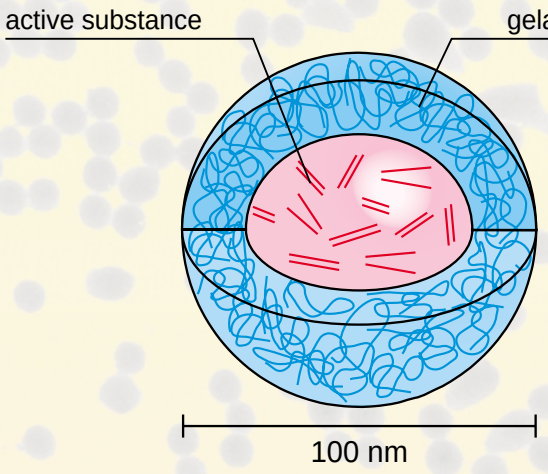
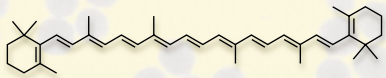
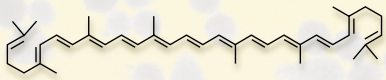
Organic Nanoparticles in Aqueous Phase

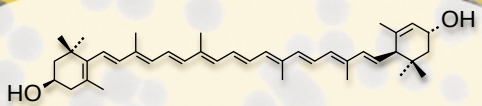




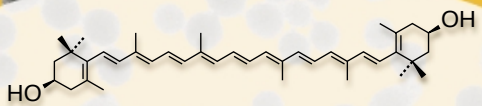
β-carotene $C_{40}H_{56}$



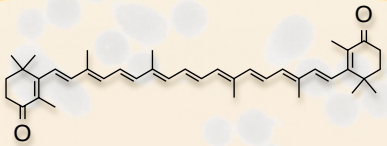
lycopene $C_{40}H_{56}$



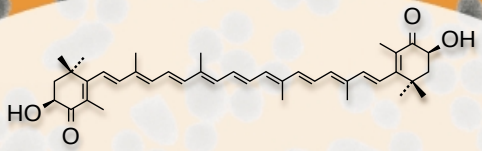
lutein $C_{40}H_{56}O_2$



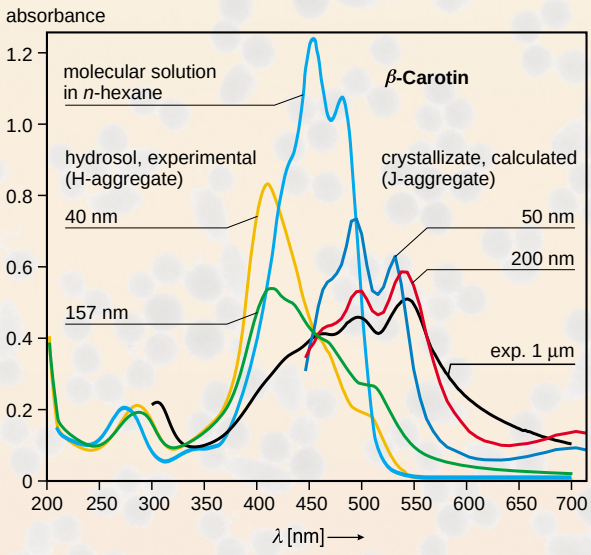
zeaxanthin $C_{40}H_{56}O_2$



canthaxanthin $C_{40}H_{52}O_2$



astaxanthin $C_{40}H_{52}O_4$



Organic Nanoparticles in the Aqueous Phase—Theory, Experiment, and Use

Dieter Horn and Jens Rieger*

Many active organic compounds and organic effect materials are poorly soluble in water, or even insoluble. Aqueous forms of application thus require special formulation techniques to utilize or optimize the physiological (pharmaceuticals, cosmetics, plant protection, nutrition) or technical (varnishes, printing inks, toners) action. The most interesting properties of nanodispersions of active organic compounds and effect materials include the impressive increase in solubility, the improvement in biological resorption, and the modification of optical, electrooptical, and other physical properties which are achievable only with particle sizes in the middle or lower nanometer range (50 – 500 nm). Hence in addition to economic and ecological constraints there are also technical demands which appear to urgently require the development of new processes for the production of organic nanoparticles as alternatives to the established mechanical milling processes. In this context attention is drawn to the recent increase in research activities which have as their objective the continuous, automatic preparation of nanodispersed systems by precipitation from molecular solution. In this review the current state of knowledge of the fundamentals of particle formation from homogeneous solution and the effect of solvent and polymer additives on the morphology and supramolecular structure of the nanoparticle will be discussed. The practical implementation of this new formulation technology will be explored in detail for the carotenoids, a class of compounds of both physiological and technical interest.

Keywords: carotenoids · disperse systems · nanoparticles · nanostructures · phase transformations

1. Introduction

The importance of nanoparticles, that is, particles with dimensions in the range of about 10 nm to a few hundred nanometers is obvious: they determine our life in the form of protein complexes and other cell components, as viruses, colloidal particles in drinking water, surface water and sea water, and as aerosols; they find use as dispersion colors and as adhesives; in industry they play an important role in the formulation of pigments and in the production of catalysts; numerous attempts are being made to deliver nanoparticulate forms of pharmaceutically active compounds specifically to the desired site of the action in the body; finally nanoparticles find use as quantum dots with special properties for electronic components. Beyond these practical aspects there is scientific interest in nanoparticles owing to their special properties which lie between the properties of molecules and those of bulk material.

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In a thorough study of the scientific literature on the topic of colloidal systems it became evident that much has been written on inorganic nanoparticles, polymer dispersions, and on the principles of particle formation in general. In contrast, surprisingly little is learnt of the mechanisms of particle formation of organic systems. Moreover, there is a clear gap between that which is reported in many textbooks on particle formation and the current state of knowledge. This unsatisfactory situation was the motivation for this contribution which on the one hand deals with the preparation and properties of organic nanoparticles, and with modern aspects of particle formation on the other.

Organic nanoparticles take on many forms (Figure 1). Here we will limit ourselves to the consideration of pharmaceutically active organic compounds and organic effect materials as they occur, for example in pharmaceuticals applications and in the form of vitamins and pigments. Many of these materials are poorly soluble in water, or even insoluble. Aqueous forms of application thus require special formulation techniques to optimize the physiological (pharmaceuticals, cosmetics, plant protection, nutrition) or technical (varnishes, printing inks, toners) action. An important target in this context is the conversion of the generally coarse crystalline synthesis product into the finest particulate dispersion possible, with

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man-made nanoparticles	active substances (pharmaceuticals)	pigments	polymer dispersions	micellar systems	supermolecules and dendrimers	protein aggregates (as nuclei for crystals)
natural nanoparticles	cell components	viruses	environmental colloids (on surfaces, in water, in air)			

Figure 1. Classification of organic nanoparticles.

particle sizes in the range of 10 to 500 nm. In principle two strategies are conceivable for this purpose: 1) the mechanical milling of the raw material by wet or dry milling processes; 2) the conversion of the products or educts dissolved in suitable solvents into nanodispersed systems by precipitation, condensation, or by specific synthesis procedures (Figure 2). In the second case the undesirable solvent must often then be removed. The differentiation between precipitation and condensation processes makes it clear that in the actual precipitation process further additives such as surfactants and polymers assume the role of surface-active colloidal stabilizers; in the condensation process these additives themselves form the nanoparticulate phase (pseudolatexes) which contains the active compound or effect material bound by adsorption or absorption. The synthetic preparation of polymer dispersions[1] as a special class of organic nanoparticles of considerable economic significance will not be discussed here. Other procedures which have also found

