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REVIEW ARTICLE

Drug Microencapsulation by PLA/PLGA Coacervation in the Light of Thermodynamics. 1. Overview and Theoretical Considerations

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Abstract □ Phase separation of poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA), often called "coacervation" in the pharmaceutical field, is one of the classical methods for peptide drug microencapsulation in biodegradable polyesters. Although numerous studies have used this technique, the underlying physicochemical mechanisms of polyester coacervation under conditions of microsphere production have not been well-described yet. Moreover, the quality of microencapsulation in terms of drug loading efficiency and residual organic solvents is often not entirely satisfactory and depends greatly on the specific drug and polymer used. The first part of this contribution reviews briefly the scientific and patent literature on PLA/PLGA coacervation. Then, the underlying physicochemical principles of polyester coacervation are discussed and relevant thermodynamic models presented. More specifically, attempts were made to clarify the necessary characteristics of polymers, solvents, and coacervating and hardening agents for successful phase separation and microsphere formation. These basic considerations may contribute to a better understanding of the boundary conditions crucial for efficient drug microencapsulation by polyester coacervation.

Introduction

Major efforts are presently devoted to improve existing or develop new microencapsulation techniques for peptide drugs in biodegradable polyesters, such as poly(lactide) (PLA) or poly(lactide-*co*-glycolide) (PLGA). The generally recognized problems of this technology lie in the limited core loading, i.e., <15%, the moderate and sometimes low loading efficiencies, the variable burst release of insufficiently entrapped drug and the unacceptable organic solvent residues. All these essential characteristics are virtually impossible to predict and can vary with the polymer type, drug, solvent, and technique used for microencapsulation and final processing. The innumerable studies in this field have been extensively reviewed with respect to technological aspects and related pharmaceutical applications.[1−6] One of the main methods for drug microencapsulation into PLA/PLGA is that of polymer phase separation. In the field of drug microencapsulation into PLA/PLGA, the term coacervation is commonly used for polymer phase separation induced by the addition of a coacervating agent to the polymer solution. Typically, addition of poly(dimethylsiloxane) (PDMS, silicone oil) to PLA/PLGA dissolved in dichloromethane (DCM) yields the so-called coacervate droplets, which are solidified in a hardening agent, such as hexane or octamethylcyclotetrasiloxane (OMCTS), to produce the final microspheres. Both terms of phase separation and coacervation will be used interchangeably in this contribution.

Although coacervation has been used in various studies for peptide microencapsulation,[7,8] the physicochemical basis of this method is still not well-described. Some essential requirements for PLA/PLGA coacervation for microsphere formation were disclosed in earlier patents[9,10] and in more recent scientific reports,[11−17] which considered the distinct macroscopic stages and the kinetics of coacervation. Besides, relatively little information is available on the physicochemical principles of PLA/PLGA coacervation for microspheres formation and drug microencapsulation,

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although fundamental aspects of coacervation were covered thoroughly a long time ago by the classical studies of Bungenberg and Kruyt for ionic systems[18] and by Dobry and Boyer-Kawenoki for nonionic systems.[19] Basic thermodynamic conditions for polymer−solvent interactions and polymer phase separation were initially worked out by Flory for binary systems,[20] by Scott[21] and by Bamford and Tompa[22] for ternary systems, and finally reviewed beautifully by Flory.[23] Current knowledge of interactions occurring in polymeric solutions has been described more recently.[24] Thermodynamic aspects have also been considered in the coacervation of ethylcellulose.[25−27] The approach taken in these studies was based on the solubility parameters of Hansen.[28] Finally, a new thermodynamic model has been recently proposed to quantify drug−polymer−solvent interactions and to predict, in terms of a scale, aqueous protein encapsulation in PLA/PLGA microspheres prepared by spray-drying.[29]

In this contribution (part 1), the most relevant studies on PLA/PLGA coacervation for microsphere formation will be reviewed, and various thermodynamic models likely to be helpful for better understanding this process will be discussed. In part 2,[71] experimental physicochemical data on PLA/PLGA coacervation will be presented and interpreted in thermodynamic terms. With this information, a rationale for drug microencapsulation by coacervation will be provided, which is intended to improve microsphere quality.

Brief Overview on PLA/PLGA Coacervation for Drug Microencapsulation

From our own experience and from literature, five major concerns prevail in microencapsulation by coacervation: (i) production of free-flowing, nonagglomerated microspheres, (ii) control of particle size, also in the lower micrometer range, (iii) use of toxicologically acceptable solvents, (iv) lowering solvent residues in the microspheres to levels conforming with regulatory standards, and (v) encapsulation of drugs with a high efficiency and resulting in a low burst release. Most of the studies generally address one or several of these issues, either by technological optimization or by physicochemical approaches. Technological developments and parameters in PLA/PLGA coacervation will only be briefly reviewed here, since the main objective of this contribution is to discuss the applicability of physicochemical and thermodynamic parameters in PLA/PLGA microsphere preparation.

Terminology—The term coacervation was introduced in 1930 by Bungenberg de Jong and Kruyt for a process in which aqueous colloidal solutions are separated into two liquid phases, a colloid-rich phase, i.e., the coacervate, and a colloid-poor phase.[18] At that time, this terminology was primarily used for aqueous gelatin solutions. Accounting for different phase separation mechanisms, coacervation was subdivided into simple and complex coacervation. In simple coacervation, the polymer is salted out by electrolytes, such as sodium sulfate,[30,31] or desolvated by the addition of water-miscible nonsolvents such as ethanol.[31] On the other hand, the process of complex coacervation is described as a phase-separation process which occurs in the mixture solution of oppositely charged polyions.[32]

