); (**ii**) dissolution-controlled systems; (**iii**) erosion-controlled systems; and (**iv**) swelling-controlled systems and hydrogels; (**v**) chemically controlled systems; (**vi**) constant or zero-order release; and (**vii**) other delivery systems.

Drug diffusion can occur:[199] (**i**) through polymer matrix; (**ii**) through water-filled pores/cavities; or (**iii**) through both, in parallel and/or sequence. The significance of the initial burst has not been entirely ignored, only less theories have been put forth to fully describe the phenomenon.[200]

Mathematical evaluation. The models can be selected for ideal formulation meeting the USP requirements according to the determination coefficient and the *'goodness-of-fit'* test, employing the following set of equations known in the literature (Table 4).

Model VI is used to describe the release from swelling-controlled systems[201]; its modifications were introduced by Kim-Fassihi,[202] Peppas-Sahlin,[203] and Colombo[204] who suggested that the distance of dissolved gel layer thickness of the polymer is the most important parameter influencing drug release.

Table 4. List of the generally used mathematical models

I	first-order[205]	homogeneous dissolution, the release is independent of the amount of drug
II	zero-order[205]	coated dosage forms or membrane controlled dosage forms
III	Higuchi square root time[206]	diffusion-controlled model, drug is dispersed in a uniform polymeric matrix system[122]
IV	Hixson-Crowell cube root[207]	water-soluble drugs are in porous matrices,[208] release rate is limited by the drug dissolution rate and not by the diffusion through the polymeric matrix
V	Baker-Lonsdale[209]	drug is dissolved uniformly in the matrix,[28] (e.g. the $W_1/O/W_2$ technique)[165]
VI	Korsemeyer-Peppas[210]	the diffusion is the main drug release mechanism, *n* value is used in order to characterize different release mechanisms
VII	Hopfenberg[210]	surface-eroding devices with several geometries
VIII	Nernst equation[211]	dosage forms that do not change during the release process
IX	Weibull distribution[208]	empiric model, it presents some deficiencies and has been the subject of some criticism; applied to almost all kinds of dissolution curves

Other methods

The following methods are also frequently used in microparticle technology.

X-ray diffraction (XRD). Wide-angle (WAXS) and small-angle (SAXS) methods are used to get information on helical polymers, and i.e. in detecting large periodicities in structures such as lamellae, respectively. XRD can be used to quantify the crystalline drug content in microsphere.[183] The amorphous nature of the polymers can be confirmed.[212] DSC and XRD studies reveal the existence of drug-polymer interactions.[99] The first complete analysis of NSAID-loaded ethylcellulose microparticle matrix structure by TG, DSC, HPLC, and XRD was presented in 1991.[213]

NMR measurements. It can show if a rigid microsphere structure is formed due to ionic interaction between the drug and the polymer.[54] To verify that a peptide drug is not modified chemically during microencapsulation, analytical one- and two-dimensional NMR spectroscopy is used.[83]

Electron Microscopy. Freeze-Fracture Electron Microscopy shows information about the *internal* structure of the microparticles. Atomic force microscopy can be used to study the surface morphology and the porosity of the microspheres.[87] Confocal laser scanning microscope (CLSM) can be used to observe protein distribution within microspheres because proteins themselves show fluorescence in many cases[163] or a fluorescent marker can be added to the organic phase.[25] Confocal fluorescence microscopy (CFM) can reveal the drug distribution in microspheres prior to and after drug release.[76] Transmission electron microscopy (TEM) analysis was used to characterize the histopathology of the ileum after oral administration of drug-containing microparticles to rats.[214] CLSM and TEM were used to investigate the ability of pig ileal Peyer's patch segments to transport microspheres from GI lumen across the mucosa.[23]

Helium pycnometry is used to determine the density of the microparticles, the porosity and pore size distributions can be measured by mercury intrusion porosimetry.[73] For **surface charge measurements**, the zeta potentials of suspension of microparticles and nanoparticles can be studied using a Zetasizer.[58,82,146] **Biological activity** assay is used to achieve the maximum degree of retained biological activity after the microparticle preparation process. Caco-2 cell studies is used as ex vivo drug dissolution measurement, microparticles may bind to Ca^{2+} ions which could increase the paracellular permeability of epithelial cell monolayers by opening the tight junctions.[125]

3. MATERIALS AND METHODS

3.1. MAIN INGREDIENTS

API

Diclofenac sodium (DS, Ph.Eur. 5.) (the model hydrophilic drug) is a widely used potent NSAID used for the long-therm treatment of (chronic) degenerative joint diseases, it has both analgesic and antipyretic properties[215] and this is one of the approved NSAIDs available for parenteral delivery.[99] It has weak acidic properties (pKa 4.2), the solubility in PBS is 6 $mg \cdot ml^{-1}$ (pH 7.2).[216] It has low oral bioavailability (60%), low therapeutic index, short plasma half-life (1.1-1.8 h),[217] and a C_{max} value within the interval 1.5-2.5 h, requiring prolonged treatment. The polymorphysm,[218,219] the melting characteristics and decomposition have been performed.[184,218-223] DS-containing dosage forms were characterized by different spectroscopic techniques as NMR,[224] IR and FTIR,[117,215] and Raman[189] in the literature.

Copolymer used

Ammonio Methacrylate Copolymer (AMC) (Type B, MW 150.000; (Ph.Eur. 5./NF.) Eudragit® RS) was selected as the biocompatible, but non-biodegradable frame-forming material of the microspheres, based on the low permeability and pH independent release properties.[34,225,226]

3.2. ADDITIVES

The present thesis was designed to evaluate the effects of four polar cosolvents on the microsphere characteristics. Me_2CO, MeCOEt, *n*PrOH and *n*BuOAc were mixed individually with CH_2Cl_2 as the organic solvent of the multiple emulsion. The characteristic physicochemical properties of the cosolvents are listed in Table 5.

The nonionic surfactants (sorbitan mono-oleate, HLB = 4.3, W_1/O emulsifier; and polyoxyethylene 20 sorbitan mono-oleate, HLB = 14.9, O/W_2 stabilizer); plasticizer (PEGS) and protective colloid (PVA) were of pharmacopoeial grade (Ph.Eur. 5).

Table 5. Physicochemical properties of the organic solvents used[a]

Used solvents	ICH Class	B. p.[b] (°C)	Density ($g \cdot ml^{-1}$)	Polarity index	Log P	Visc.[c] (mPas)	Solub.[d]	Saturation[e]
CH_2Cl_2	2	39.5	1.317	3.1	1.511	0.475	1.3	Rapid
Me_2CO	3	56.5	0.785	5.1	0.234	0.360	miscible	Mixing
MeCOEt	3	79.6	0.800	4.7	0.736	0.415	29.0	-
*n*PrOH	3	97.2	0.807	4.0	0.559	2.072	miscible	Mixing
*n*BuOAc	3	125.0	0.882	3.9	1.822	0.730	0.7	Rapid

[a] physicochemical data from chemical databases; [b] boiling point (°C);
[c] absolute viscosity data from preliminary measurements (relative density of water = 1.000);
[d] solubility in water ($g \cdot 100\ ml^{-1}$); [e] saturation at maximum cosolvent concentration (75% w/w) in the aqueous phase.

3.3. PREPARATION OF PREFORMULATIONS AND MICROSPHERE SAMPLES

3.3.1. Conventional solvent evaporation technique

In the preformulation study different **films** were prepared by the solvent casting method.[IV] DS was dissolved at various ratios DS/AMC in EtOH and this solution was added to the AMC dissolved in CH_2Cl_2. The optically clear solvent mixtures were then cast and heated at 30 °C in vacuum for 48 h. The final membranes were vacuum-dried for 24 h and stored (desiccator, 4 °C).

Physical mixtures were prepared for TA and RS investigations with ratio DS/AMC = 1:6.

Model mixture was prepared as cast film for RS. Ethanolic solutions of DS and AMC in CH_2Cl_2 were mixed, followed by vacuum drying. In contrast with the physical mixture, the model mixture allows the preparation of a solid solution of DS in the AMC matrix.

SE-microspheres:[I,V] In the $W_1/O/W_2$ emulsion–solvent evaporation method,[4] the aqueous solution of DS (W_1) in the lipophilic solvent (containing AMC, plasticizer, and the W/O emulsifier) was emulsified at RT by high-shear mixing.[84] The W_1/O emulsion was then dispersed into the W_2-phase containing the O/W emulsifier and protective colloid, using a homogeniser. Solvent evaporation and solidification of the microspheres proceeded at RT and normal atmospheric pressure, under continuous stirring.[112] microspheres were collected by centrifugation under cooling. Drying was performed by vacuum filtration; microspheres were washed with distilled water, followed by freeze-drying (-80 °C). The final products were stored under controlled humidity conditions at 4 °C.

3.3.2. 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.

DS-containing SD-microspheres:[V,VI] 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). The microspheres were freeze-dried for 24 h and stored under controlled humidity conditions at 4 °C.

DH-containing SD-microspheres:[III] an aqueous solution of CHT containing 1% CH_3COOH or 1% HCl was prepared. The process was performed at the same conditions (inlet temperature: 150 °C; air flow: 10 $l \cdot min^{-1}$; pump rate: 3.5 $ml \cdot min^{-1}$). The microspheres were prepared by dissolving DH (DH/CHT ratios of 1:1, 1:1.5 and 1:2) in the CHT solution prior to spray-drying.

3.4. MICROSPHERE CHARACTERIZATION

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.

Design of experiment (DOE). To evaluate the contribution of each factor with different levels on responses, factorial based design was conducted, using Statistica for Windows® software (v.7.1). Tests for significant differences were made by analysis of variance (*one-way ANOVA*) ($p < 0.05$). The responses (Y_i) were expressed as a second-order polynomial equation (quadratic model) for each batch.

Rheological measurements.[I,VI] The absolute viscosity (mPas) of the organic solvent mixtures (η_1) was determined by using a *capillary viscometer*. The dynamic viscosity (mPas) of the organic phase (η_2) and that of the W_1/O emulsion (η_3) were measured with a *rotational viscometer*, at a constant shear rate of 130 $l \cdot s^{-1}$ ($n = 5$). Each reading was taken after equilibration.

Morphological study[I,II,III,VI]**.** After preparation, microscopic observations of multiple emulsions were made (without dilution) with a LEICA image analyser at 100× magnification. SEM was used to determine the surface characteristics and the external morphology of the DS-containing microspheres.

Particle size analysis[I,VI]**.** The microspheres were first suspended in distilled water, and sized by laser diffractometry (Malvern Mastersizer) (n = 5). Parameter D [4.3] was used to describe the PS.

Drug entrapment (E) and encapsulation efficiency (EE)[I,V,VI]**.** E (%, w/w) was determined with EDXRF instrument from pressed microsphere samples (n = 7). EE was expressed in percentage, compared to the theoretical drug content (100%).

Thermoanalytical measurements[I,III,IV,V] were performed using the same thermal program (25-400 °C heating range; 10 °C min^{-1} heating rate).TG (mass loss (%, w/w) vs. temperature), and DTG (derived mass loss vs. temperature) curves were plotted. DSC measurements: accurately weighed portions (n = 2) of the samples were subjected to the thermal program (-5-350 °C heating range, 10 °C min^{-1} heating rate) under a dynamic flow of N_2 and Ar. The thermograms and the changes in enthalpy (ΔH, $J \cdot g^{-1}$) were recorded.

Raman spectroscopy measurements[IV,V]**.** The DS, AMC, physical and model mixtures and the microspheres were characterized (n = 3). For the characterization of DS, the region between 1650–1530 cm^{-1} was used, because there were no Raman lines belonging to any other components in this area.

Analysis of residual organic solvent and cosolvent[I,VI]**.** The levels of residual organic solvent and cosolvents within the freeze-dried microspheres were determined by GC analysis (static head-space method), with a set of standard organic solvent concentrations (n = 3).

Cumulative drug release and release profiles[I,VI]**.** 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 $1 \cdot min^{-1}$. The samples (n = 7) were replaced with fresh PBS solution. The amount of DS liberated was determined using UV spectrophotometer, after filtration (0.45 μm) (S.D. < 7 %). Six types of kinetic models (zero- and first-order, Higuchi square root of time, Hixson-Crowell cube root, Baker-Lonsdale, and Nernst equation were applied to process the *in vitro* data. In the course of the release profile analysis, the amount of DS released within the first 30 min could not be interpreted with certainty due to the initial burst for particular batches.