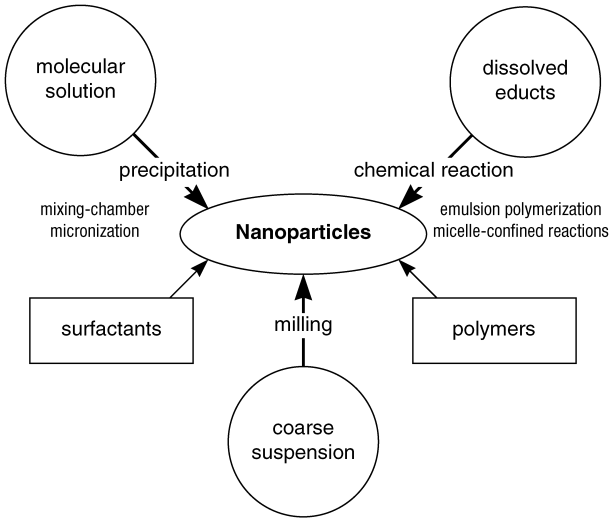

Figure 2. Methods for the preparation of nanoparticles.

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J. Rieger

Dieter Horn, born 1936 in Höchst/Odenwald, studied chemistry at the Technical University Darmstadt and the University of Heidelberg, where he gained his doctorate in physical chemistry in 1967 working with Prof. Klaus Schäfer. He then moved to the University of California, Berkeley, where he worked in the physical chemistry group of Prof. George C. Pimentel in the Department of Chemistry. As a member of the NASA Mariner 6 and 7 project team he was responsible for the development and application of analytical methods for the quantitative interpretation of infrared spectroscopic data of the atmosphere and surface of the planet Mars transmitted by the space probes. He joined the main laboratory of BASF AG in autumn 1970 where he first worked on the physics of organic pigments. A colloidal and biophysical work group was built up under his leadership, with research priorities in the preparation of organic nanoparticles and the development of laser optic methods for the characterization of dispersed systems. In 1987 he was appointed head of the polymer physics/solid-state physics research department in the Plastics Laboratory, with research interests in the physics and the physical chemistry of polymeric structural and functional materials, polymeric effect materials, and dispersed active compounds. For his fundamental contributions to applied colloid science he has been awarded the Steinkopff Prize of the Kolloid-Gesellschaft and the Bonhoeffer-Eucken-Scheibe lectureship of the Deutsche Bunsen Gesellschaft für Physikalische Chemie.

Jens Rieger, born 1958, gained his doctorate at the University of the Saarland working with Prof. Arno Holz in theoretical physics on random walks. In 1989 he joined the department for polymer physics/solid-state physics in BASF AG in which, now as senior scientist in charge of the area "Structure Formation", he is working on the following topics: deduction of the structure-property relationships in polymeric, colloidal, and hybrid systems, development of methods for the time-resolved tracking of structure formation processes in complex systems on all length scales, control of crystallization and particle formation processes by polymers. In addition he is exploring the potential of high-performance radiation sources for polymer and colloidal physics (small-angle neutron scattering, X-ray microscopy, SAXS, and WAXS at the synchroton).

extensive use in the preparation of inorganic nanoparticles,[2] such as sol–gel processes,[2, 3] synthesis in microemulsion templates,[2, 4] and aerosol procedures[5] have hitherto played no part in the preparation of organic nanoparticles from the standpoint discussed here. Any discussion of nanoparticles by aggregation of block polymers[6] or by the targeted synthesis of extended molecules such as dendrimers[7] and other branched systems[8] will also be omitted.

To discuss the mechanisms of particle formation it is useful to describe the current state of knowledge—initially independently of the nature of the system formed. Figure 3 gives an initial view of the complexity of particle formation and highlights a number of the outstanding questions. As will become clear, five essential points need to be emphasized within this context.

1. There is a far deeper understanding of the formation of inorganic particles than of organic particles.
2. Interesting advances in protein crystallization have been achieved recently which also affect the classical area of nucleation theory.
3. There is still a considerable need for research in the area of nucleation theory, for example, where the interaction of several components during particle formation is concerned, whether it be the simultaneous precipitation of two materials or the control of particle morphology by the use of additives.
4. Certain particle sizes are necessary to achieve certain effects, these can be obtained either through the "physical chemistry" of the particle-forming system or by the use of additives (protective colloids). For an efficient procedure in both cases the structure-formation processes must be understood on all length scales.
5. Little is known about the molecular processes which take place during the mixing of two starting solutions for producing the state of supersaturation which initiates particle formation.

The preparation of colloidal systems has occupied scientists for a long time.[9, 10] Numerous monographs and review articles have been devoted to this topic, a few of which are mentioned here by way of example.[2, 3, 11–15] Several points arise from a perusal of the literature on this topic: a strongly phenomenological approach is frequently encountered, that is, attempts are made to deduce a mechanism of formation from the structure of the product, often a purely descriptive treatment is considered satisfactory. If one looks beyond textbook knowledge on "supersaturation, nucleation, growth" a "zoo" of theories and interpretations is encountered which must be taken into account if there is a desire to understand at a basic, that is, molecular, level how a nanoparticulate system is formed. This knowledge is of prime importance as only with a knowledge of the mechanistic aspects of particle formation can the process be manipulated specifically, that is, controlled—whether it be by variation of the process parameters or by the use of suitable additive molecules. This area of research is truly interdisciplinary since chemists, physicists, and engineers, each with their specialist knowledge are in demand. However, within academia this cooperation is nowhere near as wide-spread as would appear appropriate for an optimal treatment of this problem.