The use of water-insoluble, mainly nonionic polymers in pharmaceutical technology was the reason for extending the terminology to nonaqueous polymer systems. For better differentiation, this type of coacervation is often called organic phase separation.[33] Depending on the desolvation process, the nonaqueous polymer phase separation is also referred to as temperature-induced[34] or nonsolvent-induced coacervation. Unfortunately, in the pharmaceutical field, the latter term is indistinguishably used for two types of polymer phase separation processes: (i) coacervation induced by addition of a low molecular weight nonsolvent for the polymer and (ii) coacervation induced by polymer 1−polymer 2 incompatibility. The confusing terminology was further complicated by coining different terms for the phases produced during polymer phase separation: "coacervate phase", "polymer-rich phase", "colloid-poor phase", "equilibrium phase", "equilibrium liquid", "continuous phase", "lean phase", "polymer-poor phase", or "supernatant". Attempts to define principles and classification of coacervation processes for drug microencapsulation have been made with illustrated examples.[33,35−37] Nonetheless, these unnecessary terminological complications could have been avoided by adopting the original terminology used by Flory in his classic textbook.[23] On the basis of thermodynamic considerations of phase equilibria, Flory distinguished between binary systems consisting of one polymer dissolved in a solvent and ternary systems consisting either of a single polymer component in a binary solvent mixture or of two polymeric components in a single solvent. For binary systems, phase separation can be typically induced by changing the temperature. For ternary systems consisting of a single polymer, phase separation can be induced by increasing the amount of nonsolvent above a critical volume fraction for a given polymer concentration; for ternary systems consisting of two polymeric components in a single solvent, as is the case with the system described in this contribution, phase separation can occur when the volume fractions of both polymers are above a critical value. Thus, in a system composed of dichloromethane as common solvent and the polymers PLA (or PLGA) and PDMS, phase separation can be observed above a critical total polymer concentration (see the model proposed by Scott[21] described below). Naturally, for all polymer phase separation processes, temperature and polymer molecular weight affect the critical conditions.

For microencapsulation purposes, polymer phase separation represents only a first step in a series of important events. In a typical system containing a solvent, PLA/PLGA (polymer 1) and PDMS (polymer 2), the parameters temperature, polymer concentration, and molecular weight must be adjusted carefully to obtain a PLA/PLGA-rich phase in the form of droplets (coacervate droplets) which are physically stabilized in the PLA/PLGA-poor surrounding phase. Thus, interfacial properties and viscosity will play a predominant role. Moreover, the system should provide favorable conditions for the engulfment of dissolved or dispersed drugs in the PLA/PLGA-rich coacervate phase, without affecting the physicochemical properties of the active compound. This again is a matter of interaction energies at the molecular level or of interfacial properties at the macroscopic level. Finally, the physically stabilized and drug containing coacervate droplets have to be transferred into a hardening agent, where solidification of the polymeric microspheres takes place.

To avoid any terminological misunderstanding of these complex processes, the following terms will be used interchangeably hereafter:

microsphere forming polymer = polymer 1 (e.g. PLA, PLGA)

coacervating agents

= polymer 2 (e.g. PDMS) for ternary systems of polymer 1/polymer 2/solvent

= nonsolvent or poor solvent (e.g. glyceride) for ternary systems of polymer/solvent/nonsolvent

hardening agent = nonsolvent for hardening coacervate droplets to form microspheres (MS) (e.g. hexane, OMCTS)

coacervate (phase or droplets) = phase rich in polymer 1 (e.g. PLA, PLGA)

continuous phase or equilibrium liquid = phase poor in polymer 1

Incidentally, the term nonsolvent for coacervating agent should not be understood in a thermodynamic sense. Practically suitable low molecular weight coacervating agents show generally a very limited swelling capacity for the microsphere-forming polymer and may therefore be better described as poor solvents.

Technological Developments—The principle of organic phase separation for preparing nonbiodegradable polymeric microspheres for drug delivery was already described in patents in the 1960s.[38,39] With the increasing availability of high molecular weight biodegradable polyesters, as produced by ring-opening polymerization,[40] this class of biomaterial attracted great interest for the microencapsulation of peptide and protein drugs or hormones.[41] The use of polymer coacervation to prepare polyester microspheres was described probably for the first time in 1979.[9] Toluene, 2-propanol, and heptane were typically used as solvent, coacervating agent, and hardening agent, respectively, although other mixtures were also mentioned. This procedure was subsequently modified by numerous authors, who varied one or several components of the coacervation system. Table 1 summarizes studies on drug microencapsulation by coacervation focusing on technological aspects. The majority of drug microencapsulation studies by coacervation were obviously dealing with peptide and protein drugs (Tables 1–3). In the early 1980s, the phase separation process was used for microencapsulating aqueous peptide solutions, such as nafarelin acetate.[7,8,42] Together with the goserelin acetate loaded, cylindrical PLGA implant Zoladex,[43,44] these microspheres represented the first industrial development of a controlled peptide release system for parenteral application. Since then, a great variety of peptide and protein compounds have been investigated including octa-[45] and decapeptides,[7,46–50] the 24-amino acid peptide atriopeptin III,[51] the 32-residue peptide calcitonin,[10,46] and also 68–150 kDa proteins such as serum albumins[52–54] or tetanus toxoid.[55,56]

The list of studies (Table 1) reveals that a common subject of research work in the field of microencapsulation was dedicated to the development of microsphere products free of agglomerates and having a suitable particle size distribution for parenteral or intraocular administration. Therefore, some of the most common variables examined were the temperature of coacervation[9] and hardening[10] and the type of ternary solvent/coacervating agent/hardening agent system.[46,47,51,57–61] These studies followed the classical coacervation process, where a coacervating agent was added to a polymer/drug dispersion. In a more sophisticated process, a PLA solution was injected into mineral oil, whereby particles of any desirable size were allegedly produced by varying the diameter of the injection equipment.[62] Recently, a modified PLGA phase separation was proposed for preparing small-sized ovalbumin-loaded microspheres by emulsifying the peptide/polymer mixture into a silicone oil–surfactant solution.[52,53] This method resembled a procedure previously used and named the solvent extraction/precipitation technique.[63,64] Other modifications of the classical coacervation procedure were made in studies on zidovudine microspheres[65] and on nafarelin acetate nanoparticles.[66] Both investigations combined phase-separation with solvent-evaporation processes to obtain freely flowing powders.