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.[54] 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 DS, the thermal stability and the properties of drug-containing AMC film (using TA), and determination of the possible interactions between the DS and the AMC (using RS). A specific objective was to determine an appropriate DS/AMC ratio for the drug entrapment.[IV]

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.

4.1.1. Thermoanalytical measurements

DSC profile of DS and AMC

The physical state of the drug in the preparation depends on its solubility in the polymer matrix. When the preparation method is suitable to dissolve the drug molecularly, a *solid solution* may arise. In the solid solution form, drug–polymer interactions are the most probable reason for plasticization of the polymer. This can appear as lowered polymer T_g. Another opportunity is the formation of *metastable molecular dispersion*, where the recrystallization rate of the drug depends on the viscosity of the polymer matrix and the strength of the drug-polymer interactions, this type can exist under certain storage conditions for a few days to a few years until total recrystallization.[186] A further possibility is the formation of a *solid dispersion* of the crystalline drug in the matrix as a drug crystal nucleus.

When heated in the presence of air, DS decomposes below the T_m;[227] therefore the DS was subjected to thermal program in a controlled atmosphere (N_2 and Ar).[IV] The DS had a characteristic, well-shaped calorimetric profile, revealing endothermic peaks at ~285 and ~290 ºC (T_m), and a single exotherm at ~306 ºC, followed by a decomposition process (~323 °C) (Fig. 2B), in accordance with the literature.[222] TG analyses (in air) showed a mass loss of 21% in two steps between 270 and 400 °C, corresponding to the decomposition of the initial DS (Fig. 3, Table 7). In the TG measurements, no change was observed up to 270 °C; this can therefore be the temperature upper limit of the spray-drying.

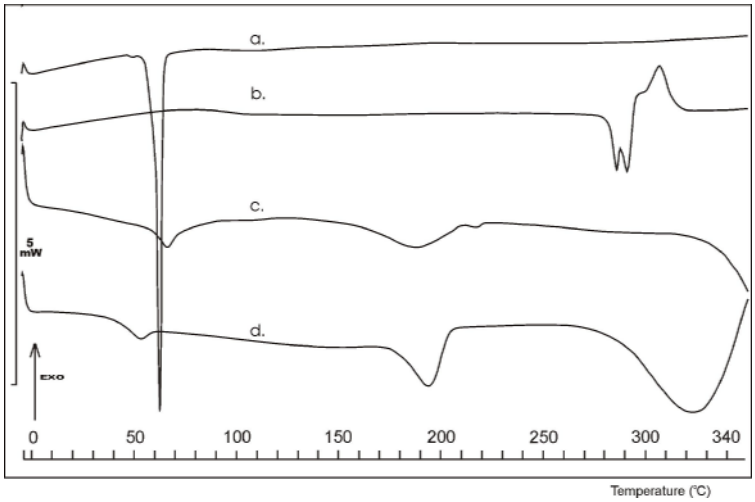
Table 6. Thermal events and enthalpies (ΔH) of the initial ingredients (1), physical mixtures (2); and microspheres (3) (mean values; n = 2)

No.	Appearance	1st event		2nd event		3rd event	
		T_1 (°C)	ΔH_1 (J·g^{-1})	T_2 (°C)	ΔH_2 (J·g^{-1})	T_3 (°C)	ΔH_3 (J·g^{-1})
1	AMC	66.2 (T_g)	8.6	188.0 (T_m)	9.1	-	-
	PEGS	62.5 (T_m)	214.2	-	-	-	-
	PVA	53.2 (T_g)	8.9	193.6 (T_m)	37.7	322.2 (T_m)	155.1
2	AMC+PEGS	64.0	35.8	187.8	18.5	-	-
	AMC+PVA	66.2	3.0	191.0	14.5	-	-
	AMC+DS	66.3	3.0	187.4	11.3	-	-
	AMC+DS (model mixture)	46.3	2.6	218.7	4.2	-	-
	AMC+PEGS+PVA	66.0	28.1	190.2	20.5	327.5	0.51
	AMC+PEGS+PVA+DS	64.8	19.4	191.3	15.3	-	-

T_1: peak maximum of first event (PEGS T_m + AMC T_g); T_2: peak maximum of second event (AMC T_m + PVA T_m); T_3: peak maximum of third event (PVA T_m).

Figure 2.

DSC profiles of the initial ingredients:
(A) PEGS;
(B) DS;
(C) AMC and
(D) PVA.



The form of AMC used was amorphous, due to the absence of complete stereoregularity and the presence of bulky side groups, the T_g was at 55-60 ºC[130,228] (Fig. 2C, Table 6). The DSC curve of

AMC revealed an endothermic peak at ~66 ºC (T_g) and a broad endotherm at ~188 ºC (T_m) (Table 6). The T_m at 217.5 ºC indicated two different crystalline form of copolymer present, followed by a decomposition process above 320 ºC. As a consequence, using a spray-dryer, it is not worth increasing the inlet temperature above 180 °C because of melting. There was no mass loss up to 300 °C, but 75% was experienced between 300-400 °C, due to the evaporation of the decomposition fragments of the copolymer without burning (Fig. 4, Table 7).

Figure 3. TG and DTG profiles of DS

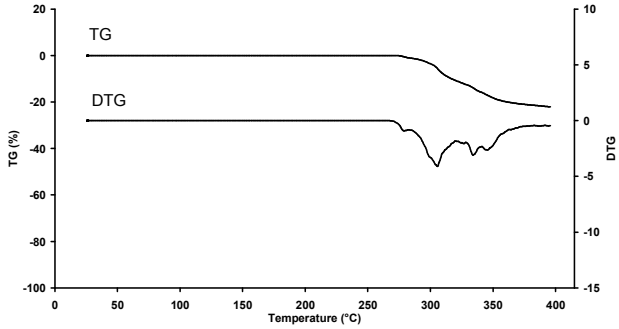
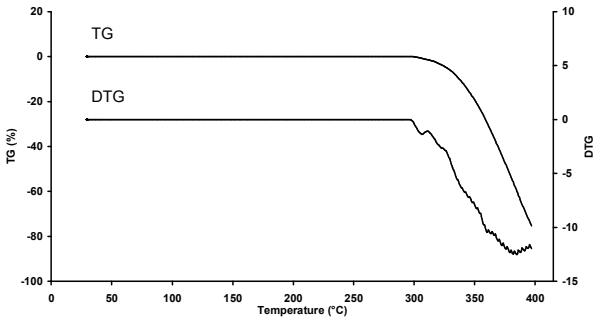


Figure 4. TG and DTG profiles of AMC



DSC profiles of the other ingredients

The plasticizer (PEGS) had a weak endotherm at ~49 ºC (T_g) and a single well-shaped characteristic endothermic peak at ~62 ºC (T_m) (Fig. 2A). The PVA exhibited characteristic thermal events: T_g at ~53 ºC, a broad T_m at ~193 ºC and a wide endotherm at around 322 ºC (Fig. 2D).

DSC profiles of physical mixtures and the model mixture

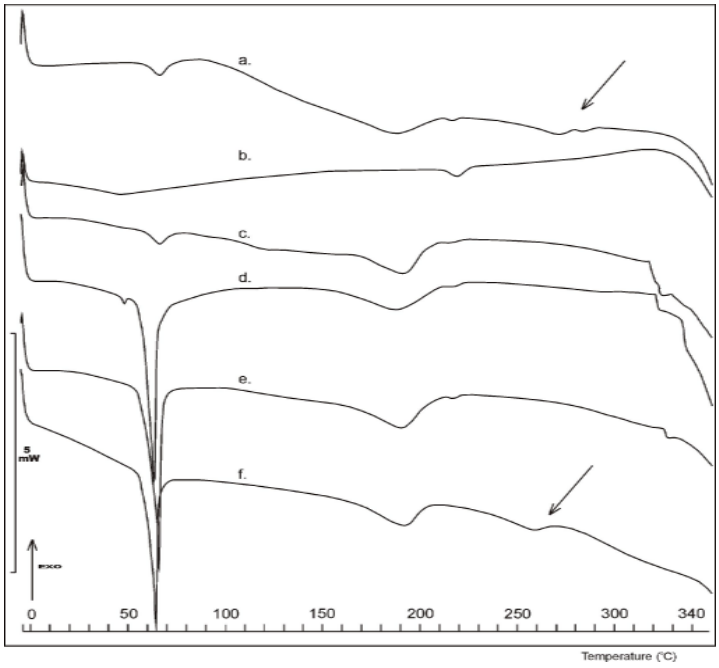
The positions and enthalpies of the AMC T_g and T_m events can be influenced by other components present. Different *physical mixtures* were therefore prepared and analysed to identify the matrix interference and to assign the endothermic events of the microsphere products. Figure 5 shows the DSC curves of the physical mixtures of AMC with the drug, PVA and plasticizer separately and in combinations, the main endothermic events observed are listed in Table 6.

The ammonio and ester groups of AMC are capable of interacting with anionic drugs such as DS through hydrogen-bonding, electrostatic and dispersion forces, resulting in a decreased T_g of AMC. In the physical mixture (DS/AMC = 1:1), the drug could not plasticize copolymer, the T_g value of AMC did not change significantly (Fig. 5A). The T_m of drug changed from ~293 ºC to ~269 and 281 ºC, which revealed the existence of drug crystals, and the possibility of the interactions. In the TG curve, mass loss occurs in three steps, 15% (235-300 ºC), 27% (300-360 ºC), and 5% (360-400 ºC), due to decomposition and burning of DS and decomposition of AMC[II] (Table 7).

To prepare the molecular drug dispersion in the matrix, a *model mixture* (DS/AMC = 1:6) was formulated. In contrast with the physical mixtures, the preparation of the model mixture involved thermal treatment (vacuum-drying + heating). The thermal treatment of the polymer (curing) above T_g could alter the structure due to the internal structural changes (the moving of side-chains, and a shift from a glassy to a more flexible rubbery state) and the T_g of AMC therefore disappears.[229] This could characteristically decrease the drug release,[230,231] and the porosity.[179] The AMC+DS model mixture exhibited distinct thermal events: a broad and very weak T_m (46 ºC), and a T_m of AMC (~218 ºC), without the T_m of DS (Fig. 5B). The DS melted and dispersed in the fused AMC; it should be responsible for the absence of the DS T_m, which implies that drug solubility in the copolymer was ensured at this ratio DS/AMC, and therefore also in the microspheres.

Figure 5.

DSC profiles of physical mixtures:
(A) AMC+DS;
(B) AMC+DS – model mixture;
(C) AMC+PVA;
(D) AMC+PEGS;
(E) AMC+PEGS+PVA; and
(F) AMC+PEGS+DS+PVA.



For the *AMC+PVA* physical mixture, common T_g and T_m were seen at ~66 and ~191 ºC, respectively (Fig. 5C), demonstrating that miscible polymers can exhibit a common, single T_g between the T_gs of the components.[232] When plasticizer was added to the copolymer, the characteristic sharp T_m and the T_g of AMC overlapped (~64 ºC). The total enthalpy might be influenced and increased due to the very sharp enthalpy of the plasticizer. The addition of DS (alone) to AMC did not change the kinetics of the copolymer degradation, whereas the addition of PVA (Fig. 5C) or plasticizer or both to the copolymer (Fig. 5E) resulted in an abrupt and decreased T_D (> 310 ºC). These phenomena suggested that the latter components exerted a destabilizing effect on the copolymer.