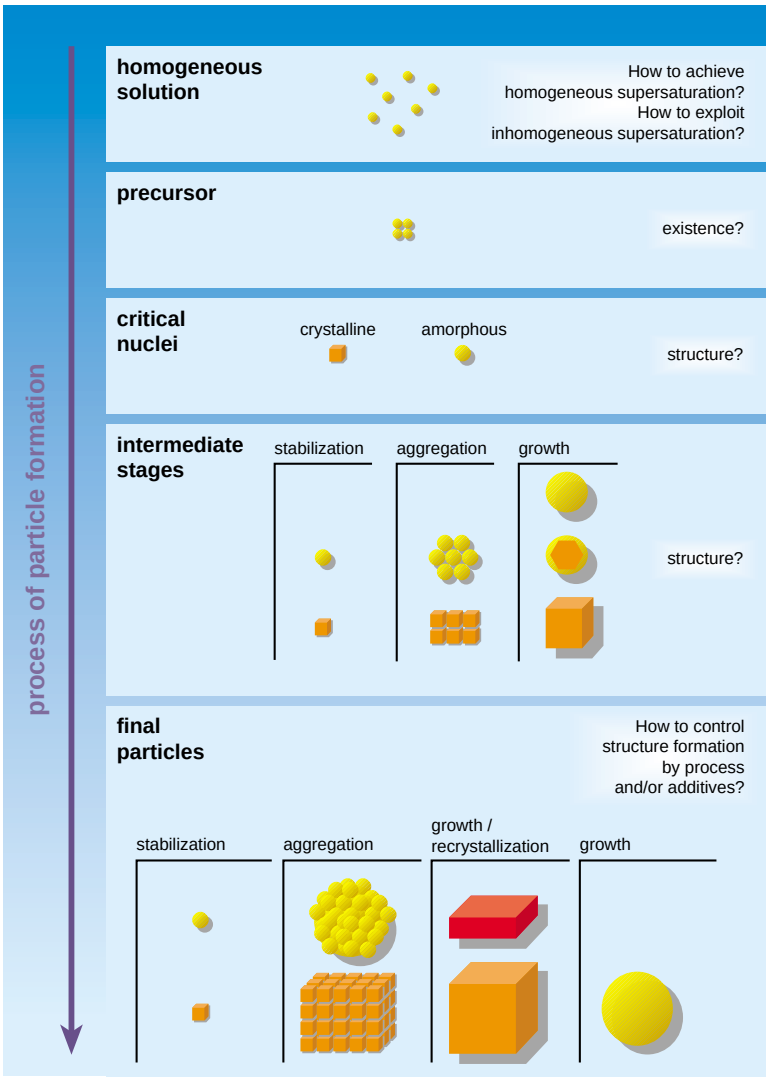

Figure 3. Stages of particle formation and open questions (see text for further explanation).

The article is arranged as follows: in Section 2 the current state of knowledge of the fundamentals of particle formation is discussed, however, the classical theory of nucleation is treated only briefly. Emphasis is placed upon more recent thoughts on mechanisms of particle formation including computer simulations, as well as on the question of to what extent the processes occurring during initiation of particle formation—mainly by the mixing of two educts—are understood? Finally, how the particle formation process can be controlled by the use of (mainly polymeric) additives will be discussed. In Section 3 processes for the preparation of organic nanoparticles are considered. In Section 4 the properties and areas of application of these particles where the nanoparticulate state is a requirement will be introduced. Finally open questions on this topic will again be addressed.

2. Theoretical Approaches

2.1. Classical Nucleation Theory

Textbook knowledge may be summarized in the following way:[11, 14, 16, 17] A multicomponent system exists initially as a single phase. By modification of boundary conditions such as temperature and pressure or by homogenous mixing with a further component the free energy changes in such a way that phase separation is energetically more favorable. The approach taken assumes that particles (atoms, ions, molecules) of the one component coalesce and form nuclei of the separating phase, initially it is immaterial whether it is, for example, the separation of a solid phase in the liquid medium (the subject matter of this article), condensation from the gaseous phase, or bubble formation in a liquid (foaming). The free energy of a spherical nucleus with radius r can be described in respect of the single-phase state to a first approximation as in Equation (1) (the subscripts S and V

$$\Delta G = \Delta G_s + \Delta G_v = 4\pi r^2\gamma + 4/3\pi r^3 \Delta g_v \quad (1)$$

refer to surface and bulk volume, respectively) where γ stands for the surface tension between the two phases and Δg_v for the difference in free energy per unit volume between the two phases. The two terms on the right of the equation have opposite signs so that ΔG as a function of r passes through a maximum (Figure 4). The critical nucleus radius r^* is defined

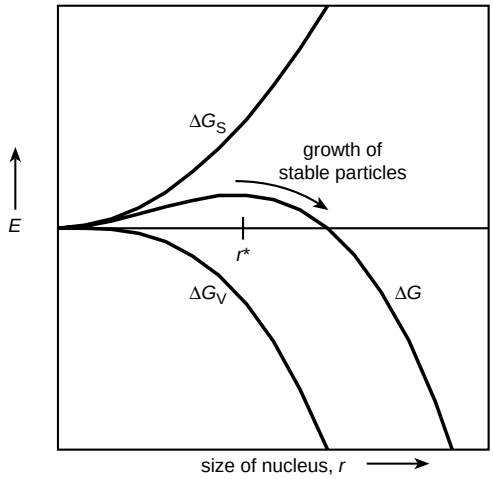

Figure 4. Energy diagram to explane the nucleation process (ΔG: free energy of a particle with radius r, ΔG_s: surface energy, ΔG_v: bulk energy, r^*: radius of the critical nucleus). The particle sizes fluctuate because of statistical processes. Particles with a radius $r < r^*$ redissolve, those with $r > r^*$ grow further.

by the position of the maximum of the free energy and given by Equation (2). Particles with a radius smaller than r^* redissolve, whilst particles which by reason of statistical fluctuations exceed this size are stable and can grow further.

$$r^* = -2\gamma/\Delta g_v \quad (2)$$

Within the scope of a quasi-equilibrium approach (Arrhenius) the rate of nucleation that is, the number of nuclei which form per unit time and volume is described by Equation (3), in which A is determined by the frequency of the molecular

$$J = A\exp(-\Delta G^*/kT) \quad (3)$$

processes and k and T have the usual meanings. The rate of nucleation is thus Equation (4), where in accordance with Equation (5) the supersaturation $S = c(r)/c^*$ is coupled with the particle radius; v is the molecular volume, $c(r)$ denotes the solubility of a particle with radius r, and c^* the equilibrium solubility.

$$J = A\exp(-(16\pi\gamma^3 v^2)/(3k^3T^3[\ln S]^2)) \quad (4)$$

$$kT\ln(S) = 2\sigma v/r \quad (5)$$

In classical colloid chemistry a further model concept is used especially to explain monodispersity in certain systems (Figure 5):[18] the concentration of a dissolved substance continues to rise, for example, by release in a reaction until the critical nucleation concentration is reached. At this point a shower of nuclei are formed which begin to grow. In this way the concentration falls momentarily below the critical threshold so that no new nuclei can form. The nuclei already formed grow until the concentration of the still-dissolved material has fallen to the equilibrium concentration.

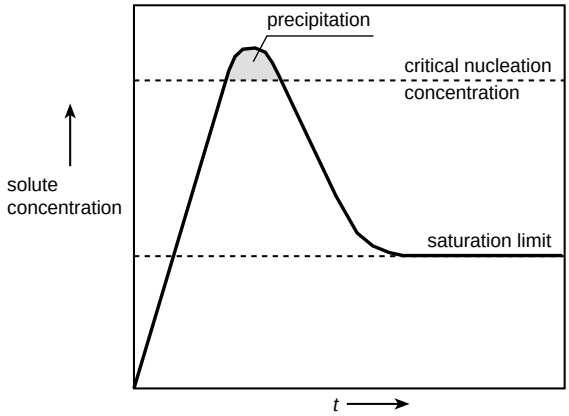

Figure 5. Schematic representation of the concentration relationships in controlled particle formation according to the model representation of LaMer, ref. [18] (for full explanation see text).

2.2. More Recent Knowledge

In the following, new ideas on particle formation from supersaturated systems will be presented in chronological order from the stage of the homogeneous starting state to that of the colloidal particle. An assessment has been intentionally omitted as there is rapid development in this area. For reasons of space complete citation has to be omitted; a comprehensive review of this topic is in preparation.