A few studies also compared the coacervation process with other microencapsulation methods such as solvent evaporation[48,63,64,66–68] or extended the phase-separation process on other water-insoluble polymers such as poly-

Table 1—Reports on Technological Developments of Drug Encapsulation by PLA/PLGA Coacervation[a]

solvent	coacervating agent	hardening agent	special coacervation conditions	focus of the study	ref
			Nonpeptide Drugs		
DCM	mineral oil	mineral oil	injection of polymer solution into mineral oil	control of particle size by needle diameter	62
chloroform	heptane	heptane	coacervation at 4 °C, hardening in cold heptane overnight	pilocarpine eyedrop suspension with prolonged miotic effect	60
toluene	isopropanol, cyclohexane	heptane	coacervation at −65 °C	prevention of agglomeration	9
ethyl acetate	diethyl ether	diethyl ether	carboxymethylcellulose/PLA mixture	oral sulfamethizole delivery system	57
DCM	DCM/PDMS	petroleum ether	combination of coacervation and solvent evaporation	increased zidovudine entrapment efficiency	65
DCM	diethyl ether	diethyl ether	addition of nonsolvent over 3 h	entrapment of ciprofloxacine for intraperitoneal use	59
toluene	BR	heptane	transfer of coacervate at low temperature into cold hardening agent	prevention of agglomeration; entrapment of cyclazocine, digitoxin, naltrexone	10
			Peptide and Protein Drugs, Cells		
DCM	PDMS	heptane	hardening at ambient temperature	controlled release of nafarelin by varying polymer type and M_w	7, 8, 42
DCM	BR in DCM	hexane, cold	Tween 85 as emulsifier/stabilizer in PLGA; APIII used as solid powder	stability of APIII in PLA/PLGA under different conditions	51
toluene	BR in toluene	heptane	hardening of coacervate at low temperature; entrapment of micronized calcitonin/gelatine solid solution	effect of temperature, spreading behavior, and viscosity of coacervate on calcitonin entrapment	10
DCM/acetone	caprylate triglyceride	caprylate triglyceride	solidification by diffusion and evaporation of solvent	nafarelin loaded nanoparticles prepared in an oily system	66
DCM	PDMS	heptane	emulsification of the polymer solution into PDMS by the aid of Span 85	novel phase separation process yielding microspheres with zero order OVA release	52, 53
not specified	not specified	not specified	modified process without specifications	encapsulation of FITC-conjugated BSA and TT	99
DCM	PDMS	OMCTS	encapsulation of TT, BSA, and a synthetic peptide as micronized powders and aqueous solutions	key parameters controlling coacervation and drug encapsulation	54, 56, 70, 71, 77
EtAc	PDMS	heptane	encapsulation of double micronized cellular *S. enteritidis*	development of a *S. enteritidis* vaccine with PLA microspheres	72
DCM	PDMS	heptane	thrombin and BSA used as model proteins	encapsulation of an aqueous phase	16, 17
DCM	PDMS with Span 85	petroleum ether	injection of a BSA/PLGA suspension into PDMS by a 22 G needle	effect of preparation technique on microspheres and degradation	63, 64

[a] Abbreviations used: APIII, atriopeptin III; BR, poly(butadiene); BSA, bovine serum albumin; DCM, dichloromethane; EtAc, ethyl acetate; FITC, fluorescein isothiocyanate; OMCTS, octamethylcyclotetrasiloxane; OVA, ovalbumin; PDMS, silicone oil; PLA, poly(lactide); PLGA, poly(lactide-*co*-glycolide).

Table 2—Reports on Toxicological Improvements in Coacervation Processes[a]

solvent	coacervating agent	hardening agent	special coacervation conditions	focus of the study	ref
DCM	PDMS	triglycerides, 2-propanol, sesame oil	ratio of solvent:hardening agent = 1:33; adjusting residual nonsolvent by the washing process	biocompatible hardening agents; modifying triptoreline release by residual hardening agent	58
DCM	PDMS	OMCTS, hexamethyldisiloxane	ratio of solvent:hardening agent = 1:20 to 1:76; hardening during 2 h	minimizing residuals by using volatile hardening agent	50
DCM, EtAc	PDMS	OMCTS, hexane	empty and BSA loaded coacervates	effect of loading and drying conditions on residuals and morphology	70
DCM	PDMS	fatty acid esters	ratio of solvent:hardening agent = 1:20; hardening during 90 min	biocompatible hardening agents	61
DCM	PDMS, sesame oil	heptane	varying the ratio of solvent:coacervating agent; hardening during 3 h	minimizing residual heptane	47
DCM	PDMS	PDMS of low viscosity	partial evaporation of solvent before hardening	optimal combination of evaporation and hardening	68
DCM	PDMS	FCHC, FHC	hardening at 15 °C	replacement of heptane by FCHC or FHC	46

[a] Abbreviations used: BSA, bovine serum albumin; DCM, dichloromethane; EtAc, ethyl acetate; FCHC, fluorochlorohydrocarbons; FHC, fluorohydrocarbons; OMCTS, octamethylcyclotetrasiloxane; PDMS, silicone oil.

(glycolic acid), poly(hydroxybutyrate),[69] poly(1,4-butylene-*co*-succinate),[48] or poly(ϵ-caprolactone).[68] Although the obtained results may have favored one or the other technique, this is of course not generally valid, but only holds true for the particular drugs and process conditions used.

It is probably fair to say that the coacervation technology offers interesting possibilities for peptide and protein microencapsulation. However, its main drawback lies in the residual solvents and coacervating and hardening agents remaining in the microspheres that compromise biocompatibility. Great efforts have been made to use solvents and, especially, coacervating and hardening agents that are toxicologically acceptable and to minimize the residues of processing liquids in the final microspheres (Table 2).