When first PVA and then DS was added to the AMC+ plasticizer physical mixture, the weak T_g of plasticizer (~49 ºC), and the second T_m of AMC (~217 ºC) disappeared (curve not shown). The

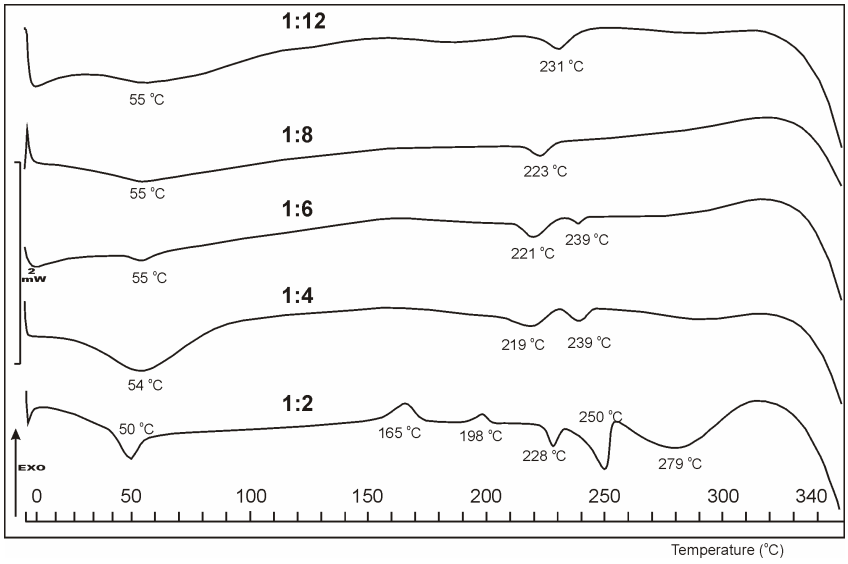
T_m of the low-melting polymorph of DS was clearly visible in the DSC spectrum of the drug-containing AMC+PEGS+DS+PVA physical mixture (250-270 °C) (Fig. 5F).

Films with different ratios DS/AMC

On increase of DS/AMC ratio (1:12→1:2), the first endotherm (AMC T_g) was observed between 50-55 ºC (Fig. 6, Table 7). The area under the curve increased and T_g decreased slightly with increasing ratio DS/AMC. The 2nd T_m of the copolymer appeared at DS/AMC = 1:4 (~239 ºC) and its ΔH value increased with increasing DS/AMC ratio (1:6 vs. 1:2), implying that AMC chain structure changed by the increased drug amount. At DS/AMC ratios of 1:12-1:6, the distinctive endotherm characteristic of the DS was absent, the drug being partly molecularly dispersed inside the AMC matrix as a solid solution. When DS dissolves in the AMC matrix, the ammonio groups of the AMC supposedly form hydrogen-bonds to the carboxylic group of the DS. The segment-segment interactions between the copolymer chains are weakened by these bonds and a consequent *plasticizing effect* can be observed, with increased permeability, which leads to a lowered T_g value of AMC. These observations indicate that the AMC-DS complex is less prone to be crystalline than the initial copolymer.

Figure 6.

DSC curves of films with ratios DS/AMC of 1:12, 1:8, 1:6, 1:4 and 1:2



The endothermic range of crystalline DS melting was noteworthy at DS/AMC = 1:2 (~279 °C). The relatively high drug content existed in a particular dispersion state instead of a molecular dispersion, due to the reduced solubility in the polymer matrix,[231] and therefore two exotherms (165 and 198 ºC) appeared before the DS T_m (Fig. 6). The order of magnitude of the interaction between the DS and the AMC was higher, a lower AMC T_g was observed (65→50 °C).

In the TG curves of the drug-containing films, the processes shifted simultaneously.[II] The mass loss from the TG curves were around 0-14% (range 1) and 18-75% (range 2), which can be

attributed to the decomposition and ignition of the DS and evaporation of the AMC (Table 7). The mass losses were higher than expected, as the forces between the drug molecules were lower due to the good dispersity in the copolymer matrix, leading to better sublimation of the melted drug.

Table 7. Temperatures of peaks in DSC curves and the mass losses in the TG curves

Material	DS-content (%, w/w)	AMC T_g (°C)	AMC T_{m1} (°C)	AMC T_{m2} (°C)	DS T_{m1} (°C)	DS T_{m2} (°C)	Range 1 (25–300 °C) (%, w/w)	Range 2 (300–400 °C) (%, w/w)	Total up to 400 °C (%, w/w)
AMC (initial)	0	65	187	218	-	-	0	-75	-75
AMC (recryst.)	0	-	189	218	-	-	---------- ---	---------- --	---------- ---
DS/AMC 1:12	7.6	55	231	-	-	-	-9	-65	-74
DS/AMC 1:8	11.1	55	223	-	-	-	-9	-67	-76
DS/AMC 1:6	14.2	55	221	239	-	-	-10	-63	-74
DS/AMC 1:4	20.0	54	219	239	-	-	-13	-56	-69
DS/AMC 1:2	33.3	50	228	250	279	-	-14	-46	-60
Physical mixture	50	66	187	-	269	281	----------	----------	----------
DS (initial)	100	-	-	-	293	308	100	-3	-18
DS (recryst.)	100	-	-	-	291	308	----------	----------	----------

4.1.2. Raman spectroscopy

The spectra of DS, films with DS/AMC ratios of 1:2, 1:4 and 1:6 (B-D) and AMC (E) in the range of 1675-1025 cm^{-1} are illustrated in Fig. 7. The changes in relative intensities of the characteristic wavenumbers of DS were due to decreasing drug content, however the difference between spectra C and D did not reveal the double drug amount. The changes in the phenyl and carbonyl vibrations of the DS in the region 1630–1550 cm^{-1} differed in spectra B-D, which is in accordance with the literature,[179,[IV]] the band at 1590 cm^{-1} is not distinct from the 1581 cm^{-1} peak, but forms a shoulder. The shoulder at 1163 cm^{-1} disappeared, while the band become broader. In the spectra, the traces of crystalline drug could be identified, which was in accordance with the DSC results.

Figure 7 reveals dominant bands of DS. The three characteristic peaks at 1581, 1590 and 1608 cm^{-1} are due to the $O^1C^8O^2$ asymmetric stretching and to ring 1 and 2 stretching vibrations, respectively. The increase in the bandwidths at ratio DS/AMC = 1:2 means a decrease in the vibrational relaxation time due to the weak interaction of $O^1C^8O^2$ of DS with the ammonio group of AMC.

Figure 7.
Raman spectra of
(A) DS and
films with ratios DS/AMC of
(B) 1:2;
(C) 1:4;
(D) 1:6;
(E) AMC.

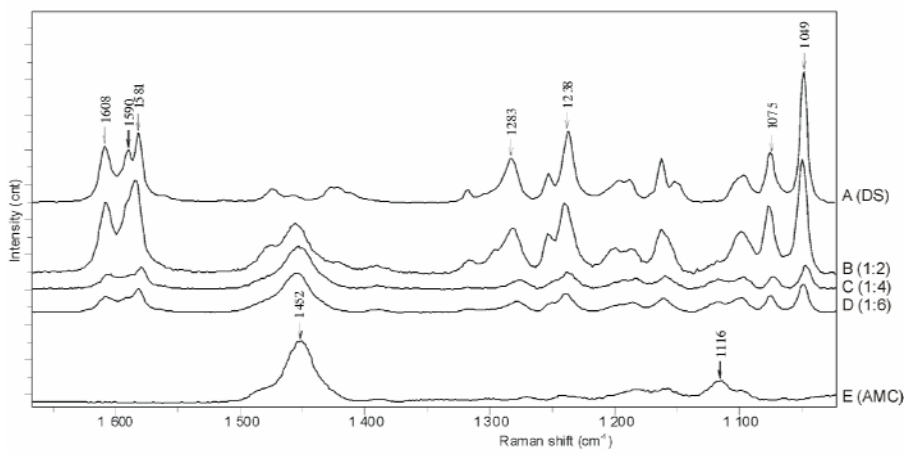
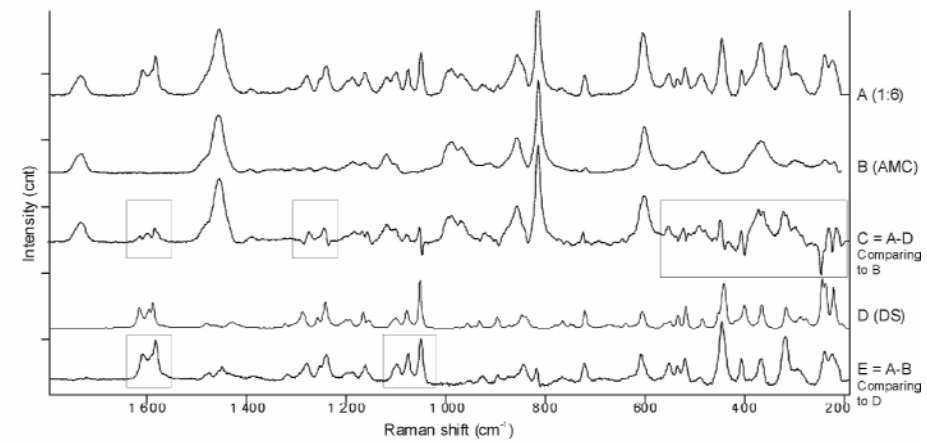


Figure 8.

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



To observe the changes in the peak shapes and positions in the overlapping regions required *subtraction* of the spectra from each other. According to the DSC measurements, the film with ratio DS/AMC = 1:6 contained less crystalline drug, and it was therefore chosen to prepare the difference spectra and analyse them. The spectrum of the DS (Fig. 8D) was subtracted from the spectrum of the film (DS/AMC = 1:6) (Fig. 8A), and the result (Fig. 8C) was compared with the spectrum of AMC (Fig. 8B). The Raman spectrum of AMC did not change in spectra A and B (Fig. 8C) and spectra B and C. In the marked regions (1650-1530 cm^{-1}, 1300-1250 cm^{-1}, 1150-1050 cm^{-1} and 570-200 cm^{-1}), the differences arose from the changes in drug content. For determination of the changes in DS, the difference spectrum of the model mixture (A) and AMC (B) was calculated. The result (E) was compared with the DS spectrum (D); the differences could be well observed in the regions overlapping with AMC bands (1500-1400 cm^{-1} and 850-800 cm^{-1}). The intensity ratio of the peaks of ratios DS/AMC between 1120 and 1030 cm^{-1} was 1:1:6, the intensity of 1:2 ratio was higher (Fig. 7). A significant intensity increase and shape alteration could be observed in the group around 300 cm^{-1}. There was no significant difference in the characteristic peak of the carbonyl group of the AMC (1736 cm^{-1}) (Fig. 8), which belongs to the trimethyl-ammonioethyl methacrylate segment; it was in accordance with the literature.[123] This confirmed that the strength of possible interactions between the carbonyl group of the copolymer and the drug

decreased at DS/AMC = 1:6. The changes between spectra D and E indicate that the crystalline state of drug was changed, while the broadening and merging effects suggest partly molecular dispersity for the drug.

4.1.3. Conclusions of the preformulation study

(1) TA studies confirmed that DS can behave as a plasticizer in DS-AMC 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 DS/AMC 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.

(2) RS: 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 DS and AMC chains seemed too weak to have an additional retaining effect of drug from dissolution. These investigations facilitated the selection of the appropriate DS/AMC 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.[V] The formulations were designed by varying the independent variables as the preparation methods and the concentrations of four polar cosolvents, which were distinguished by the log P value. 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) (Tables 8, 9) was performed to determine the significance of differences in the main DSC events of the microspheres. The factors selected as *independent variables* were: the ratio of 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 8 shows the levels and actual values of the

independent variables. Thus, Me_2CO (batches A1-A8), *n*PrOH (A9-A16), MeCOEt (A17-A24), or *n*BuOAc (A25-A32) were mixed individually with CH_2Cl_2 as organic solvent. Thermal events 1-3 (°C) (**Y_1**), ΔH values ($J \cdot g^{-1}$) (**Y_2**), and EE (%)(**Y_3**), as *dependent variables* were examined.