2.2.1. Precursors

A fundamental assumption of classical nucleation theory is that prior to production of the supersaturation that initiates

particle formation the system can be treated as homogeneous, that is, it is assumed that the components are present in a molecularly dispersed state. Numerous experiments suggest, however, the existence of precursors, that is, species which form from the reacting components, the size of which is still below that of a critical nucleus. This is not meant to suggest transient clusters arising from fluctuations in nucleation, but long-living species which are not considered as part of classical nucleation theory.[11, 19, 20]

2.2.2. The Structure of Clusters and Nuclei

In classical nucleation theory it is assumed that the critical nuclei are spherical and that the structure of the nuclei and their surface can be described by that of the corresponding macroscopic phase, that is, that γ and Δg are defined by the corresponding values of the bulk phase. In many cases this assumption in respect of Δg_v does not apply, most clearly when the nuclei comprise only a small number of molecules, as has been observed with, for example proteins.[21] In turn this point has consequences for the assumptions which are made for the surface tension. Many theories have been proposed for the structure of particle surfaces to take into account that, for example, a density gradient can exist in the transition from particle interiors to the dispersed medium.[22] Moreover, that γ is dependent upon the curvature of the particle surface is not considered in classical nucleation theory. This deficiency was recognized early on and has been redressed in a number of theories.[23, 24] Questions of bulk structure are discussed in detail in Section 2.3. In a few cases the assumption of sphericity also does not apply.[25, 26]

In most theories on particle formation it is assumed that the clusters and nuclei do not interact and that the corresponding rate equations describe particle formation by the incorporation or loss of individual basic elements (atoms, ions, monomers, molecules). There are indications, however that subcritical clusters contribute to nucleation by incorporation and that cluster coagulation plays a role in particle formation.[27]

2.2.3. Spinodal Versus Binodal Decomposition

In the description of the particle formation processes classical nucleation theory is applied almost without exception in the sense that in the phase diagram the supersaturated system exists in the binodal, that is, metastable region (Figure 6). However, with sufficiently high supersaturation the borderline to spinodal decomposition is crossed, and phase separation occurs spontaneously, that is, *without* actual nucleation. That a differentiation must be made between binodal and spinodal demixing (particle formation) is well known in polymer physics.[28] In the case of low molecular systems it has indeed been maintained that: "Spinodal decomposition has never been observed in solutions made up of a solid solute and a liquid solvent because of the large width of the metastable zone that must be crossed without nucleation being induced".[29] It has to be assumed, however, that this citation is attributed to the fact that spinodal demixing in low molecular weight systems is very difficult to detect because of the short time scales involved; the structures formed initially convert rapidly into more compact structures (particles) because of the large surface energy.[28, 30] Investigations in which a particle-forming system was quenched and investigated immediately after establishment of supersaturation supports the assumption that initially during the precipitation of low molecular substances and correspondingly high supersaturation spinodal demulsifying with subsequent conversion into particles also takes place.[31] By simulation of the Brownian movement of particles with suitable interaction potentials it has been shown that dependent upon the level of supersaturation structure-formation mechanisms which are in agreement with the concept of binodal and spinodal demulsifying do occur.[32] The sharp differentiation between binodal and spinodal demulsifying is thus an artifact of highly simplified theories; in most real systems the transition between the two demulsifying mechanisms is gradual.[17, 28]

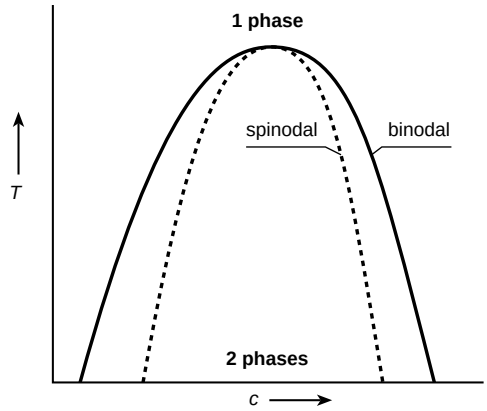

Figure 6. Phase diagram of a two component system with miscibility gap. The region between the binodal and spinodal corresponds to the metastable region in which, according to the classical theories, particle formation takes place through nucleation and growth, whereas the system within the spinodal demixes spontaneously.

2.2.4. Particle Formation in Multicomponent Systems

There is a whole series of more recent theoretical approaches on the formation of multicomponent particles from supersaturated systems. There are, for example discussions on such fundamental questions as to how the surface energy is to be described, how heterogeneous nucleation is influenced, and which pathway the system takes within the realm of the two parameters "number of particles of type A and type B which are contained within the respective critical nucleus".[33] Simulations on the structure of two-component clusters as a function of the interaction parameters are discussed in Section 2.3. In respect to the questions of cardinal interest here, "how surfactants or polymers affect particle formation behavior in the aqueous phase?" the work described is of limited use, however. The points within this context that deserve special attention will be treated in detail in later sections.

2.2.5. What Does Protein Crystallization Teach Us?

In recent years the classical area of particle formation theories has received new stimuli from the area of protein crystallization.[34] With light-scattering experiments it was found that the ability of a whole series of proteins to crystallize (instead of to precipitate amorphously or to gel) correlates with the second virial coefficient.[35, 36] This coefficient is correlated with the interaction potential between individual molecules in solution. It can be shown that the crystallization behavior of the proteins is approximately explained by a simple interaction potential and a study of the resulting complex phase diagrams (see also the discussion in Section 2.3).[34, 36–38]

2.2.6. The Collapse of Polymer Molecules

Owing to the importance for an understanding of the formation of pseudolatex particles, which will be discussed later in the experimental section (Section 3) the collapse transition of individual polymer molecules by changes on solvent quality (by mixing with an antisolvent or by change in temperature) is of interest. The phenomenon is itself well known[39] and—as with low molecular weight systems—may be discussed on the basis of the phase diagram shown in Figure 6,[40] in which, in the case of solvent mixtures, the temperature is replaced by a corresponding quantity which describes the interaction of the polymers with the solvent. That all aspects of the collapse transition are in no way fully known is demonstrated by work carried out in recent years on the dynamics of the collapse transition, the interaction between individual chain collapse and macroscopic phase separation, and conformation statistics at the collapse transition.[41] The relationship between this fundamental work and end-use related experiments on particle formation by the precipitation of polymers[42] is currently not discernible.

2.3. Computer Simulation of the Early Stages of Particle Formation

With the aid of computer simulation it has meanwhile become possible to obtain an insight into structure formation during the early stages—even if initially only with the most idealized systems. For fundamental investigations of the behavior of many-body systems the so-called Lennard–Jones α-β potential (LJ α-β) for the description of the interaction of two particles is widely used [Eq. (6)]. With the help

$$V(r) = -a/r^{\alpha} + b/r^{\beta} \quad (6)$$

of this potential (with $\alpha = 6$ and $\beta = 12$) the homogeneous nucleation of particles from the liquid phase has been studied.[44, 45] With use of the LJ 6-12 potential at moderate cooling, that is, low supersaturation, it has been possible to establish how in the pre-critical phase aggregates with liquid-like structure are formed initially which on exceeding the critical size are present mainly in face-centered cubic (fcc) form. A more precise investigation of the critical nuclei showed that the core exhibits an fcc structure, whereas the shell is liquidlike. In a layer between these two structures a body-centered cubic (bcc) structure is realized. Simulations of this type give an insight into the causes for the so-called Ostwald rule which states that the phase which forms by nucleation is not necessarily that with the thermodynamically most stable (crystal) structure but that which in respect to its free energy is closest to the melt. A series of approaches to explain this phenomenon have been published without as yet any consensus being reached.[12, 46]

In investigations of the LJ 3-6 system with which the phase behavior of protein molecules may be approximately described ten Wolde and Frenkel observed—in addition to phenomena which correspond to the classical ideas of crystalline nucleation—how under modified boundary conditions amorphous nuclei are also formed in which crystalline cores form on further growth.[45, 47] This pathway to particle formation is associated with a lower nucleation barrier. The authors speculate that this result may be put to use to grow better protein crystals for structure determination.