A toxicologically interesting, but environmentally problematic, approach was the replacement of the commonly used hardening agents hexane, heptane, or petroleum ether by fluorochlorohydrocarbons (FCHC and FHC).[46] In another attempt to minimize the amount of residuals, a low ratio of solvent:coacervating agent (PDMS) was claimed to be a key parameter to control the amount of the residual hardening agent heptane in histreline-loaded PLGA microspheres.[47] These authors also suggested new coacervating agents such as sesame oil. The suitability of volatile siloxanes such as OMCTS or hexadimethylsiloxane as hardening agents was shown in a study using the peptide drug triptoreline.[50] The residual amount of siloxane in the final product was 2−5%. These findings were in agreement with results obtained from studies on empty and bovine serum albumin (BSA)-loaded microspheres manufactured in our laboratory.[70] As will be shown in detail in part 2,[71] the amount of DCM and OMCTS residues depend greatly on the amount of PDMS used for coacervation. Supposedly biocompatible hardening agents for PLGA microspheres are fatty acid esters such as isopropyl myristate, for which 5% residuals have been found.[57] A comparable approach was made with sesame and neutral oils for hardening.[58] In the latter study, the authors claimed that the release pattern of encapsulated drugs such as triptoreline acetate could be controlled by the amount of residual hardening agent remaining in the microspheres after washing with 2-propanol. However, it remains unknown whether the relatively high amount (11%) of residual propylene glycol octanoate/decanoate or the washing with 2-propanol influenced the microsphere quality. From our own experience, 2-propanol lowers the glass transition temperature, T_g, of the polymer and softens the polymer matrix (unpublished results).

Despite the numerous reports concerning the problem of residual hardening compounds, only minor attention has been paid so far to the question of residual coacervating agents in microspheres. NMR and FTIR experiments conducted in our laboratory on empty and BSA-loaded microspheres revealed PDMS residuals of <0.5%.[70] Other investigators, however, found residual PDMS on the order of 4−5% in the final product.[15]

In light of these reports, the problem of residual hardening compounds as a major drawback of the coacervation method seems to be solvable. Conversely, residual dichloromethane (DCM) remains a serious concern. The very few studies having addressed this aspect in the coacervation of PLGA/PLA showed that drying of the microspheres was a crucial step to reduce residual DCM.[47,70] For low molecular weight polymers, drying near the glass transition temperature of the polymer was most efficient to lower DCM below 100 ppm, but this seemed rather ineffective for high molecular weight polymers.[70] Our group tested ethyl acetate as alternative polymer solvent and postulated

Table 3—Reports on Physicochemical and Thermodynamic Approaches to Drug Encapsulation by Aliphatic Polyester Coacervation[a]

physicochemical and thermodynamic parameters studied	experimental approach	ref
Polymer characterization		
type and M_w distribution of polymer; polymer hydrophobicity	GPC; ternary phase diagram established from microscopic observation	13, 14, 73
hydrodynamic volume and radii of gyration of polymer coils in solvent	laser light scattering	12
polymer solubility	solubility parameters	71, 73, 82
Process and parameters of phase separation		
temperature and nonsolvent induced polymer phase separation; kinetics of liquid–liquid (PDLA) and solid–liquid demixing (PLLA)	cloud point titration; phase diagram; turbidity measurement; DSC	15, 100,[b] 101,[b] 103
definition of the consecutive stages of polymer phase separation	ternary phase diagram from microscopic examination	10, 13, 14, 71, 77
consistency of coacervate at different temperatures and solvent/nonsolvent combinations	microscopic examination of coacervate	10
nonsolvent viscosity	microscopic examination	13, 14, 68
volume, solvent content, viscosity of coacervate and continuous phases; solubility parameters of the phases	isolation of the coacervation phases	10, 14, 71, 77
Interfacial properties		
wettability and spreading of coacervate and continuous liquid on solid powder	microscopic examination	10
interfacial properties of coacervate and continuous liquid on protein powder	contact angle measurements	54
interfacial properties of coacervate and continuous liquid on aqueous solutions	tensiometric measurements	17, 54, 73
characterization of the final product		
surface and internal porosity	SEM; TEM; mercury intrusion porosimetry	16

[a] Abbreviations used: DSC, differential scanning calorimetry; GPC, gel permeation chromatography; M_w, weight average molecular weight of polymer; PDLA, poly(D,L-lactide); PLLA, poly(L-lactide); SEM, scanning electron microscopy; TEM, transmission electron microscopy. [b] These studies described phase separation processes for foam preparations.

a relation between the amount of residual solvent and the interaction capacity with the coacervating and hardening agent. Ethyl acetate was also used by others, although residual EtAc was not measured.[52,72] Undoubtedly, the search for toxicologically safer and technologically more suitable solvents and coacervating and hardening agents should remain a prime goal in coacervation technology.

Physicochemical and Thermodynamic Approaches—The aforementioned issue of undesired residual compounds and the delicate stability and activity properties of some peptide drugs may require a more detailed characterization of the PLA/PLGA coacervation process to optimize microsphere quality. Commonly, successful drug encapsulation is considered to be primarily governed by the three basic requirements, namely (i) separation of a liquid coacervate phase, (ii) adsorption of coacervate droplets onto the drug particle surface, and (iii) coalescence and spreading of coacervate droplets on this surface. Various investigations were aimed at acquiring a better knowledge of the consecutive steps in phase separation processes and of the importance of the actual interfaces. Typically, the physicochemical behavior of coacervation phases prepared from various solvent/coacervating agent/hardening agent mixtures and their ability to spread over and engulf dispersed drugs (solid or liquid) were examined.[10] The interfacial tension was recognized as an important factor influencing the wettability of and spreading on a drug particle surface. However, the process of engulfing of a drug particle was found to depend strongly on the coacervate viscosity. From microscopic examination, a suitable viscosity range for the coacervate and continuous liquid was defined so that drug-loaded, spherical microspheres were produced.