Table 8. Levels and values of the independent variables (non-randomized)

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

Table 9. Microsphere batches according to the levels and values of the independent variables

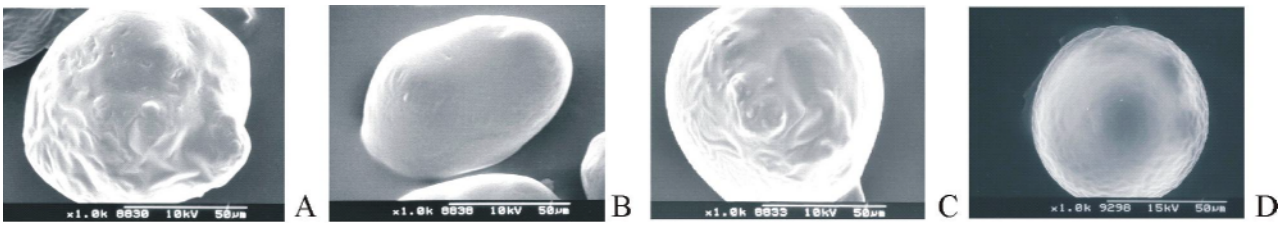
	X_1	X_2	X_3		X_1	X_2	X_3		X_1	X_2	X_3		X_1	X_2	X_3
A1	-1	-1	-1	**A9**	-0.3	-1	-1	**A17**	+0.3	-1	-1	**A25**	+1	-1	-1
A2	-1	-1	-0.3	**A10**	-0.3	-1	-0.3	**A18**	+0.3	-1	-0.3	**A26**	+1	-1	-0.3
A3	-1	-1	+0.3	**A11**	-0.3	-1	+0.3	**A19**	+0.3	-1	+0.3	**A27**	+1	-1	+0.3
A4	-1	-1	+1	**A12**	-0.3	-1	+1	**A20**	+0.3	-1	+1	**A28**	+1	-1	+1
A5	-1	+1	-1	**A13**	-0.3	+1	-1	**A21**	+0.3	+1	-1	**A29**	+1	+1	-1
A6	-1	+1	-0.3	**A14**	-0.3	+1	-0.3	**A22**	+0.3	+1	-0.3	**A30**	+1	+1	-0.3
A7	-1	+1	+0.3	**A15**	-0.3	+1	+0.3	**A23**	+0.3	+1	+0.3	**A31**	+1	+1	+0.3
A8	-1	+1	+1	**A16**	-0.3	+1	+1	**A24**	+0.3	+1	+1	**A32**	+1	+1	+1
A3	-1	-1	+0.3	**A11**	-0.3	-1	+0.3	**A19**	+0.3	-1	+0.3	**A27**	+1	-1	+0.3

A3, A11, A19 and A27: drug-free SD-microspheres

4.2.1. SEM evaluation of the basic composition microspheres

The basic composition SE- and SD-microspheres were prepared with CH_2Cl_2 alone. The basic composition SE-microspheres were all nonporous and spherical in shape as expected (Fig. 9A), 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.

Figure 9. Basic composition microspheres: (A) drug-containing SE-microspheres; (B) drug-free SE-microspheres; (C) drug-containing SD-microspheres; (D) drug-free SD-microspheres.



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. 9B). The basic composition SD-microspheres displayed spherical particles with

a smooth surface, without agglomeration, an uneven shape, or drug crystals on the surface (Fig. 9C). The drug-free basic composition SD-microspheres exhibited an intact and smooth surface (Fig. 9D).

4.2.2. Thermal investigation of the microspheres

Influence of the cosolvent log P and concentration

The temperatures and enthalpies (ΔH) of T_1-T_3 events (Table 10) of the microspheres were evaluated. Figure 10 shows representative DSC profiles of the SD- and SE-microspheres. The first endothermic event (T_1) (=plasticizer T_m and AMC T_g – overlapped) could be observed in the interval 39-47 °C, situated between 17-25 °C before that for the AMC+PEGS+DS+PVA physical mixture (65 °C). The miscibility/compatibility in the molten state of AMC, PEGS and PVA and therefore the increase in the chain mobility of the copolymer molecules and the decrease in the cohesive interactions between the copolymer chains were confirmed by the T_g depression. The dissolved state and the plasticizing effect of drug can also increase the mobility of the AMC monomers and weaken the copolymer chain segment-segment interactions; as a consequence, the T_g and other thermal events decreased.[131] Similar tendencies were noted for the T_2 event (common AMC and PVA T_m) observed at 179-188 °C; the effect of the dispersed drug was confirmed by the 2-11 °C difference of T_2 from the AMC+PEGS+DS+PVA physical mixture (191 °C). The T_3 event (PVA T_m), which could be observed only in the DSC curves of the SD-microspheres, was at 320-324 °C. The reason for this was that crystalline PVA was present only in the SD-microspheres, formed from residual PVA, revealed at around its T_m (322 ºC). The thermograms of the batches did not indicate any sharp thermal event corresponding to the melting of drug crystal domains, indicating the mainly molecular drug dispersion. The residual humidity could exert a plasticizing effect,[233] but the solvent elimination was proved by the low (1% w/w) mass loss of the product between 42 and 98 °C.

The *drug-free SD-microspheres* displayed a similar behaviour to that of the drug-containing ones, nonetheless, the plots revealed pronounced shifts in the T_1 and T_2 events, while the difference was negligible for T_3 (Table 10). In comparison with the drug-containing SD-microspheres, the T_1 and T_2 events moved towards higher temperature, with a difference of 7-16 and 2-11 °C, respectively. The effects of the independent variables on the thermal events were not significant, though the EE values correlated well with the independent variables. As a consequence of the rapid partitioning of the cosolvents from the organic phase of the W/O/W emulsion, and therefore the increased W_1/O emulsion viscosity and faster emulsion droplet hardening, the EE improved (Table 10), in accordance with the literature.[177,234]

Figure 10.

Typical DSC profiles of the microspheres:

(A) A1 (100% DCM, spray-drying);
(B) C0 (50% DCM, spray-drying, drug-free);
(C) C5 (50% DCM, spray-drying);
(D) A2 (100% DCM, SE);
(E) without batch number (50% DCM, SE, drug-free);
(F) C6 (50% DCM, SE).

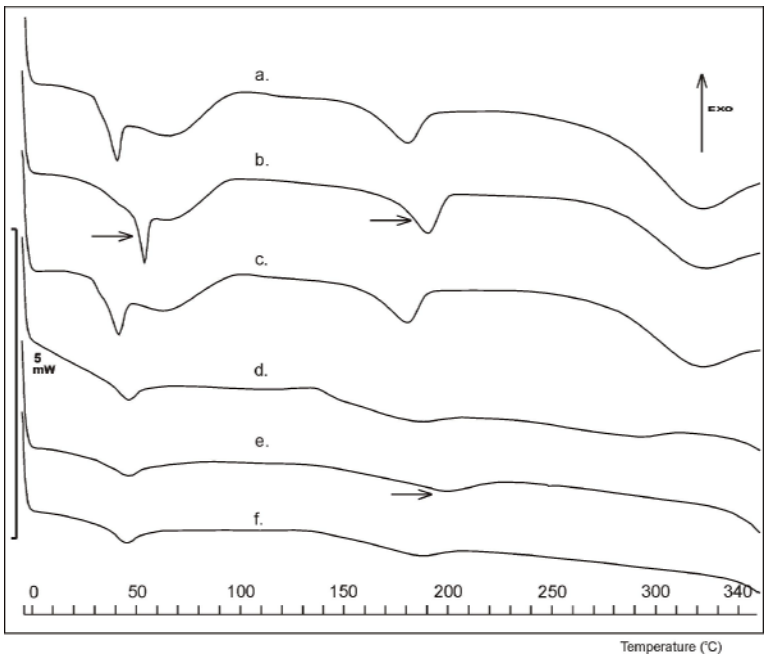


Table 10. Design study layout with the observed responses

Microspheres		1st event		2nd event		3rd event		EE
Batch	Prep.	T_1 (°C)	ΔH_1 (J·g^{-1})	T_2 (°C)	ΔH_2 (J·g^{-1})	T_3 (°C)	ΔH_3 (J·g^{-1})	(%)
A1-4	SD	41-42	5-7	179-181	12	321-323	23-29	15-33
A5-8	SE	43-47	7-8	181-187	2-11	---	---	21-70
A3[a]	SD	54	9	190	9	334	15	---
A9-12	SD	39-43	6-7	180-181	12	322-324	22-29	15-32
A13-16	SE	44-46	6-10	185-188	7-11	---	---	21-40
A11[a]	SD	54	6	190	15	323	23	---
A17-20	SD	40-44	6-7	180-184	10-13	322-324	11-29	15
A21-24	SE	44-46	7-13	183-188	8-11	---	---	21-59
A19[a]	SD	55.8	9	189	12	326	21	---
A25-28	SD	40-44	4-7	180-183	12-13	322-325	14-29	15-27
A29-32	SE	44-46	3-12	185-187	6-11	---	---	20-40
A27[a]	SD	50.2	4	186	13	324	29	---

Axx[a]: drug.free microspheres; $\mathbf{T_1}$: peak maximum of first event (PEGS T_g + AMC T_m); $\mathbf{T_2}$: peak maximum of second event (AMC T_m + PVA T_m); $\mathbf{T_3}$: peak maximum of third event (PVA T_m).

Influence of the preparation method

The solvent removal process is diffusion controlled and any factors that effect solvent diffusion such as viscosity and concentration gradients can influence microsphere preparation. All the SE-microspheres displayed an analogous trend, with broad and weak endothermic peaks and frequently lower ΔH values; representative DSC profiles are given in Fig. 10. Comparison of the microspheres prepared by the different techniques revealed that the T_1 and T_2 events of the SE-microspheres began at around the temperature where the spray-drying thermal events ended (Table 10). The DSC profiles of the drug-free and drug-containing SE-microspheres were also identical,

except for the T_2 event of the drug-free microspheres, indicating the absence of the plasticizing effect of the drug. When the SE-technique was used, the characteristic T_3 event (PVA T_m) was not observed (the SE-technique allows the elimination of residual PVA in the course of the preparation process). Furthermore, reduced enthalpy (ΔH) values were obtained with the SE-technique because there was more time for the englobing of the plasticizer and the formation of the copolymer matrix structure. These thermograms did not exhibit any thermal event corresponding to DS melting. In spite of the longer preparation time, the SE-microspheres had higher EE values.

For the SE-microspheres, the characteristic endothermic events (55-80 ºC, residual moisture) did not appear, because of effective freeze-drying (for 48 h).[233] In spite of the efficacy of the spray-drying, the SD-microspheres contained traces of absorbed moisture, indicating that the duration of the process for complete drying might be too short.

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

4.2.3. Raman spectroscopy

The fingerprint region of DS (1700-1550 cm^{-1}) was selected for closer investigation (Fig. 11). The drug-free and drug-containing SE- and SD-microspheres showed spectra with similar structures, containing broad bands. The spectrum of the model mixture (Fig. 11E) could be regarded as the superposition of the spectra of DS and AMC. As compared with the model mixture, the corresponding Raman bands of the SE- and SD-microspheres were unchanged (811 cm^{-1}), or were broader (854, 1452, 1736 cm^{-1}), indicating mutual interactions of these functional groups. Broadening was seen, whereas there was no dramatic shift in the band of the carbonyl group of the trimethyl-ammonioethyl methacrylate segment of AMC (1736 cm^{-1}), which is responsible for control of the swelling and water permeability of the copolymer matrix.[123] In the spectra of the drug-free and the drug-containing SD-microspheres, no difference was observed in the positions of the absorption bands. The shape of the band at 1452 cm^{-1} altered only in the case of the SD-microspheres, the reason was the disturbing effect of the plasticizer. The typical characteristic DS bands were at 1582, 1590 and 1608 cm^{-1} (Fig. 11F), which were also detected in the spectra of the model mixture (DS/AMC ratio 1:6; Fig. 11E) and the drug-containing microspheres (Figs 11A, 11C), but not in that of the drug-free microspheres (Figs 11B, 11D). There was no dramatic shift in the band of DS at 1581 cm^{-1}; the absence of the band at 1590 cm^{-1}

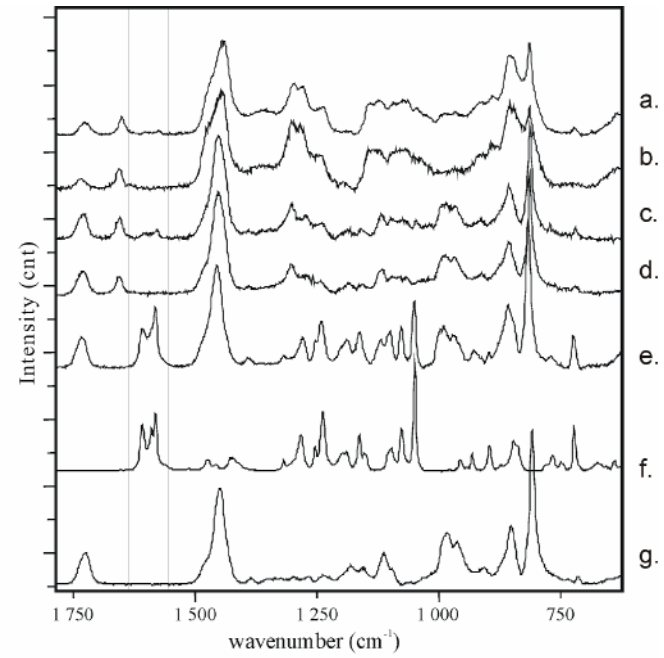
in the spectrum of the model mixture and especially in that of the SD-microspheres suggested the possibility of weak interactions.