More recently the first attempts to describe the structure of *multicomponent* nuclei have been made. A complete morphology diagram for the structure of two-component clusters as a function of the respective interaction parameters has been determined for LJ 6-12 particles (Figure 7).[48]

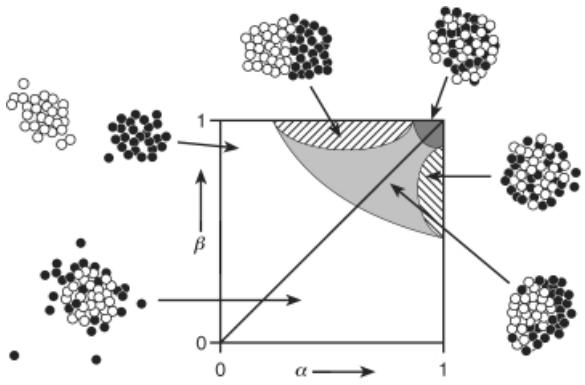

Figure 7. "Morphology diagram" of two-component clusters where α represents the strength of the interaction between the two ("black" and "white") particles and β the strength of cohesion between the "white" particles in comparison to the corresponding value for the "black" particles (according to ref. [48], Copyright©, American Institute of Physics, 1994).

2.4. Particle Growth

2.4.1. Crystallization

The investigation of the growth of crystals is a well established area of research with a countless amount of published work. For this reason reference will be made at this juncture to only two still current books, refs. [11, 49] Beyond this, however, a few points are worth special attention.

1. In the interim it has become possible to follow the growth mechanisms in situ by means of space and time resolved AFM (atomic force microscopy) at moderate supersaturation.[50, 51] In this way a number of model concepts of crystal growth are confirmed, while at the same time new aspects

also come to light, for example, discontinuous growth arising from non-equilibrium effects.[51]

2. During the preparation of oxide films from the aqueous phase it was observed that a film is formed by deposition of crystallites with dimensions in the range of a few nanometers.[52] This gives rise to the question of whether crystal growth at high concentrations takes place according to classical concepts or whether crystals could form by the deposition of preformed precursor crystallites?
3. In the previous Section the Ostwald rule was discussed. For the situation where the transition from one structure to another energetically more favorable takes place by solid-phase transformation the interesting question arises of how this transition may be controlled or suppressed? In the area of biomineralization impressive examples are known of how living organisms regulate these transitions with the aid of proteins, although the details of how this is achieved are not known.[53]

2.4.2 Aggregation

Aggregation phenomena play a critical role in the preparation of many colloidal systems so that for application in general explanations are needed for the conditions under which colloids are stable, or how colloidal particles themselves can be aggregated or flocculated in a targeted manner. Less frequently discussed is the question as to how far the colloidal particles are formed by the aggregation of precursors. There is extensive literature on the "classical" aggregation of colloidal particles, of which just a few, more recent references are cited here.[3, 16, 54, 55] In most of this work it is assumed that the kinetics of formation of aggregate populations are determined by the interaction potential between the particles, the particle size, and the flow conditions within the system. It is intuitively clear that the latter point is complex.[54, 56] However, there are also still unresolved questions of a fundamental nature regarding the interaction between colloidal particles—in contrast to the impression occasionally given in introductory contributions on the stability of colloidal systems. The question as to how far classical theories, for example the frequently used Derjaguin – Landau – Verwey – Overbeek (DLVO) theory, correctly describe interaction effects is under intense discussion.[57]

A number of systems are known in which primary particles with dimension of a few nanometers aggregate to colloidal particles, which are on the one hand compact and astonishingly monodispersed, and on the other exhibit a puzzling anisometry in some cases.[58] Different theoretical approaches have been suggested concerning the mechanisms responsible for monodispersity.[59] The special case of the aggregation step in the formation of monodispersed silica particles in the Stöber process has been discussed in theory in a series of publications.[60]

2.5. Control with Additives

Additives in the form of ions, low molecular weight molecules such as surfactants, and neutral or charged polymers (polyelectrolytes) can dramatically affect the particle formation process, desirably or undesirably. The mechanisms are differentiated as follows: nucleation by individual molecules, heterogeneous nucleation on particles or aggregates present, colloidal stabilization of structures of a mere intermediate nature in the pure particle formation process, and the influencing of crystal growth. In the ideal situation a knowledge of the basic structure – activity relationships can be used for targeted control of the particle formation process. The points described will be discussed individually below.

2.5.1. Nucleation

To what extent individual molecules and especially dissolved polymers can function as nuclei in particle formation is controversial.[61] The relationships in the case of heterogeneous nucleation, that is, nucleation on defined substrates with dimensions >1 nm are more simple.[11, 62] However, the demarcation from the former case is not fixed, for example, during the transition from the dissolved polymer to a particle of collapsed polymer chains as substrate for heterogeneous nucleation.

2.5.2. Colloidal Stabilization by Additives

The investigation of the stability of colloidal particles by adsorbed polymers/surfactants is an extensive and well-established area of research.[63] More recent work which has contributed new facets to previous knowledge will be mentioned here, where primarily electrostatic stabilization by adsorbed polyelectrolytes will be discussed.

The phenomenon of adsorption of polyelectrolytes onto oppositely charged surfaces is still the subject of detailed theoretical investigations even after years of extensive research. It has been shown, for example, how the charge density of a surface under the corresponding boundary conditions can be reduced or even reversed by oppositely charged polyelectrolytes.[64, 65] Whether or not the corresponding counterions are released into the solution during adsorption is dependent upon the physico-chemical parameters of the system.[66] Which adsorption mechanisms occur in the case of specific attractive interactions between copolymers and heterogeneous surfaces has been investigated.[67] Which conformation states may be expected during the "wrapping up" of spheres by polymers has been explained theoretically.[65, 68]

2.5.3. The Influencing of Crystal Growth

In the presence of foreign molecules crystal growth (rate, habit, polymorphism) can be influenced by two different mechanisms, both intentionally and unintentionally: ions/molecules incorporated into the particle influence the precipitation/crystallization behavior at typical concentrations of 0.1 to 1 mmol L^{-1}; ions/molecules interacting with the crystal growth faces are active at concentrations down to 10^{-7} mol L^{-1}.[69] For organic molecular crystals too, numerous instances are known in which crystal modification and habit may be influenced by additives.[70]

In the fundamental discussion on nucleus formation processes and in the discussion of the Ostwald rule the impression may be given that the type of crystal modification produced is dependent only upon the degree of supersaturation. That the nature of the fluid medium in which nucleation and crystallization occur also plays a decisive role is common knowledge in industrial crystallization processes, but described relatively rarely in the literature.[71] In addition to their function as dispersion medium the solvent molecules take on the role of a substance interacting with the growth faces of the crystals.