In other studies, the importance of the polymer molecular weight distribution on the phase separation of a noncommercial PLGA 50:50 and on its coating quality was determined.[12,13] The mechanism of coacervation induced by PDMS was determined as a polymer-incompatibility process. The onset of coacervation was related to polymer hydrophobicity and solubility, with the latter depending on the amount of oligomers present. These findings were supported by results from size exclusion chromatography and laser light scattering. The term "stability window" was introduced to characterize the range of stable coacervate droplets. It was shown that this window could be enlarged by increasing the PDMS viscosity. Finally, the entrapment and release of triptoreline from microspheres were influenced by the amount of oligomers and, hence, by the polymer hydrophobicity. This type of characterization of the phase-separation process was extended to commercial PLGAs 75:25 and PLAs of different molecular weight.[14,73] The type of determined coacervation stages were comparable to those of the noncommercial PLGA 50:50, although the location of the stability window was different. This was explained by the narrower molecular weight distribution of the commercial Resomer products lacking significant amounts of oligomers. When examining the influence of the molecular weight of PDMS on the coacervation process, phase separation occurred at an earlier stage when the molecular weight and, thus, the viscosity of the nonsolvent was increased.[14] On the other hand, a clear relationship between the polymer molecular weight and the amount of PDMS necessary for obtaining stable coacervates was found in our own experiments.[71,73] Both these findings are consistent with the thermodynamic incompatibility of the two polymers in a single solvent, as outlined in the theoretical section below. Contradictory results were found, however, on the width of the stability window. In one study, this width decreased in the order PLGA 50:50 > PLGA 75:25 > PLA, although no distinct borderline of the stable coacervate zone was identified for PLA.[14] It was concluded that more hydrophilic copolymers show a larger range of stable coacervate droplets. Conversely, our own and other authors' experiments indicated a relatively narrow stability window for PLGA 50:50, which was due to a rather quick transition from a liquid coacervate to a gel-like state, giving rise to aggregation and polymer precipitation.[12,13,71] When studying the importance of coacervate and continuous phase viscosities for the stability of dispersed coacervate droplets, no clear relationship was found between these two properties.[14] It was assumed that the interfacial tensions between the different phases of the coacervation dispersion might be more important for coacervate stabilization than the viscosity of these phases. However, other studies emphasized the importance of the viscosity of the coacervating agent for coacervate stabilization[13,68] and for the yield and particle size distribution of microspheres.[54]

An interesting piece of work was presented on critical interfacial conditions for the entrapment of a dispersed

phase in a coacervate[17] by applying a theoretical model for particle engulfment.[74] Not surprisingly, the interfacial tension between an aqueous phase and PLGA coacervate was lower than (PLGA 50:50) or equal to (PLGA 75:25) that between aqueous and continuous phases, whereas the opposite result was obtained with the less polar PLA. It was predicted that drug entrapment into the less polar PLA coacervate should fail. However, our own experiments on the encapsulation of aqueous BSA into PLA gave entrapment efficiencies of 50−70%.[54] These results clearly demonstrated the beneficial effect of the decreased interfacial tension of a protein solution as compared to that of pure water. Nonetheless, both studies demonstrated that interfacial properties must be a key parameter for efficient drug encapsulation.

Pharmaceutical technology generally uses materials and conditions for which polymer solution thermodynamics can be applied only under important restrictions. Typically, the use of complex mixtures of solutes and solvents, relatively high concentrations of solutes, and variable working temperatures do often not agree with the application of thermodynamic models. In particular, although thermodynamic aspects of coacervation have extensively been discussed in polymer sciences,[23,32,102] the practical implication for microsphere formation and drug microencapsulation has only been scarcely considered.[25,75,76] For the coacervation of PLA/PLGA, a nonequilibrium phase separation process was recently postulated.[15] It was assumed that sufficiently high precipitation rates would lead to a fast shrinking of polymer coils, so macromolecular chains could not reach an equilibrium between the two phases. Consequently, a nonequilibrium polymer distribution would occur during the coacervation process and be frozen in the hardening step of the coacervate droplets. This theory was supported by results from M_w distribution of PLA coacervates which remained practically identical with that of the native polymer.[77]

Substantial improvements in PLA/PLGA microencapsulation were claimed when solubility parameters of the polymers[78,79] or the solvents[80,81] were taken into account. Very recently, water-miscible solvents were selected for PLGA precipitation by the aid of polymer−solvent interaction parameters.[82]

These studies showed that thermodynamic concepts can be applied to describe phase-separation processes. However, such concepts become more complicated, if the drug to be encapsulated must also be taken into account. Further, the unique physicochemical and stability properties of peptide and proteins and especially their interactions with polymer solvents and coacervating and hardening agents will remain a critical parameter to be clarified in all coacervation processes.

Thermodynamic Models

We consider that knowledge of polymer solution behavior is necessary to improve our understanding of microsphere formation and drug microencapsulation by polymer phase separation. The following brief overview summarizes the Flory−Huggins polymer solution theory, the solubility parameter concept, and more recently developed thermodynamic models possibly applicable to PLA/PLGA coacervation.

The Flory Interaction Parameter in Phase Separation Induced by Nonsolvent Addition—In 1944, Flory proposed a lattice model to describe polymer solutions and introduced the interaction parameter χ.[20] This parameter increases as solvent power decreases. Hence, a thermodynamically good solvent is characterized by a low interaction parameter. In systems used for microsphere formation, most polymer−solvent mixtures show χ-values ranging from 0.2 to 0.6.[25] Moreover, the theory predicts that a polymer will dissolve in a solvent only if the interaction parameter is less than a critical value χ_c. At a given temperature, χ_c depends on the degree of polymerization (x) of the dissolved polymer.[83]

$$\chi_c = 0.5\left(1 + \frac{1}{\sqrt{x}}\right)^2 \approx 0.5 + \frac{1}{\sqrt{x}} \qquad (1)$$

For polymers of very high molecular weight, χ_c approaches the value of 0.5. Upon gradual addition of a nonsolvent (with a large χ value), phase separation occurs in the order of decreasing x, i.e., when $\chi > \chi_c$. In coacervation induced by nonsolvent addition, complete separation of the polymer material from the continuous liquid is important for high yield and to avoid polymer fractionation. The latter phenomenon was found to occur in a polystyrene−THF mixture upon temperature-induced coacervation.[84] In this light, polymers with low polydispersity are preferable. In recent thermodynamic studies, the phase behavior of amorphous poly(D,L-lactide) and semicrystalline poly(L-lactide) in several solvent−nonsolvent systems were analyzed.[100,103] Applying the Flory−Huggins theory for ternary solutions, qualitative agreement between the theoretical predictions and experimentally obtained liquid−liquid miscibility gaps was found. However, the importance of these observations for drug engulfment by polymer phase separation was not outlined.