Comparison of the DSC profiles and Raman spectra of the model mixture and the SD-microspheres allowed the assumation that, if DS is dispersed in the model mixture, then it must be present in a similarly dispersed state in the SD-microspheres because of its lower concentration, which is in accordance with the literature (DS in β-CD).[235] The measurements revealed that the preparation methods used did not significantly influence the structure of the drug. The small shifts, the absence of particular bands, and the changes in the relative intensities of the microsphere bands with respect to the Raman bands of the drug and the copolymer did not permit the exclusion of possible weak interactions, which could be responsible for the retaining effect on DS, altering drug release rate.

Figure 11.

Raman spectra of
(A) Drug-containing and
(B) Drug-free SD-microspheres;
(C) Drug-containing and
(D) Drug-free SE-microspheres;
(E) model mixture with DS/AMC = 1:6;
(F) DS and
(G) AMC

in the spectral region 1800-625 cm^{-1}.



4.2.4. Conclusions of the comparative study

(1) In TA 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. 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.

(2) RS demonstrated that only the nature of the preparation method caused significant variations in the structure of the microspheres. RS 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 AMC-based 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) (**X_1**), emulsion stirring rate (rpm) (**X_2**), the ratio DS/AMC (**X_3**), and the ratio PEGS/AMC (**X_4**). Table 11 shows the levels and actual values of the independent variables. Several parameters were examined as *dependent variables*: η (mPas) (**Y_1**), D [4,3] (μm) (**Y_2**), SSA (m^2/g) (**Y_3**), E (%, w/w) (**Y_4**) and EE (%) (**Y_5**).

Table 11. 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;

Table 12. Microsphere batches according to the levels and values of the independent variables

batch	X_1	X_2	X_3	X_4	batch	X_1	X_2	X_3	X_4
B1	-1	-0.3	-0.3	-0.3	**B9**	-0.3	-0.3	-1	-0.3
B2	-0.3	-0.3	-0.3	-0.3	**B10**	-0.3	-0.3	-0.3	-0.3
B3	+0.3	-0.3	-0.3	-0.3	**B11**	-0.3	-0.3	+0.3	-0.3
B4	+1	-0.3	-0.3	-0.3	**B12**	-0.3	-0.3	+1	-0.3
batch	X_1	X_2	X_3	X_4	**batch**	X_1	X_2	X_3	X_4
B5	-0.3	-1	-0.3	-0.3	**B13**	-0.3	-0.3	-0.3	-1
B6	-0.3	-0.3	-0.3	-0.3	**B14**	-0.3	-0.3	-0.3	-0.3
B7	-0.3	+0.3	-0.3	-0.3	**B15**	-0.3	-0.3	-0.3	+0.3
B8	-0.3	+1	-0.3	-0.3	**B16**	-0.3	-0.3	-0.3	+1

4.3.1. Effect of processing parameters on SE-microspheres

Volume ratio of W_1/O emulsion - W_2 phase (X_1 variable)

When the W_2 phase was present in lower amount, and therefore the viscosity was higher, the degree of dispersity was higher, and the emulsion was monodisperse (B1) (Table 13). The surface morphology of the microspheres was spherical, the surface being smooth with few aggregated microspheres. When the volume of the W_2 phase was higher, CH_2Cl_2 evaporated more easily from the emulsion droplets, resulting in a rapid matrix structure formation before droplet coalescence of the W_1/O emulsion. In the case of lower W_2 phase volume, faster droplet coalescence occurred before solidification. Elevation of the W_2 phase volume decreased the particle size, and a reduction in EE was also detected (37→25%), as more active agent diffused from the W_1/O emulsion to the increased W_2 phase during the second emulsification process. As a consequence of the high SSA, the interaction between the W_1 and W_2 phases proved to be stronger with smaller emulsion droplets, resulting in an increased drug migration towards the W_2 phase.

Table 13. Design study layout with the observed responses

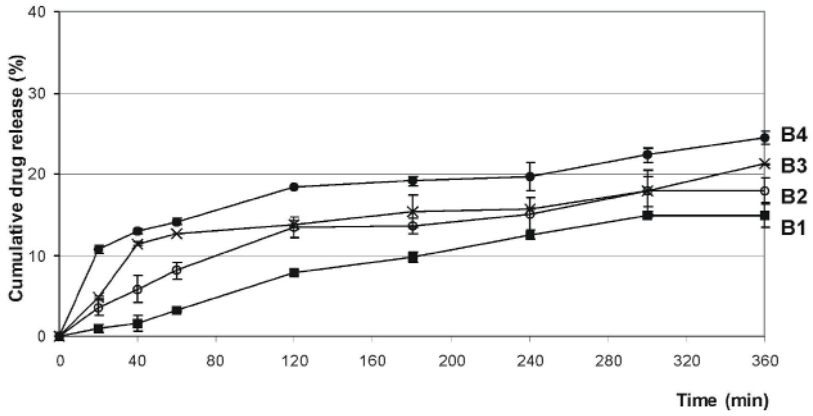
	Y_1	Y_2	Y_3	Y_4	Y_5		Y_1	Y_2	Y_3	Y_4	Y_5
	η	PS	SSA	E	EE		η	PS	SSA	E	EE
B1	29	314.7	0.021	20.0	37.1	**B9**	21	136.1	0.039	6.1	30.2
B2	29	150.5	0.033	16.7	33.3	**B10**	29	150.5	0.033	16.7	33.3
B3	29	124.4	0.037	14.6	29.2	**B11**	32	162.3	0.033	24.2	40.4
B4	29	101.9	0.046	12.0	25.8	**B12**	85	242.9	0.024	33.4	41.7
	Y_1	Y_2	Y_5	Y_6	Y_7		Y_1	Y_2	Y_5	Y_6	Y_7
	η	PS	SSA	E	EE		η	PS	SSA	E	EE
B5	31.5	249.8	0.023	17.1	38.4	**B13**	23	477.0	0.013	17.0	38.6
B6	29	150.5	0.033	16.7	33.3	**B14**	29	150.5	0.033	16.7	33.3
B7	51	115.5	0.041	6.3	15.7	**B15**	24	115.7	0.039	12.3	24.6
B8	145	220.5	0.026	20.1	30.1	**B16**	30	112.2	0.040	9.6	16.8

Responses: Y_1, η (mPas); Y_2, D [4,3] (μm); Y_3, SSA (m^2/g); Y_4, E (%, w/w); Y_5, EE (%).

Figure 12.

In vitro drug release ($W_1/O:W_2$ ratio):

B1 - 1:5;
B2 - 1:10;
B3 - 1:15;
B4 - 1:20.



During the release process, drug diffusion into the acceptor phase started with a 2 h delay, as the denser copolymer wall was able to retard the process (Fig. 12). The slow initial release (min: 14.9%, max: 22% in 6 h) reflects the time-consuming process of diffusion through a lipophilic copolymer wall, as well as the formation of pores and channels within the spheres. The dissolution

profiles of samples B1–B4 followed the Higuchi equation (R^2 = 0.911-0.976), suggesting that the drug is dispersed in uniform spherical matrix, and that the release is controlled by diffusion.

Stirring rate of the W_1/O emulsion (X_2 variable)

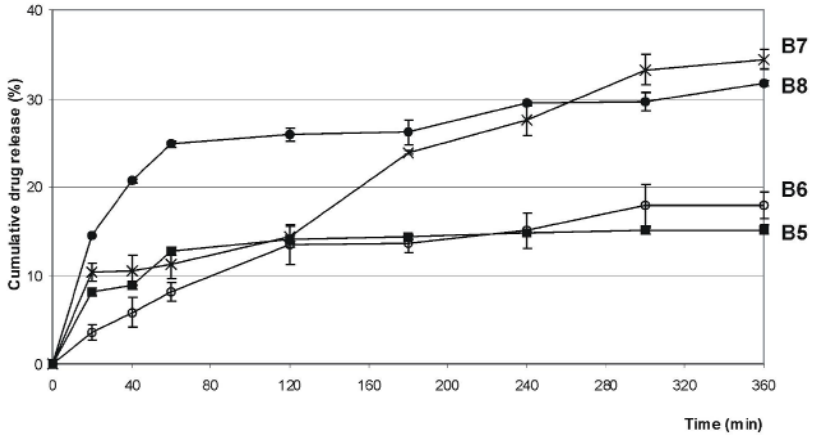
When the stirring rate was increased, the particle size decreased, and consequently the SSA increased (R^2 = 0.999) (B5–B7, Table 13). The emulsification efficiency decreased with decreasing mixing rate, while the droplet size and particle size distribution increased. As a result of the high particle size (B5: 249 μm), and accordingly the small SSA, the drug release into the acceptor phase was slower. The stirring rate had a considerable significant influence on the viscosity of the W_1/O emulsion of B8 (145 mPas). An inadequately stabilized W_1/O emulsion with very small globules was prepared at 24000 rpm, which underwent rapid coalescence. Its viscosity was extremely high (145 mPas), therefore, when the W_1/O emulsion was added to the W_2 phase, large multiple droplets formed.

The EE for B8 (particle size: 220 μm and SSA: 0.026 m^2/g) was found to be optimal (30%). In the first 1 h, a burst release effect was experienced, as a result of the disintegration of the agglomerates formed on rapid mixing, and dissolution of the adhered drug from the surface. The release of samples of B6 and B7 accurately followed the diffusion-controlled model for an inert homogeneous matrix as described by Higuchi[206] (R^2 = 0.967-0.973). The Baker-Lonsdale model proved to be the best mathematical model to describe the release from B5 and B8 (R^2 = 0.911-0.921) confirming that these microspheres were heterogeneous matrix systems.

Figure 13.

In vitro drug release (stirring rate, rpm):

B5 - 14400;
B6 - 17600;
B7 - 20800;
B8 - 24000.



Ratio DS/AMC (X_3 variable)

At low DS content (DS/AMC = 1:16), a smooth surface could be observed (Fig. 14A). With increasing drug concentration, the surface became wrinkled, and some collapsed particles formed during the solvent evaporation (Fig. 14B). The copolymer precipitated on the surface of the W_1/O emulsion droplets during preparation, before the complete evaporation of CH_2Cl_2. As a result of

further solvent diffusion, some of the particles collapsed, large pores formed and the structure of the microspheres became irregular (Fig. 14B).

With increasing DS amount, the particle size increased (136→242 μm) (Table 13). The viscosity of the W_1/O emulsion also increased, as a result of the higher E and the increased viscosity of the W_1 phase. With increasing emulsion viscosity, the particle size increased, whereas for B5-8 and B13-16 it decreased with increasing emulsion viscosity. The dispersion of these more viscous emulsions into the W_2 phases resulted larger microspheres under the same mixing conditions. The EE increased only moderately (30→41%; $R^2 = 0.942$) with increasing E (6→33%; $R^2 = 0.996$).

Figure 14. External morphology of drug -containing SE- microspheres prepared with different DS/AMC ratios: (A) B11 - 1:16; (B) B12 - 1:12.

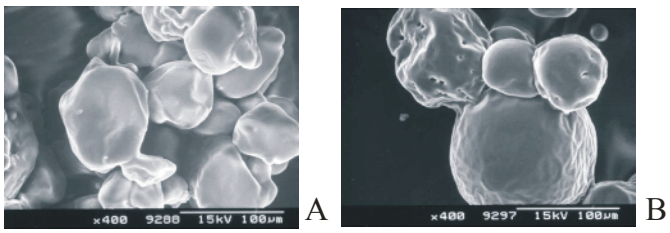
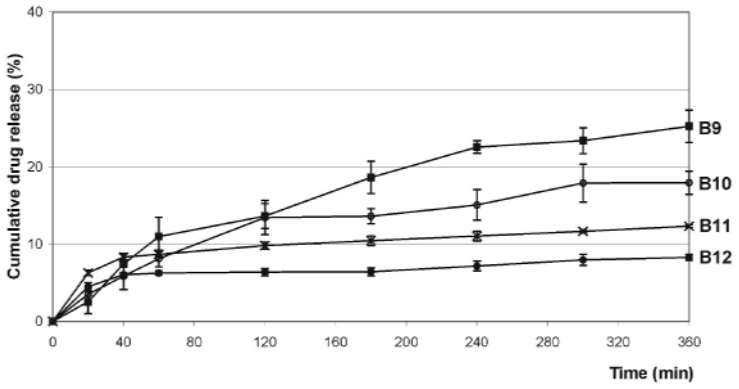


Figure 15.