Also worthy of mention at this point are the possibilities of preventing with the aid of additives an—in general—undesirable Ostwald ripening of the colloidal systems. Ostwald ripening is the growth of larger particles at the expense of smaller, where the necessary material exchange occurs diffusely through the dispersing phase.[12, 72, 73] In the development of dispersed systems in which the solubility of the phase to be dispersed is not especially small one is confronted in all cases by the question of how to prevent Ostwald ripening, that is, how a stable dispersion of small particles may be obtained. There are two approaches to the solution of this problem. Attempts can be made to "seal" the surface of the particle with adsorbing molecules so well that an exchange of the active substance is effectively suppressed. That this approach is possible was demonstrated with a nanoparticulate dispersed dye, which could be effectively stabilized with a suitable mixture of sodium dodecyl sulfate (SDS) and polyvinylpyrrolidone (PVP).[74] This stabilization did not occur with either SDS or PVP alone. Another possibility is to mix the dispersing phase with a second component (2) which is less soluble still in the liquid matrix than the actual active substance (1), but where 2 is soluble in 1.[72, 75] At the start of the ripening process the concentration ratios in all particles are the same. As soon as the particle growth process begins by diffusion of 1 the concentration ratio of 2 to 1 in the smaller particles shifts to greater values, until eventually the associated loss of mixing entropy prevents a further reduction in particle size. Reference is made here to a review article for a detailed discussion of this and other points discussed in this section, ref. [72]

2.6. Particle Formation by Mixing Two Components

The supersaturation necessary for the induction of a precipitation/crystallization reaction may be produced by a variety of methods: 1) change in temperature or pressure, 2) change in the solvent quality of the fluid phase in which the material to be precipitated is initially present by mixing with a second fluid phase which is miscible with the solvent and in which the substance is poorly soluble or insoluble, and 3) by mixing two solvents the dissolved components of which are insoluble in the reacted state. The theoretical approaches described previously do not differentiate in principle between these three cases since *homogeneous* supersaturation is always assumed at the start of the experiment. Within the context discussed here only cases (2) and (3) are of interest. During precipitation by mixing two liquid flows it is often implicitly assumed that the mixing is so intensive and so rapid down to small length scales that the system is homogeneously mixed before particle formation commences. That assumption is clearly only justified when the corresponding time constants are sufficiently different (see below). In practice attempts are made to achieve homogeneity as rapidly as possible by turbulent mixing with high energy input.

The following will describe why an understanding of the mixing behavior is critical for an understanding of the particle formation process, especially when the reaction for phase separation begins during mixing. According to Baldyga the mixing of two liquid flows can be divided into macro-, meso-, and micromixing.[76] The term macromixing includes flow phenomena on length scales of decimeters, such as those that are relevant in stirred vessels. By mesomixing is meant mixing effects on a length scale of millimeters; they are founded upon the breakdown of turbulent vortices into increasingly smaller vortices until viscous processes predominate over inertial forces. The term micromixing encompasses processes in the region of micrometers. In Figure 8 the processes taking place

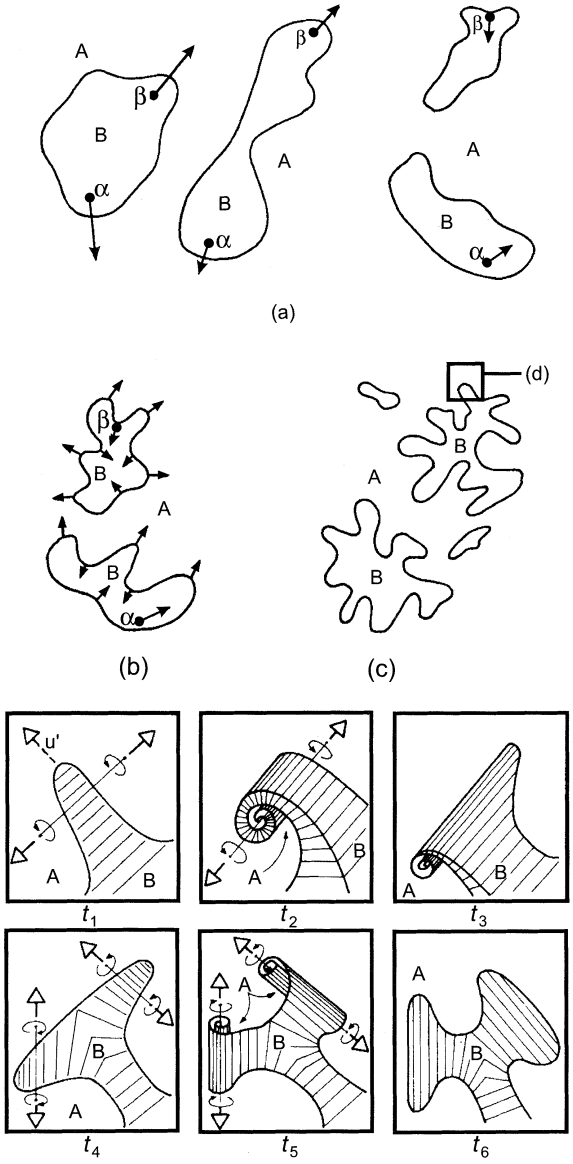

Figure 8. Schematic representation of the mixing processes of two educt flows during turbulent mixing (J. Baldyga[76]).

during turbulent mixing on the scale of micromixing are outlined. Two approaching liquid compartments envelope each other when the layers become increasingly thinner. Locally the two educt flows exist as lamellae. Respective simulations illustrate clearly this effect as well as the inhomogeneity of the turbulence (Figure 9).[77] In each case

Figure 9. Two-dimensional simulation of the mixing of two educt flows under turbulent conditions.[77]

a boundary layer initially exists between the two flows. This boundary layer is initially the site of maximum supersaturation. Whether the rate of particle formation is slower or faster than the time to complete homogeneous mixing, which on small length scales is defined by the rate of diffusion of the molecules, is critical for consideration of the particle formation process. In the first case (slower particle formation) the previously described theories may be used with the named limitations. It is otherwise in the second case (faster particle formation), here the supersaturation profiles and structures that form at the boundary surface between the two mixing educt flows must be explicitly considered. Relatively little is still known experimentally on this point. However, it must be emphasized that in a few cases the phenomenological approaches developed by Baldyga and Bourne and by Franke in the extension to aggregation processes offer a good description of the particle size distributions developing in the precipitation reactions.[76, 78, 79]

The concurrent diffusion and reaction processes have been treated in a series of theoretical studies. Sokolov and Blumen have shown, for example that supersaturation in a system reacting in laminar layers, as is realized locally in the above described liquid vortices, shows a non-classical time course: for extended times the supersaturation is proportional to $t^{-1/4}$.[80] On the basis of classical nucleation theory Dolgonosov calculated supersaturation profiles and particle concentration gradients at reactant boundary interfaces.[81] Similarly Lindberg and Rasmuson proposed that in many of the cases occurring in practice it must be considered that particle formation occurs to a considerable extent in the boundary layer between the educt solutions before the system is fully homogenized by interdiffusion.[82] In all this work it is assumed that particle formation in the boundary layer takes place in a classical manner, that is, by nucleation and growth. For the case of real particle formation processes it still has to be clarified whether or not initially—possibly strongly hydrated—precursors are formed which either aggregate to larger particles with dehydration or partially redissolve in an Ostwald ripening to contribute to actual particle formation on stable nuclei.[83]

In the use of additives for control of the particle formation process the points mentioned gain further in importance; if an additive is added to one of the two educt flows a number of interactions of additive molecules with the particle formation process is conceivable: 1) the additive molecules act as nucleation initiators, 2) the additive molecules complex some of the educt molecules and thus lower the level of supersaturation, which can lead to an increase in the induction time or to a change in the reaction mechanism, 3) the additive molecules are incorporated during particle formation. On the one hand this incorporation can possibly be used to induce a targeted crystal modification during the early stage of particle formation. On the other hand such an incorporation is undesirable when, for example, particles for pharmaceutical purposes need to be prepared in a pure state, 4) the additive molecules coat the surface of the nanoparticles being formed and by efficient dispersing fix a certain particle size distribution.