Hildebrand and Hansen Interaction and Cohesion Parameters To Describe Phase Separation Induced by Nonsolvent Addition—While the Flory−Huggins model represents probably the most complete approach to describe the thermodynamics of dilute polymer solutions and phase equilibria,[23] its applicability to concentrated solutions and multicomponent mixtures remains limited. Typically, χ is not a constant but depends on polymer concentration and molecular weight as well as on temperature. Moreover, χ cannot be determined readily when a larger number of polymer−liquid mixtures are to be investigated. The model characterizes a polymer−liquid pair and is, therefore, inconvenient for multicomponent systems. Finally, χ is a composite term influenced by hydrogen bonding. As a consequence, the solubility parameter, as defined by the square root of the cohesive energy density, is frequently used to describe polymer−solvent interactions in actual pharmaceutical systems.[85] Originally, Hildebrand and Scott introduced this parameter in 1949 for systems characterized exclusively by dispersive forces (δ).[86] In 1967, Hansen extended this concept and defined three partial solubility parameters for dispersive (δ_d), polar (δ_p), and hydrogen-bonding (δ_h) contributions.[28] An equation that has sometimes been used to estimate the solubility range of a polymer (subscript 2) in solvents (subscript 1) is

$$\Delta\delta = [4(\delta_{d1} - \delta_{d2})^2 + (\delta_{p1} - \delta_{p2})^2 + (\delta_{h1} - \delta_{h2})^2]^{0.5} \qquad (2)$$

It has been postulated that $\Delta\delta$ should not exceed 5 $MPa^{0.5}$ for good solubility.[87] This rule of thumb may be applied to solutions with constant polymer concentration. However, the stepwise addition of a coacervating agent, followed by solvent partitioning between coacervate phase and continuous liquid, changes the composition of a coacervation dispersion continuously. Under the assumptions that the continuous phase consists exclusively of solvent and coacervating agent and the coacervate phase of solvent and polymer 1, the solubility parameters of both phases may be calculated by

$$\delta_{\text{continuous phase}} = \phi_{1'}\delta_{\text{solvent}} + \phi_3\delta_{\text{coacervating agent}} \quad (3)$$

$$\delta_{\text{coacervate phase}} = \phi_1\delta_{\text{solvent}} + \phi_2\delta_{\text{polymer 1}} \quad (4)$$

where $\phi_{1'}$, ϕ_1, ϕ_2, and ϕ_3 are the volume fractions of solvent in the continuous phase, of solvent in the coacervate phase, of polymer, and of coacervating agent, respectively. For calculation of $\phi_{1'}$, ϕ_1, ϕ_2, and ϕ_3 see the Appendix. Although eqs 3 and 4 are empirical mixing equations with limited validity, they are frequently used in pharmaceutical technology to improve solvent mixtures for coating, film formation, and microencapsulation. The mean solubility parameter of the coacervation mixture can be calculated only for a one-phase system, i.e., as long as the coacervating agent is soluble in the polymer solution. As shown later, this is valid for systems where phase separation is induced by nonsolvents rather than by polymer 1−polymer 2 incompatibility.

In the original Flory−Huggins theory, χ was a pure enthalpy term. In reality, χ is best treated as a Gibbs free energy, i.e., a measure of both enthalpic, χ_H , and excess entropic, χ_S, interactions. In this context, χ should be written as

$$\chi = \chi_H + \chi_S \quad (5)$$

Generally, the common value for the entropic contribution χ_S for nonpolar systems is approximately 0.34,[85] but lower and higher values are possible. For calculating χ_S, a modification of the original method of Bristow and Watson[88] has been suggested recently.[82]

$$\left(\frac{\delta_1^2}{RT} - \frac{\chi}{V_1}\right) = \left(2\frac{\delta_2}{RT}\right)\delta_1 - \frac{\delta_2^2}{RT} - \frac{\chi_S}{V_1} \quad (6)$$

By plotting the left-hand side of eq 6 versus the solubility parameter of the solvent (δ_1), the y-intercept is equal to $(-\delta_2^2/RT) - (\chi_S/V_1)$. From the intercept and δ_2 and V_1, the parameter χ_S can be calculated. If χ_S is greater than 0.5, χ_H must be negative for full miscibility. For the latter case, χ_H is related to δ by

$$\chi_H = \frac{V_1}{RT}(\delta_2 - \delta_1)^2 + 2(1-l_{ij})\delta_1\delta_2 \quad (7)$$

where l_{ij} is a dimensionless empirical geometric mean deviation parameter.[89] However, in the absence of polar or specific interactions, eq 7 reduces to:

$$\chi \approx \frac{V_1}{RT}(\delta_2 - \delta_1)^2 \quad (8)$$

Provided the Hildebrand solubility parameters are known for both the solvent and the polymer, χ_H and χ_S can be approximated. This may help to select candidates for good polymer solvents (small polymer−solvent interaction parameter) and for polymer nonsolvents (large polymer−solvent interaction parameter). As solubility parameters of polymers cannot be determined directly from vaporization energy, various authors proposed calculation methods based on the assumed additive contribution of different functional groups to the cohesive properties.[90−94] Moreover, polymer solubility experiments in a variety of solvents of known solubility parameters may be useful to define the borderlines of good solvents and nonsolvents for polymer phase separation.