In vitro drug release (DS/AMC ratio):

B9 - 1:50;
B10 - 1:25;
B11 - 1:16;
B12 - 1:12.



In the first 1.5 h of the release, the same amount of drug was released from the samples of B9-B12, but after 2 h the curves started to deviate sharply (Fig. 15).[I] The diffusion exponent '*n*' was around 0.5 for B9 and B10, in accordance with the Higuchi diffusion model ($R^2 = 0.973$-0.979). The release profiles for B11 and B12 were almost parallel, but the kinetic studies of B12 suggested a two-step release process (Table 13).

Ratio PEGS/AMC (X_4 variable)

A lower plasticizer (PEGS) concentration resulted in larger particles, a higher EE, and denser microspheres. The SSA/particle volume ratio decreased in parallel with the increase of particle size, leading to slow drug release. Through increase of the plasticizer concentration, a significant decrease could be achieved in particle size (477→112 μm) (Table 13), ensuring a higher SSA. The

higher the plasticizer concentrations are, the more heterogeneous and coarser the surface becomes with a number of pores in the microspheres (Fig. 16), which led to an increased amount of drug release.

The more porous microspheres (B15 and B16) (Fig. 16B) exhibited a faster release (Fig. 17). The slower drug liberation from B13 was a consequence of the thick copolymer wall and the small (0.013 m^2/g) wetted surface. E reduced slightly (16.9→9.5%), but the EE decreased significantly (38→16%; R^2=0.991), with increasing plasticizer content. The decrease in EE was caused by the slower solidification of the W_1/O emulsion droplets in the W_2 phase. The longer duration of solidification of the more hydrophilic W_1/O emulsion is associated with the diffusion of a larger amount of drug from the microspheres. As the concentration of plasticizer was increased, the acceptor phase reached the internal parts of the microspheres more readily. The higher drug release (35%) of B16 resulted from the more porous structure, the low particle size, the increased SSA, and the more hydrophilic matrix, in spite of the fact that E was only 9% (Fig. 17). The SEM picture of B16 reveals a high number of aggregated amorphous microspheres,[I] as a result of the swelling characteristics of plasticizer, present in relatively high concentration, and also of the poor stability of the W_1/O emulsion droplets. The rate of drug release from B13 and B14 fitted the Higuchi model, which verified the homogeneous matrix structure and the diffusion-controlled process (R^2 > 0.958). From B15 and B16, with the highest plasticizer content, the type of release process could be appropriately described by the Baker-Lonsdale model (R^2 = 0.958-0.977).

Figure 16. External morphology of SE-microspheres prepared with different ratios PEGS/AMC: (A) B13 - 1:10; (B) B15 - 1:3.3.

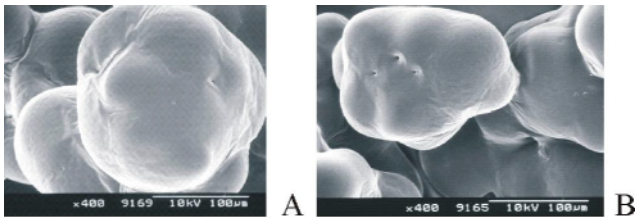
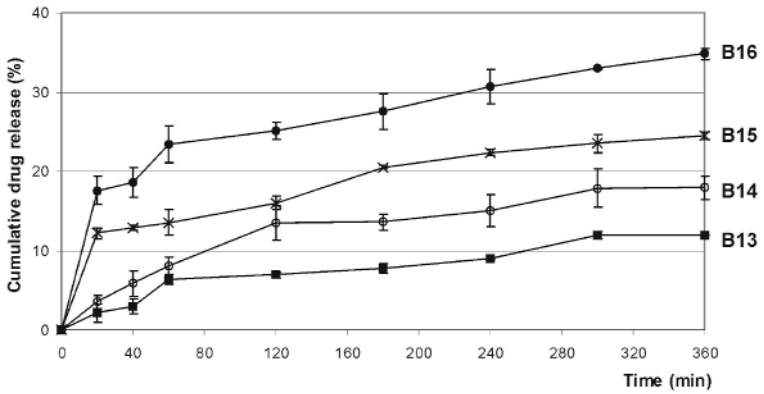


Figure 17.

In vitro drug release (PEGS/AMC ratio):

B13 - 1:10;
B14 - 1:5;
B15 - 1:3.3;
B16 - 1:2.5.



4.3.2. 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 SSA/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.[236]

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 (X_1), and the concentrations of the Class 3 polar cosolvents (X_2), and the ratio DS/AMC (X_3). Several parameters were examined as *dependent variables*: η_1 (mPas) (Y_1), production yield (%) (Y_2), particle size (μm) (Y_3), EE (%) (Y_4), and Q_6 (%) (Y_5) (Table 14). Me_2CO (batches C1-C9), MeCOEt (C10-C18) or *n*BuOAc (C19-C27) 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 (C1A-C9A) and the factorial design was also accomplished for *n*PrOH.

Table 14. 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 viscosities of the solvent-cosolvent mixtures, the organic phases, and the W_1/O emulsions (η_1) were investigated for all the batches. 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.[1] *The required parameters* were low values of W_1/O emulsion viscosity (η_1) 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. Tables 14 and 15 show the levels and actual values of the independent variables. Table 16 shows the factorial design layout for the variables and the measured values of the responses.

Table 15. Levels and values of the independent variables (non-randomized)

batch	X_1	X_2	X_3	batch	X_1	X_2	X_3	batch	X_1	X_2	X_3	batch	X_1	X_2	X_3
C1	-1	-1	-1	**C1A**	-1A	-1	-1	**C10**	0	-1	-1	**C19**	+1	-1	-1
C2	-1	-1	0	**C2A**	-1A	-1	0	**C11**	0	-1	0	**C20**	+1	-1	0
C3	-1	-1	+1	**C3A**	-1A	-1	+1	**C12**	0	-1	+1	**C21**	+1	-1	+1
C4	-1	0	-1	**C4A**	-1A	0	-1	**C13**	0	0	-1	**C22**	+1	0	-1
C5	-1	0	0	**C5A**	-1A	0	0	**C14**	0	0	0	**C23**	+1	0	0
C6	-1	0	+1	**C6A**	-1A	0	+1	**C15**	0	0	+1	**C24**	+1	0	+1
C7	-1	+1	-1	**C7A**	-1A	+1	-1	**C16**	0	+1	-1	**C25**	+1	+1	-1
C8	-1	+1	0	**C8A**	-1A	+1	0	**C17**	0	+1	0	**C26**	+1	+1	0
C9	-1	+1	+1	**C9A**	-1A	+1	+1	**C18**	0	+1	+1	**C27**	+1	+1	+1
Batches of **C0a-C0c**: 100% of CH_2Cl_2; X_3 = -1, 0 and +1, respectively. Batches of **C1A-C9A**: microspheres prepared with *n*PrOH															

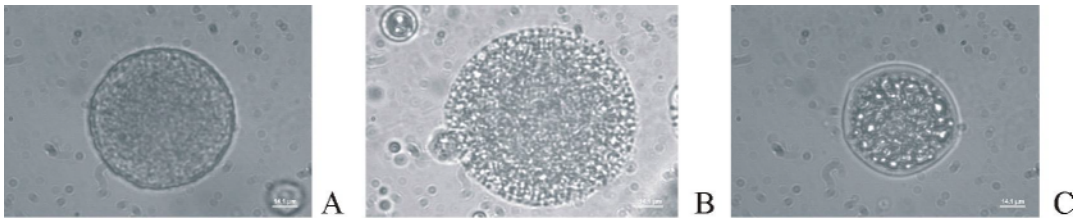
Table 16. - 3^3 factorial design study layout with the dependent variables

	Y_1	Y_2	Y_3	Y_4	Y_5		Y_1	Y_2	Y_3	Y_5	Y_5
	η	Yield	PS	EE	Q_6		η	Yield	PS	EE	Q_6
C1	12.8	74.6	120.6	37.8	3.26	**C1A**	11.8	75.6	142.1	30.9	68.9
C2	11.5	70.4	162.4	22.5	6.81	**C2A**	12.3	44.2	175.3	14.8	100.0
C3	9.60	64.2	178.6	10.5	11.71	**C3A**	12.5	58.1	183.8	11.1	100.0
C4	9.60	67.3	164.8	38.5	5.88	**C4A**	10.2	70.9	218.7	37.6	100.0
C5	8.62	68.9	170.9	23.3	10.08	**C5A**	10.5	55.5	234.5	15.3	100.0
C6	8.15	41.8	188.7	11.2	15.79	**C6A**	10.6	57.1	240.7	17.1	67.3
C7	7.04	71.5	144.6	53.3	13.68	**C7A**	8.96	69.9	157.8	15.8	100.0
C8	6.98	71.4	166.8	32.8	25.82	**C8A**	9.32	65.2	169.4	15.2	79.2
C9	6.40	62.7	184.2	17.4	28.67	**C9A**	9.60	60.2	178.8	13.4	67.1
C10	15.4	74.6	148.2	36.2	53.2	**C19**	22.7	45.1	205.4	33.3	75.2
C11	12.8	64.7	182.5	26.2	47.2	**C20**	20.2	37.5	212.3	15.1	100.0
C12	11.8	63.7	200.1	21.6	44.7	**C21**	17.6	40.3	236.1	11.3	100.0
C13	9.92	69.9	168.7	33.4	89.3	**C22**	19.5	61.1	278.3	35.1	69.5
C14	9.28	63.2	192.3	31.8	78.4	**C23**	17.9	29.3	313.4	16.5	100.0
C15	9.02	63.1	216.8	23.1	62.1	**C24**	16.6	26.1	308.6	18.8	86.8
C16	8.64	68.9	140.9	41.8	29.1	**C25**	18.6	62.1	108.8	38.4	52.8
C17	7.68	66.4	158.2	28.1	89.9	**C26**	16.6	51.9	141.4	27.1	49.6
C18	6.98	61.2	172.3	18.7	97.9	**C27**	15.4	52.5	158.1	18.9	47.1
C0A	30.9	78.1	54.6	37.3	47.7						
C0B	20.5	72.2	107.3	14.6	43.1						
C0C	18.6	66.1	130.1	9.8	38.8						

Responses: $\mathbf{Y_1}$, W_1/O emulsion viscosity (η) (mPas); $\mathbf{Y_2}$, production yield (%); $\mathbf{Y_3}$, average particle size (μm); $\mathbf{Y_4}$, Encapsulation efficiency (EE) (%) and $\mathbf{Y_5}$, cumulative drug release in 6 h (Q_6) (%).

4.4.1. Characterization of W_1/O emulsion droplets

Figure 19. Representative image analysis of multiple emulsion droplets (magnification: 100x) (X_1; X_2; X_3): (A) C4 (-1; 0; -1); (B) C5 (-1; 0; 0); (C) C6 (-1; 0; +1).



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 ratio DS/AMC; the changes due to osmotic swelling are presented in Figs 19A-C. Increase of the ratio DS/AMC (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 (C4-C6, Table 16), in accordance with the literature.[237] When the ratio DS/AMC 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.[V] Despite of the large W_1 droplet size, the copolymer precipitation rate increased due to the higher amount of cosolvent, increasing the EE.

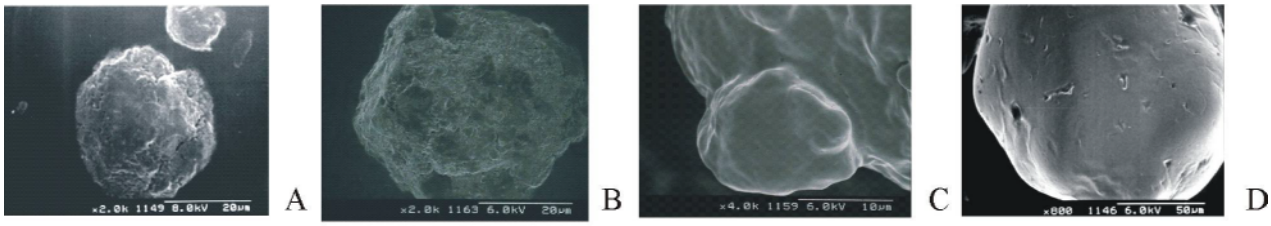
4.4.2. SEM evaluation of specific SD-microspheres

The surface of the microparticles was affected by the independent variables. If cosolvents are used (C1-C27), the rapid droplet hardening can lead to a more viscous W_1/O emulsion and uneven microspheres. Wrinkled surface and progressively increased porosity could be observed (Fig. 20). The trends observed for all the cosolvents used were similar.