Which of the mechanisms described comes into effect depends both upon the chemical interaction between the additive molecules with the molecular and particulate species and the time scales upon which the different processes (micromixing, interdiffusion of the reactants and additive molecules, aggregation of precursors, adsorption of the additive molecules to precursors and particles) occur. The significance of these points is familiar from industrial practice. So far they have been examined only sporadically. The speculations of Lannibois-Drean are especially worth a mention.[84] On the basis of experiments on the precipitation of cholesterol acetate from solvents by mixing with water she suggested the following model for the interaction of surfactants with the particles under formation: during the diffusion of water into the solvent phase first of all the solubility of the organic molecules falls so considerably that they precipitate by nucleation. The affinity of the hydrophobic surfactant segment for cholesterol acetate at these water/solvent ratios is not so strong that an irreversible adsorption would occur. This occurs only later when the solvent properties of the water/solvent mixture have further worsened by interdiffusion. During mixing/interdiffusion the system thus passes through successive solubility limits: that of the initially dissolved active substance and that of the surfactant. Clearly in respect of this point there is a need for further research to establish definitively the actual mechanisms and to understand their totality.

It is interesting to observe how different disciplines handle the set of problems described in this Section. Polemically it could be so formulated: chemists are satisfied when they can prepare a defined end state in the form of dispersed nanoparticles under defined experimental conditions. Physicists refer to the problem of turbulent mixing in terms of the final physical questions to be solved.[85] Engineers in contrast have a results-orientated approach to the problem, as documented, for example in the recently published, voluminous work from

Baldyga and Bourne.[76] Finally it is noted that in recent years computer-supported solutions to fluid-dynamics problems are gaining increasingly in importance.[77, 86]

2.7. Experimental Aspects

To understand the stages through which the formation of colloidal particles pass it is necessary to record experimentally the particle formation process by time resolution from the timepoint when supersaturation is realized. That the time-resolved description of processes is in no way trivial is recognized from the limited number of publications in this area. In most cases a description of the mechanism of particle formation is still limited to a retrospective derivation from the structure of the particle formed. That the amount of work published in this problem area is quite limited is because, unlike crystallization, particle formation in precipitation reactions occurs on a clearly shorter time scale, down to the region of milliseconds.

Where time resolution of the chosen measurement method is sufficient the stopped-flow method has proved effective: two educt volumes are led through a mixing cell into a measurement cell. At a predetermined timepoint the flow is interrupted and the particle formation process is measured by a suitable method, for example light scattering or the scattering of intense X-rays. If the measurement method requires longer measurement times the flow reactor or precipitation jet may be used.[31, 87–89] Here, both educt flows are passed through a mixing cell and then through a reactor tube or, after a defined distance, directed as a free beam into a defined atmosphere. Assuming that the system is quasistationary, that is, that over an extended period of time the precipitating system is in the same state at definite positions of the precipitation tube, at a known flow rate v in the tube the detection point x (measured from the mixing cell) at which, for example, spectroscopic or diffractive characterization methods are attached, may be converted into reaction time $\tau = x/v$. Alternatively the tube length may be varied and samples for off-line methods (especially microscopic methods) obtained, for example, by quenching liquid samples at the beam outlet.[31] It is assumed when using these systems that the time scale of the reaction process is longer than the time to homogeneous mixing of the educts in and after the mixing cell; typical mixing times lie in the range of milliseconds for the stopped-flow technique and in the range of microseconds with special flow cells.[88] Hitherto there have been few investigations of processes *during* mixing. More recent experiments show, however, that precursor structures which break down to nanoparticles are already forming at the boundary interface of the two turbulently mixing educt flows.[31]

In the following a number of experimental methods will be described briefly with which particle formation processes may be investigated on length scales of nanometers. Static and dynamic light scattering are established techniques for the investigation of colloidal systems.[90] More recently correlation spectroscopic methods such as fluorescence correlation spectroscopy (FCS) and Raman correlation spectroscopy (RCS) have been developed for the characterization of nanodispersed systems.[91] Concentrated dispersions can also be characterized reliably by fiber optic quasielastic light scattering (FOQELS).[92] Small-angle X-ray and neutron scattering (SAXS and SANS) are also classical methods for the characterization of colloidal systems[93] and have been used in many investigations for the determination of primary particle size and aggregate structures. Investigations on the following systems are cited as examples: lysozyme clusters as precursor for critical nuclei (SANS),[94] precursor formation in zeolite crystallization (SAXS),[95] aggregation and compaction in the formation of SiO_2 and TiO_2 particles (SAXS),[96] hydrolysis and condensation of metal alkoxides,[89] and particle formation in quinacridone and boehmite precipitation,[31] in the latter two cases the above-described precipitation tube technique was used.

UV/Vis spectroscopic methods can be used principally for online analysis of particle size development by means of particle size dependency of turbidity spectra and, where possible absorption spectra (see Section 4.3). A quantitative evaluation is made difficult, however, because model concepts relating to particle size distribution, particle shape, and refractive index differences must be available.

In conclusion a number of techniques for the time-resolved investigation of particle formation processes are listed about which only a few reports are currently available. The earliest stage of particle formation in the hydrolysis of metal alkoxides was studied with the laser-induced liquid beam ionization/desorption (LILBID) technique.[97] An equally exotic technique is X-ray microscopy with which aqueous systems can be investigated under normal pressure with time resolution in the minute range and a space resolution of about 30 nm.[83] Finally an interesting use of the analytical ultracentrifuge[98] has been reported: this method allows the particle size distribution of precursors in the crystallization of lysozyme and CdS to be determined.[99] For completeness the pulse-radio technique is mentioned with which the formation and growth of colloidal metal clusters in an aqueous environment may be studied with a time resolution in the submillisecond range.[100]

3. Preparative Methods for the Production of Organic Nanoparticles

As discussed at the outset, nanodispersed systems can be obtained in two ways (Figure 2): 1) by mechanical milling of the raw material by wet or dry milling processes, or 2) by precipitation or condensation of the products or educts dissolved in solvents with subsequent separation of the unwanted solvent. In both variants additives such as surfactants and polymers take over the function of boundary layer active, colloidal stabilizers or—in the condensation procedure of method (2)—also form the nanoparticulate phase itself which contains the active compound or effect substance, bound by adsorption or absorption.