The Flory Interaction Parameter χ To Describe Phase Separation Induced by Polymer 1−Polymer 2 Incompatibility—Theoretical treatment of polymer phase separation based on polymer 1−polymer 2 incompatibility requires an extension of the χ-parameter concepts to two polymers. For simplicity, we consider first a mixture of two different polymers in an assumed equilibrium without solvent. Then, the critical polymer 1−polymer 2 interaction parameter $(\chi_c)_{2a,2b}$ can be expressed by

$$(\chi_c)_{2a,2b} = 0.5\left(\frac{1}{\sqrt{x_{2a}}} + \frac{1}{\sqrt{x_{2b}}}\right)^2 \quad (9)$$

where x_{2a} and x_{2b} are the degrees of polymerization of the two polymers considered. In comparison to the critical polymer−solvent interaction parameter (eq 1), the critical polymer 1−polymer 2 interaction parameter is generally several orders of magnitude smaller. Consequently, in most cases, polymers will be immiscible. In the more realistic situation of a ternary system consisting of two polymers and a solvent—as encountered in polymer coacervation—the model presented by Scott[21] defines the critical conditions for phase separation, if $|\chi_{1,2a} - \chi_{1,2b} \ll 1$ and if $\sqrt{x_{2a}} < \sqrt{x_{2b}} < x_{2a}^2$, which is usually the case. Under these conditions, eq 9 becomes

$$(\chi_c)_{2a,2b} = 0.5\left(\frac{1}{\sqrt{x_{2a}}} + \frac{1}{\sqrt{x_{2b}}}\right)^2\left(\frac{1}{1-\phi_1}\right) \quad (10)$$

If the polymer 1−polymer 2 interaction parameter is estimated from the solubility parameters (eq 8), the critical volume fractions of the solvent (ϕ_1) and of both polymers (ϕ_{2a}, ϕ_{2b}) can be calculated:

$$\frac{\phi_{2a}}{1-\phi_1} = \frac{\sqrt{x_{2a}}}{(\sqrt{x_{2a}} + \sqrt{x_{2b}})} \quad (11)$$

$$\frac{\phi_{2b}}{1-\phi_1} = \frac{\sqrt{x_{2b}}}{(\sqrt{x_{2a}} + \sqrt{x_{2b}})} \quad (12)$$

Unless the solubility parameters of both polymers are similar, the polymers are likely to be incompatible, leading to polymer phase separation, because $\chi_{2a,2b}$ exceeds the critical value $(\chi_c)_{2a,2b}$, even if the solvent has a high dissolving capacity for both polymers. The predominant parameters for polymer incompatibility have been outlined some time ago.[19] From more recent experiments using high molecular weight polymers dissolved in various solvents, polymer 1−polymer 2 incompatibility was found to be the rule and polymer 1−polymer 2 miscibility the exception,[95] depending on the polymer concentration and asymmetry of the $\chi_{1,2a}$ and $\chi_{1,2b}$ values.[104]

New Approaches To Characterize Polymer Coacervation—Recently, Drago[96] and Hô[97] have proposed new interaction models using, in addition to δ_d and δ_p, the two Drago parameters E and C, accounting for electrostatic and covalent Lewis acid−base interactions, respectively. For the time being, the four parameters δ_d, δ_p, E, and C are considered as the best descriptors for evaluating interaction energies between different components having acid−base characteristics such as the polyesters studied here.[97] Unfortunately, unlike Hansen's δ_h parameter, E and C values are not yet available for a large number of solvents. For this reason, calculations of the interaction energies between components with unknown parameters are often based on values obtained from materials with comparable molecular structure and properties.[29] According to the aforementioned model and adapted for the present systems, the interaction energy ($\Delta_{int}E$) between two components (1 and 2) can be calculated as follows:

$$\Delta_{int}E = -nV_1\delta_{d1}\delta_{d2} - nV_1\delta_{p1}\delta_{p2} - (E_1E_2 + C_1C_2) \quad (13)$$

If the components 1 and 2 are completely miscible, $n = 2$, whereas for two-phase dispersions $n = 1$. Alternatively, an extension of the original model of Hô[97] has recently been reported.[29] With the use of dissolution and melting enthalpy terms, of the vaporization energy term and of the cavity term, it was possible to estimate the interaction energy between polymer and solvent. Although this approach was studied in a spray-drying process, the basic information gained should also be applicable to other techniques such as coacervation.

In a coacervation process induced by the addition of a coacervating agent, three types of interactions may play a role, i.e., polymer−solvent ($\Delta_{int}E_1$), solvent−coacervating agent ($\Delta_{int}E_2$), and polymer−coacervating agent ($\Delta_{int}E_3$), as illustrated in Figure 1. We can reasonably assume that $\Delta_{int}E_1$ and $\Delta_{int}E_2$ respresent the controlling forces of phase separation. Thus, for phase separation to occur, we postulate that $\Delta_{int}E_2$ must be greater than $\Delta_{int}E_1$. Depending on the mutual importance of $\Delta_{int}E_1$, $\Delta_{int}E_2$, and $\Delta_{int}E_3$, polymer desolvation attains different stages, which may be defined by the following expressions:

$$\Delta_{int}E_1 \gg \Delta_{int}E_2 + \Delta_{int}E_3 \quad (14)$$

$$\Delta_{int}E_1 < \Delta_{int}E_2 + \Delta_{int}E_3 \quad (15)$$

$$\Delta_{int}E_1 \ll \Delta_{int}E_2 + \Delta_{int}E_3 \quad (16)$$

Equation 14 reflects a two-phase system with only weak polymer desolvation, which does not lead to a viscous coacervate phase. Equation 15 describes moderate polymer desolvation resulting in a highly viscous though still fluid coacervate phase, suitable for microsphere formation, and eq 16 defines the situation of a strong polymer desolvation and precipitation of solid polymeric material. The first two processes are also described as liquid−liquid demixing, and the final process as liquid−solid demixing.[103]

From a practical point of view, an interesting approach was made recently by Van Oss, who defined the conditions for simple and complex coacervation by the interfacial interactions between similar and dissimilar molecules.[35,98] In this model, the solubility, s, of a polymer (2) in a solvent (1) depends on the free energy of interfacial interaction (ΔG_{212}) of the polymer molecules immersed in the solvent (1):

$$RT \ln s = f(\Delta G_{212}) \quad (17)$$

Clearly, the solubility increases with increasing positive values of ΔG_{212}. In other words, the more the polymer molecules repel each other when immersed in a liquid, the more they will tend to disperse in that liquid, i.e., the more soluble they will be. With increasing polymer concentration, repulsion between the macromolecules becomes stronger. If ΔG_{212} is negative, the solubility decreases due to molecular attraction. In terms of interactions, ΔG_{212} can be composed either entirely of apolar Lifshitz−van der Waals (LW) forces or of a combination of LW and Lewis acid−base forces. Hence, the free energy of interfacial interaction, ΔG^{IF}, is composed of an apolar contribution, ΔG^{LW}, and an acid−base contribution, ΔG^{AB}. In general terms, the free energy of interfacial interaction of two molecules of the same species 2 immersed in solvent 1 can then be represented by the expression