Figures 20A-D show *the most critical cases* when microspheres were prepared at high cosolvent concentration (X_2: +1) and at a high ratio DS/AMC (X_3: +1). As compared with the microspheres prepared with the less water-soluble cosolvents (MeCOEt and *n*BuOAc), the use of Me_2CO and *n*PrOH (both water-miscible with rapid saturation in the W_2 phase) led to a dense microsphere structure, in which, despite of the pores and the depressed surface, the drug release could ensure a sustained profile. Batches C1-C9 (CH_2Cl_2+Me_2CO, Table 15) were regularly shaped, but minor or gross distortions could also be observed (C9, Fig. 20A). Cavities appeared due to the rapid diffusion of Me_2CO and thus the fast precipitation of the copolymer. A similarly depressed surface was observed when the CH_2Cl_2+*n*PrOH mixture was used (C1A-C9A): the microspheres appeared shrivelled, and resembled flowers of gypsum, especially at elevated *n*PrOH concentration (C9A,

Fig. 20B). This phenomenon could be attributed to the coalescence of the W_1 droplets and the early hardening of the copolymer, due to the high water-solubility of *n*PrOH.

Figure 20. SEM evaluation of microsphere products (X_1; X_2; X_3): (A) C9 (-1; +1; +1); (B) C9A (-1A; +1; +1); (C) C18 (0; +1; +1); (D) C27 (+1; +1; +1).



When MeCOEt, as a less water-soluble cosolvent, was added to CH_2Cl_2 (C10-C18), more spherical particles with distorted surface morphology were observed, and there were several aggregated microspheres (C18, Fig. 20C). In fact, the formation of these 'groups of particles' arose from the fusion of the semifinished microsphere walls at the interface, as the emulsion droplets could not be divided during spray-drying. The cosolvent *n*BuOAc, which is the most analogous to CH_2Cl_2, ensured the lowest microsphere hardening rate. As compared with the batches prepared with CH_2Cl_2 alone (C0a-C0c), when microspheres with a smooth surface were formed, C19-C27 contained uneven microspheres with a rough surface (C27, Fig. 20D), due to the additional cosolvent effect. The relationship between the emulsion droplet characteristics and the external morphology of the particles suggested that the surface properties and the pore formation were affected considerably by the use of polar cosolvents.

4.4.3. Effect of processing parameters on SD-microspheres

The present quantitative factorial design study allowed the mathematical evaluation of the effects of the processing parameters. The effects are presented by coefficients shown in Table 17.

Table 17. Coefficients for the mathematical models

Resp.	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}	R^2
X_1 = -1 (Me_2CO)											
Y_1	13.27	4.80	-2.25	-0.86	0.29	-0.26	0.37	-0.77	-0.32	-0.45	**0.9988**
Y_2	59.62	-6.02	1.47	-3.19	3.93	5.27	-1.11	4.46	-3.19	-3.32	**0.9449**
Y_3	185.07	25.80	-17.69	11.69	-23.29	-1.96	-1.17	-2.77	30.69	8.36	**0.9875**
Y_4	29.28	-0.73	2.93	-5.52	-0.66	3.72	-1.60	0.27	-0.79	-5.04	**0.9784**
Y_5	50.43	28.06	-4.56	-1.53	-14.34	-3.64	0.74	14.89	5.02	7.27	**0.9057**
X_1 = -1A (*n*PrOH)											
Y_1	14.17	4.01	-2.02	-0.710	-0.098	-0.709	0.222	-0.607	-0.389	-0.416	**0.9966**
Y_2	60.15	-8.91	0.747	0.057	2.18	-1.28	-3.16	0.915	-1.48	-6.65	**0.9250**
Y_3	193.04	14.22	-21.36	10.01	-20.85	1.44	-1.21	-8.19	37.76	8.63	**0.9904**
Y_4	27.75	2.661	0.338	-3.182	3.091	-0.749	-0.925	3.064	1.569	-5.37	**0.9101**
Y_5	68.56	-6.70	-5.69	-5.12	-8.50	3.76	-0.38	-10.06	5.32	8.48	0.6795

Investigation of the viscosity of the multiple emulsion (Y_1 response)

The precipitation of the polymer, and hence the microsphere formation, depends on the diffusion-controlled solvent removal process, the organic phase viscosity and the cosolvent concentration.[238] The viscosity is of great importance: the batches with the highest η ensured microspheres with a wrinkled or porous surface, with a low production yield, low EE value, but higher particle size and Q_6 value. It was observed that log P of the cosolvent (X_1) was more of a controlling factor in the viscosity of the examined phases; however, the cosolvent concentration (X_2) and the ratio DS/AMC (X_3) had significant complementary, but opposite effects.

The rate of extraction of the polar cosolvent from the W_1/O emulsion to the W_2 phase is higher than that for CH_2Cl_2; thus, the organic phase viscosity increases rapidly and polymer precipitation therefore occurs earlier. The increase of the X_2 factor level resulted in a decreased organic phase viscosity, and therefore an increased mixing efficiency. This tendency also held true for X_3, keeping X_2 constant. The W_1/O emulsion, prepared purely with CH_2Cl_2 (C0A-C0C, Table 16), had higher η (18.6-30.9 mPas) than those of the emulsions prepared with the cosolvents examined (6.4-22.7 mPas). The high viscosity of *n*PrOH as compared with the other cosolvents did not exert a positive effect on the microsphere formulation. The lipophilic components dissolved in the CH_2Cl_2+MeCOEt mixtures led to a stronger viscosity dependence than when pure CH_2Cl_2 and MeCOEt were mixed. At constant X_2, η decreased with increasing X_3, similarly in the case of Me_2CO (Table 16). The η decreased to a larger extent at constant X_3 with increasing X_2; this change was statistically significant ($R^2 = 0.976$, $p = 0.002$).

Constant X_2 and increasing X_3 resulted in a decreased η, while the same tendency could be observed at constant X_3 and increasing X_2. Both the linear and the quadratic effects of the independent variables on η were statistically significant ($R^2 = 0.998$, $p < 0.008$, Table 17). X_1 had the main (positive) effect on η (b_1: 4.80), but the increased levels of X_2 and X_3 decreased it, and a synergistic interaction between X_2 and X_3 (b_{23}: 0.37) was also observed. The required effect is a low η_3, which could be ensured by low (-1) X_1, and high (+1) level of X_2 and X_3.

Investigation of the microsphere production yield (Y_2 response)

The production yield ranged from 26.1 to 75.6% (Table 16), depending notably on the process parameters, and the viscosity and stability of the $W_1/O/W_2$ emulsions to be dried. The increase of η_3 led to a decrease in the efficacy of the spray-drying and consequently in the production yield. The production yield decreased in parallel with the increase of X_1 and X_3 (Tables 16 and 17). Low and medium (-1 and 0) levels of X_1, high (+1) level of X_2 and low level (-1) of X_3 resulted in a higher production yield (65-72%). Log P was confirmed as the limiting factor, the linear (b_1) and quadratic (b_{11}) effects of X_1 had the greatest influence (-6.02 and 4.46, respectively) ($R^2 = 0.944$)

(Table 17). It was observed that the use of *n*BuOAc and the high (+1) level of X_3 affected the production yield most adversely. Increase of X_3 caused a decrease in the production yield, due to the low stability of the W_1/O emulsion. Low (-1) level of X_3 demonstrated the highest production yield, indicating that this ratio could be used successfully at high cosolvent concentration (75% w/w) to achieve the convenient production yield (> 65%) during spray-drying.

Investigation of the particle size (Y_3 response)

The width of the particle size distributions was expressed by the *SPAN* parameter, which overall varied from 1.04E+00 to 4.84E+00, reflecting a homogeneous size distribution.

Generally, a high solvent extraction rate can lead to rapid solvent elimination, and therefore fast microsphere formation and a higher particle size.[91,147] X_1 had the *highest* effect on particle size (Table 17); its increase afforded the *same* sequence as for the boiling points (Me_2CO < MeCOEt < *n*BuOAc) and resulted in an increased particle size, while their water-solubilities exhibited the opposite sequence (Table 5). Microspheres with higher particle size were produced when *n*BuOAc was used, which can be explained by the increased η. With *n*PrOH instead of Me_2CO, the increase of particle size revealed a different sequence (MeCOEt < *n*PrOH < *n*BuOAc), because *n*PrOH has a higher viscosity than that of MeCOEt, resulting in a more viscous W_1/O emulsion. The high η_3 made it difficult to form small multiple emulsion droplets, and therefore particle size could not be reduced as reported earlier.[86,239] C0A and C0B had relatively high η (20.5 and 30.9 mPas), but the lowest particle size (54 and 107 μm), indicating the joint effect of the independent variables (Table 16).

The effects of all the factors, and the quadratic effect of X_2 (b_{22}: 30.69) were found to be significant; the microspheres obtained at DS/AMC = 1:16 (X_3; +1) were characterized by the maximum particle size in every case. The X_1X_2 interaction had the strongest effect on particle size (b_{12}: -23.29). There was a tendency for increasing amount of drug in the W_1 phase to lead to a decreased production yield and an increased particle size, which proved to be opposite effects. High (+1) level of X_2, and low (-1) level of X_1 and X_3 decreased particle size. When *n*BuOAc was used (X_1; +1) at medium concentration (X_2; 0), microspheres were formed with the maximum particle size, around 300 μm, because the increase in the CH_2Cl_2-cosolvent viscosity resulted in merged droplets or in a reduction of the efficiency of disruption of the W_1/O emulsion into droplets. The trends observed for the various batches were practically the same: particle size at constant X_2 increased with increasing X_3, while at constant X_3 and increasing X_2, particle size increased up to 50% w/w cosolvent content, and dropped at 75%. The negative sign of the X_2 effect (b_2: -17.69) confirmed this incident.

Investigation of the EE value (Y_4 response)

The value of EE is the result of a sensitive balance between two main key factors as opposite effects, the *rate of CH_2Cl_2+cosolvent migration* to the W_2 phase and the *duration of AMC precipitation*.

On the basis of preliminary studies,[I,II] the maximum DS/AMC = 1:16 (X_3; +1) was chosen, a ratio that can ensure the molecular dispersion of the drug in the copolymer matrix. EE varied in the ranges of 10.5-53.3% (Table 16). With the CH_2Cl_2+Me_2CO and CH_2Cl_2+MeCOEt mixtures, in spite of their water miscibility, the dependent variables could be balanced more effectively, leading to higher average EE values (Table 16) than with CH_2Cl_2 alone. A high (+1) level of X_2 and a low (-1) level of X_3 led to the maximum of EE, which confirmed that the polar cosolvent can leave the W_1/O emulsion faster, resulting in the fast solidification of the copolymer and in more drug in the W_1 droplets. Moreover the droplets might remain in the liquid form for a longer period of time when *n*PrOH (highest viscosity) and *n*BuOAc (lowest water-solubility) were used, leading to a greater drug leakage, which was reflected in the decreased EE values, however the more viscous W_1/O emulsion could be less likely fragmented, resulting drug retention and higher EE.[148]

EE indicated a good fit (R^2 = 0.978, Table 17). Cosolvent log P at low and medium (X_1; -1, 0) levels, cosolvent concentrations at medium or high (X_2; 0 and +1) levels and low ratio of DS/AMC (X_3; -1) yielded microspheres with the highest EE. The appreciable effects of X_2 (b_2: 2.93) and X_3 (b_2: -5.52) on EE indicated main effects that differed in magnitude and mathematical sign.

Investigation of the cumulative release (Q_6) (Y_5 response)

Q_6 varied in the ranges of 3.2-100.0% (Table 16). The release pattern was found to be complex. The goodness of fit for the kinetic models used ranked in the sequence of Hixson-Crowell < Baker-Lonsdale ~ Higuchi < Nernst. The Nernst dissolution profile best followed the release profile of batches C0A-C0C; after a slow dissolution the release rate reached a plateau. The absence of a burst effect could be due to the preferential location of drug inside the deep sections of the copolymer matrix. For batches without a burst effect, the Baker-Lonsdale and Higuchi models were found to provide best fit. Batches reaching a plateau after 2 h conformed to the Hixson-Crowell model.