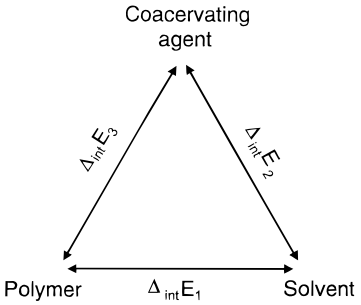

Figure 1—Interactions between the components present in a coacervation mixture.

$$\Delta G_{212}^{IF} = -2(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_1^{LW}})^2 - 4(\sqrt{\gamma_2^+\gamma_2^-} + \sqrt{\gamma_1^+\gamma_1^-} - \sqrt{\gamma_2^+\gamma_1^-} - \sqrt{\gamma_2^-\gamma_1^+}) \quad (18)$$

where the second part of the right-hand side describes the Lewis acid−base interactions. Considering the interfacial interactions in a ternary system between two unlike molecules of materials 2 and 3 immersed in a solvent 1, Van Oss postulated:

$$\Delta G_{213}^{IF} = (\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}})^2 - (\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_1^{LW}})^2 - (\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}})^2 + 2[\sqrt{\gamma_1^+}(\sqrt{\gamma_2^-} + \sqrt{\gamma_3^-} - \sqrt{\gamma_1^+}) + \sqrt{\gamma_1^-}(\sqrt{\gamma_2^+} + \sqrt{\gamma_3^+} - \sqrt{\gamma_1^+}) - \sqrt{\gamma_2^+\gamma_3^-} - \sqrt{\gamma_2^-\gamma_3^+}] \quad (19)$$

For all exclusive van der Waals interactions, the interfacial interaction energy between materials 2 and 3 immersed in a liquid 1 reduces to

$$\Delta G_{213}^{LW} = \gamma_{23}^{LW} - \gamma_{21}^{LW} - \gamma_{31}^{LW} \quad (20)$$

which can be rewritten as

$$\Delta G_{213}^{LW} = (\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_1^{LW}})(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}) \quad (21)$$

In phase separation induced by polymer 1-polymer 2 incompatibility, unlike polymer molecules repel each other, and consequently the conditions for coacervation are as follows:

$$\Delta G_{213}^{IF} > 0 \quad (22)$$

In apolar systems, this prerequisite is fulfilled only when $\gamma_2^{LW} > \gamma_1^{LW} > \gamma_3^{LW}$ or when $\gamma_2^{LW} < \gamma_1^{LW} < \gamma_3^{LW}$. In polar systems, however, phase separation becomes only predictable by solving eq 19.

Incidentally, contrary to simple coacervation, complex coacervation in a ternary system occurs only if the polymer molecules attract each other, and hence, the interfacial free energy becomes negative.

The apparent advantage of the Van Oss theory lies in the accessibility of the surface tension parameters. For a given solute, γ_2 and γ_3 can be determined from contact angle and Young's equation. Moreover, these parameters can also be derived theoretically from polymer group contributions.[87] On the other side, it remains questionable if the Van Oss model is valid for systems where hydrogen bonding plays an important role, as interfacial tension measurements are unlikely to account for H-bonding forces.

Conclusions

The here-described approaches and models to characterize PLA/PLGA microsphere formation by polymer phase separation may help the formulation scientist to become

aware of and understand better the complexity of this process. Although we emphasized the importance of thermodynamic interactions, we also would like to point out that these interactions represent only one face of the multifaceted technology. If the standard process conditions, as described in the literature, have to be adapted to the requirements of specific materials or production scale/facilities, we recommend the following strategy.

Characterize the microsphere forming polymer (polymer 1) with respect to molecular weight distribution, residual monomers and solvents, glass transition and melting temperatures, solubility and related characteristics (intrinsic viscosity, osmotic pressure, swelling, cloud points, etc.) in a variety of solvents differing in polarity and H-bonding characteristics; consider also the solubility and stability of the drug to be microencapsulated in these solvents

Determine or approximate experimentally or by calculation interaction parameters (Flory, Hildebrand, Hansen) of the most promising materials

Determine the most promising phase separation mechanism, inducible in binary systems by nonsolvent addition or temperature change and in ternary systems by nonsolvent addition or polymer 1−polymer 2 incompatibility; establish phase diagrams to define the liquid−liquid miscibility gaps

Determine interfacial tension and viscosity of the separated phases, as well as the wettability of the drug for encapsulation by the phases

Define optimal hydrodynamic conditions for good dispersibility of the coacervate droplets in the continuous phase.

Finally, from a theoretical point of view, the polymer 1−polymer 2 incompatibility mechanism should be most suitable for replacing toxicologically and environmentally harmful solvents and coacervating agents by more acceptable compounds, as the nature of the solvent in this case is of minor importance.

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Appendix

The volume fractions of solvent in the continuous phase, $\phi_{1'}$, of solvent in the coacervate phase, ϕ_1, of polymer, ϕ_2, and of nonsolvent, ϕ_3, is expressed by

$$\phi_{1'} = \frac{n_{1'}V_1}{n_{1'}V_1 + n_3V_3} \quad \text{and} \quad \phi_3 = 1 - \phi_{1'} \tag{25}$$

$$\phi_1 = \frac{n_1V_1}{n_1V_1 + n_2V_2} \quad \text{and} \quad \phi_2 = 1 - \phi_1 \tag{26}$$

where $n_{1'}$, n_1, n_2, and n_3 are the number of moles of solvent in the continuous phase, of solvent in the coacervate phase, of polymer, and of nonsolvent, respectively; V_1, V_2, and V_3 are the corresponding partial molar volumes (with $V \approx \bar{V}$).

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