Initial burst. The absence of an initial burst was observed for batches C1-C9; the rapid Me_2CO diffusion could lead to a denser copolymer matrix, eliminating the burst release, and thus the rate of drug diffusion was attenuated (Q_6: 3.2-28.6 h, Table 16). In contrast, a high burst release was observed for C1A-C9A and C19-C27. This rapid initial release might be of functional importance in providing an initial dose during drug delivery. Pore diffusion, disruption or disintegration of the matrix, as expressed in the burst effect, became more predominant at high EE.

Me_2CO. Due to the relatively low Q_6 values, the CH_2Cl_2-Me_2CO mixture could be useful when *sustained release for a longer period* is the required dissolution profile. At constant X_2, an increase of X_3 was found to improve the dissolution of drug appreciably (Tables 16 and 17). The release profiles of C1, C3 and C5 proved linear, conforming the Higuchi equation ($R^2 > 0.973$). C6-C9 followed the Hixson-Crowell release profile ($R^2 > 0.932$) without a burst effect. This confirmed dissolution rate limitation of drug release from microparticles and revealed to no dramatic changes in the structure of them meanwhile.[207]

MeCOEt. Batches C10-C12 (X_2; -1) fitted the Baker-Lonsdale model ($R^2 > 0.941$), describing release profiles from matrices with uniform drug distribution, while the release profile of C14-C16 (X_2; 0, +1) fitted the Nernst model ($R^2 > 0.962$). C13 and C17-C18 (X_2; 0, +1) did not meet our requirements (max. 80% in 6 h).

***n*PrOH.** The *n*PrOH and *n*BuOAc have the highest boiling points and viscosities of the cosolvents used; since the rate of evaporation of the solvent depends on its boiling point, the influence of their slow evaporation combined with the higher viscosity was more evident for these batches, resulting in microspheres with a large SSA, low EE, a porous nature and hence a high release rate with initial burst. The amount of dissolved drug increased up to 4 h and reached a plateau or 100%.

For C1A, C6A, C8A and C9A, the Nernst model described the drug release kinetics best ($R^2 > 0.956$). The release kinetics of the other batches did not meet the requirements set according to our aims (max. 80% drug release in 6 h). A possible reason for the high drug release could be the formation of large pores and deep channels, explained by the specific extraction of *n*PrOH from the W_1/O emulsion, which may act in this way as an effective pore-forming agent.

***n*BuOAc.** Q_6 was accompanied by a burst release effect, followed by the sustained release of 70-86% over 6 h. The release from C20, C21 and C23 exceeded our aims, which might be caused by the frequency and size of the pores. C19, C22 and C25 (X_3; -1) satisfied the Nernst equation ($R^2 > 0.977$). For C26, C27 (X_2: +1), the Hixson-Crowell model ($R^2 > 0.912$) fitted the dissolution.

X_1 proved statistically significant in its linear (b_1: 28.06), quadratic (b_{11}: 14.89) and interaction (b_{12}: -14.34) effects. X_2 and X_3 also had significant, but lower effects on the release rate.

The effects of X_1 and X_3 on the drug release rate are reflected by the following representative release profiles. Figure 21 depicts the effects of X_1 and X_2 on the cumulative release for batches C1, C7, C10, C16, C19 and C25, when ratio DS/AMC was kept constant. In spite of their different release behaviour, the production yields (62-74%), particle size (108-205 μm), and EE (33-53%) values of these batches were similar; thus, the nature and concentration of the cosolvents appeared to determine the drug release. Figure 22 demonstrates the effects of X_3 on the cumulative drug

release for batches C7, C8, C9, C25, C26 and C27. The similar release profiles indicated that the release rate could be modified only slightly by varying the ratio DS/AMC.

Figure 21.

Effect of cosolvent log P (X_1) and concentration (X_2) on rate of drug release (X_1; X_2; X_3): C1 (-1; -1; -1), C7 (-1; +1; -1), C10 (0; -1; -1), C16 (0; +1; -1), C19 (+1; -1; -1), and C25 (+1; +1; -1).

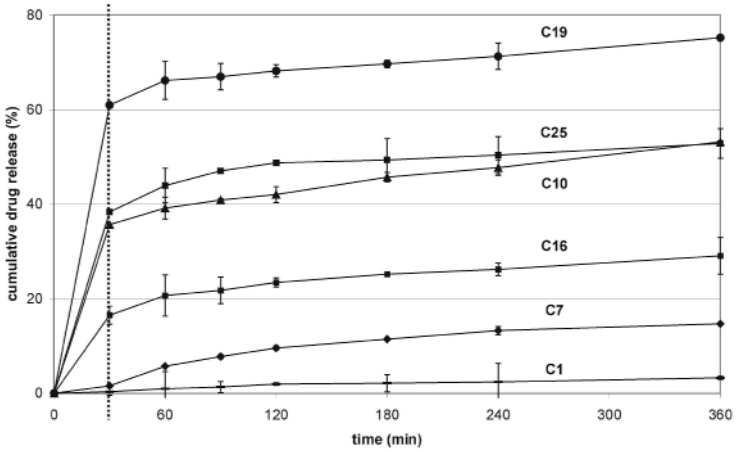
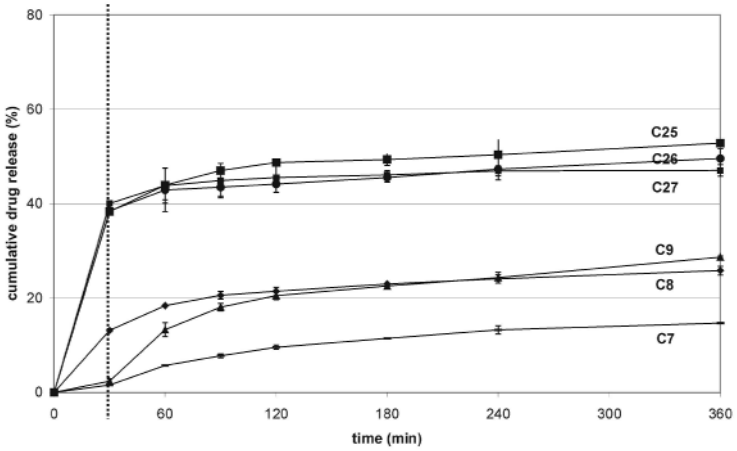


Figure 22.

Effect of ratio DS/AMC (X_3) on rate of drug release (X_1; X_2; X_3): C7 (-1; +1; -1), C8 (-1; +1; 0), C9 (-1; +1; +1), C25 (+1; +1; -1), C26 (+1; +1; 0), and C27 (+1; +1; +1).



4.4.4. Conclusion of the characterization of the SD-microspheres

The individual and joint effects of independent variables on the properties of AMC-based SD-microspheres were investigated. Table 18 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 μm). 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 ratio DS/AMC (X_3): For optimization of the microsphere characteristics, the ratio of 1:32 (X_3: -1) proved effective (Table 18). 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 18. 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	All	All
Y_2	High production yield	-1 / 0	+1	-1
Y_3	Low particle size	-1	+1	-1
Y_4	High EE	All	+1	-1
Y_5	Q_6 20-80% in 1-6 h	-1	+1	All

4.6. ORGANIC SOLVENT AND COSOLVENT RESIDUE IN THE MICROSPHERES

A relatively low amount of organic solvent residue can be achieved by increasing the drying temperature of the product approaching the polymer T_g, but the amount of the organic polar cosolvent residue depends even more on its affinity to the polymer. The concentration limit (ppm) and PDE of CH_2Cl_2 are 500 ppm (USP XXIII) or 600 ppm (ICH)[154] and 6.0 mg day^{-1}, respectively In all the *SE-microsphere* samples demonstrated in this thesis, the CH_2Cl_2 residue was < 5 ppm, which meets the requirements. The maximum residual CH_2Cl_2 content in the *SD-microspheres* prepared with 100% w/w CH_2Cl_2 (C0A-C0C, the 'worst case' of the SD-microspheres) was 808.5 ppm (S.D.: 3.81%), which was higher than the limits. These data showed that the duration of spray-drying as compared with the common SE-technique with longer post-drying could be too

short to eliminate the residual CH_2Cl_2 from the microspheres; similar observations were published earlier.[60]

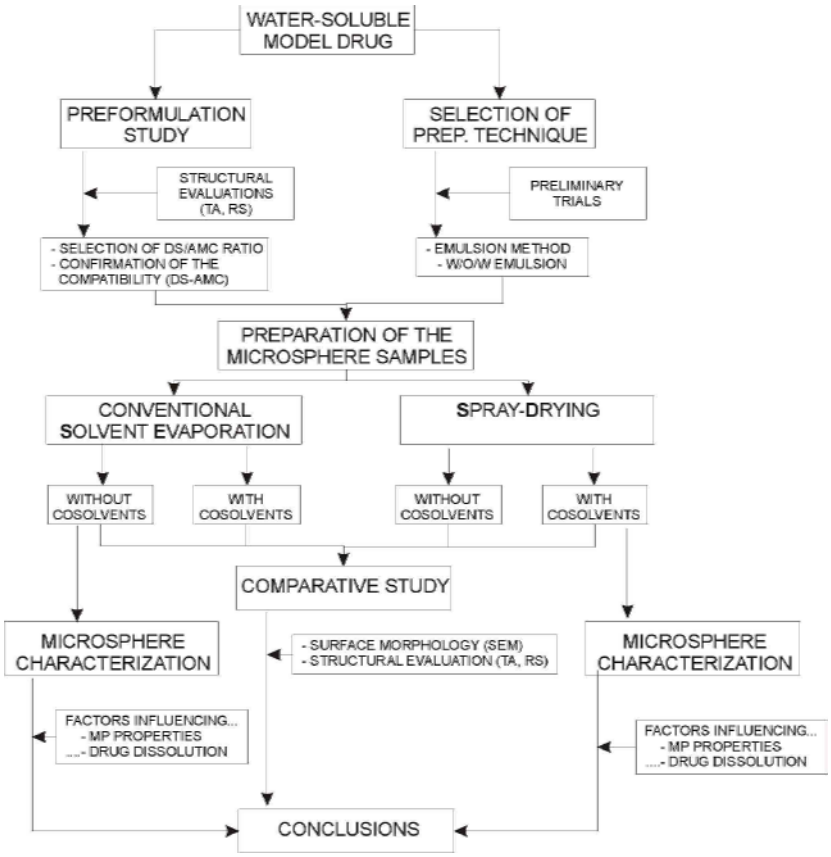
Class 3 solvents, used as cosolvents in this work, have a concentration limit of 5000 ppm (PDE = 50 mg day^{-1}) (ICH).[154] The maximum concentrations of cosolvent residues in the *SD-microspheres* prepared at high (+1) value of X_2 were 441.5 (Me_2CO), 1796.4 (MeCOEt), 442.5 (*n*PrOH) and 954.0 ppm (*n*BuOAc) (S.D.: 1.77-6.12%), which met the requirements. These results confirmed that the amount of cosolvent residue did not depend on the boiling point. The reason for the relatively high residual amounts of MeCOEt and *n*BuOAc was their higher lipophilicity, and thus the slower saturation of the W_2 phase.

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 23 shows the summary of the optimization steps of this PhD work.

Figure 23.

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



Preformulation study

Physical mixtures of AMC with DS, PVA and PEGS separately and in combinations and a DS/AMC model mixture were prepared for preformulation measurements. 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 ratio DS/AMC can be selected from a wide range in the formulation optimization of SE- and SD-microsphere preparation, in conformity with the therapeutic aim.

TA of DH-containing microspheres suggested the molecular dispersion of DH in the CHT matrix. Based on the investigations, the ratio DH/CHT = 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 prepared 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 μm.

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 ratio DS/AMC proved effective in the optimization process. Linear relationships were observed between the independent (log P and concentration of the cosolvents, and the ratio DS/AMC) 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 aimed to choose, keeping Q6 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 ratio drug/copolymer = 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 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.

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LAUS DEO

Dissertatio scripta est Szegedini Anno Domini MMVIII

8. ANNEX

I.

II.

III.

IV.

V.

VI.