Milling processes[101] are in principle unsuitable for the production of nanodispersed systems with narrow size distribution since with decreasing particle size it becomes

increasingly more difficult to use the applied mechanical energy in the form of shearing and cavitation forces[102] for particle milling without simultaneously inducing particle agglomeration.[101, 103] Moreover, the unavoidable milling element abrasion which contaminates the end product and is difficult to separate, especially in active compound formulations, is often undesirable or not tolerated.[104] The considerable practical significance of milling processes lies mainly in that in pigment and dyestuffs formulations the achievable particle size distributions in the lower micrometer region, in special cases also below, satisfy technical demands.[101, 105]

In spite of these disadvantages milling processes do find widespread use in the formulation of poorly soluble active compounds[106–108] since alternative technologies which could deliver nanoparticulate products are essentially still in the development stage. These include in particular the described precipitation processes from homogeneous solution which with suitable process control not only allow the preparation of extremely fine particulate dispersions, but also allow a continuous and, in respect of process parameters, easily controllable method of production. These technical advantages also make precipitation processes particularly attractive from an economic viewpoint.

With the theoretical principles discussed in Section 2 in mind the precipitation processes are illustrated below. Various methodological variants for the production of nanodispersed polymer dispersions by physical condensation processes will also be described briefly.[109–111] Figure 10 gives a structured methodical overview of precipitation and condensation processes for the production of organic nanoparticles in aqueous media. Starting from a molecularly dispersed solution of the "active compound" three groups or processes which allow a restriction of particle growth to the nanometer region can be differentiated.

1. With lipophilic solvents (processes I and II) particle dimensioning occurs through an emulsion step as an intermediate stage. The particle size distribution of this oil in water (o/w) emulsion is adjusted mechanically by homogenization.[101, 103, 106] The conversion of the emulsion into a nanodispersion is then carried out by separation of the solvent by evaporation or diffusion procedures. The size distribution of the nanodispersion is determined by that of the o/w emulsion, the mean particle size by the concentration of the substrate in the emulsion phase.
2. If hydrophilic, fully water-miscible solvents are used (processes IV and V) particle formation occurs by precipitation[103] either according to the principles of nucleation and growth outlined in Section 2 or, at extremely high supersaturation, by spinodal phase separation. In each case an agglomeration step can follow these elementary processes (Section 2.4.2).[112]
3. With use of amphiphilic solvents or solvent mixtures (process III) nanoparticle formation takes place through a transient emulsion phase which forms spontaneously and which then transforms into a nanodispersive state.

Only with process groups (2) and (3) is size distribution controlled by the level of the adjustable supersaturation as well as by surfactant additives, which possibly intercede specifically in the elementary steps of nucleation, growth, phase breakdown, and agglomeration. The individual process variants differ in respect of the adjustment of the temporal supersaturation profile as well as in the choice and function of the additives introduced.

In the structuring indicated in Figure 10 only in the limiting cases I and V does particle formation takes place by pure

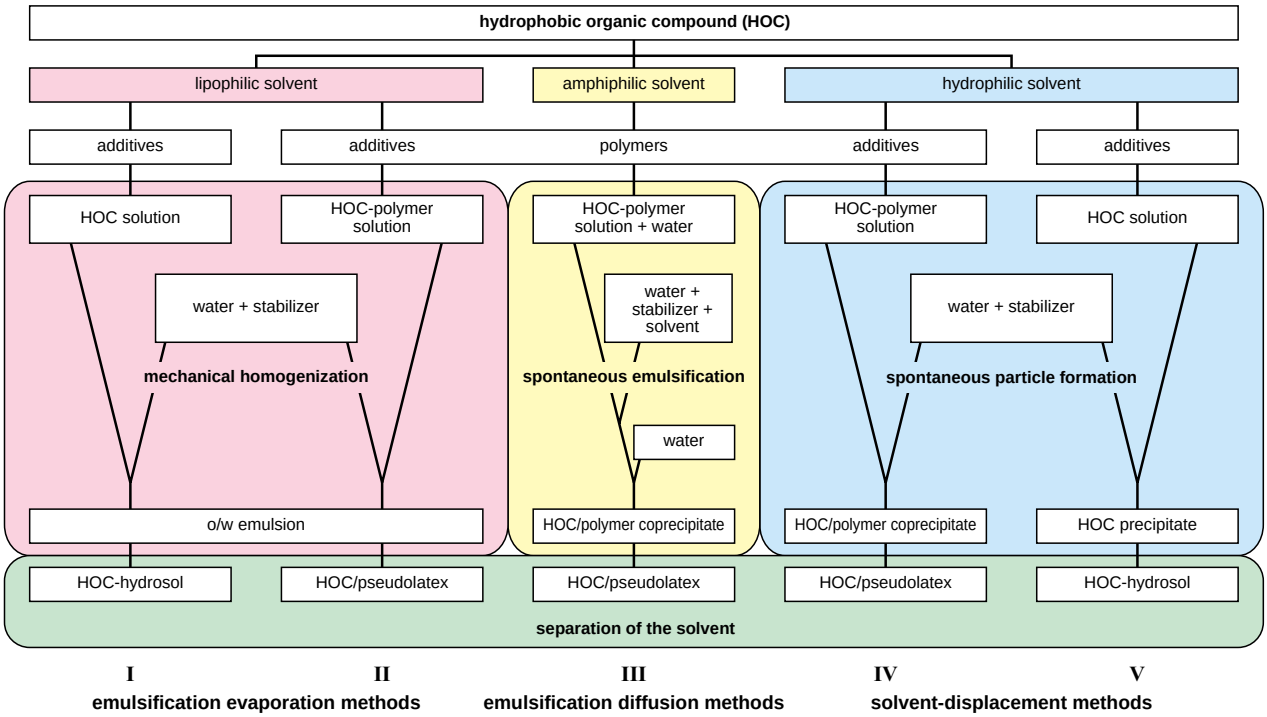

Figure 10. Precipitation and condensation processes for the preparation of organic nanoparticles. More detailed explanations in Figures 11–15 and the text.

precipitation processes (in respect of active compound). In contrast, with variants II, III, and IV processes are described which lead to so-called pseudolatex systems through physical condensation of dissolved macromolecules, that is, the "support" is primarily precipitated.[111] The physico-chemical elementary processes of particle formation and the bonding of dissolved active compounds onto or into the particulate phase are still essentially unexplained. The terms nucleation from a homogeneous supersaturated phase and particle growth normally used in the description of the elemental steps in precipitation reactions are here of limited suitability in describing the complex relationships in particle formation. It should be noted that processes I – III contain an emulsification step prior to the actual particle formation.

Process variants I – V are illustrated below with selected examples. In all processes polymers, surfactants, and surface-active protective colloids play a significant role in particle formation, even if in quite different functions. Detailed knowledge of molecular-specific possibilities for influencing particle formation, especially for the targeted adjustment of particle size and particle morphology, is only available in individual cases.

3.1. Nanoparticle Formation by the Emulsification – Evaporation Process from Lipophilic Solution

3.1.1. Hydrosols of Active Compounds (Process I)

Processes of this type belong to the classical methods for the preparation of water dispersible nanoparticulate hydrosols of water-insoluble active compounds. They were developed especially for the formulation of carotenoids.[113–115] Preparation of the nanoparticles is carried out by dissolving the active compound together with an emulsifier, for example, ascorbyl palmitate, in a suitable solvent, for example, chloroform or methylene chloride, then emulsifying this solution with an aqueous solution of a protective colloid, for example gelatin, and removing the solvent by distillation (Figure 11). The actual precipitation/crystallization takes place in the emulsion droplet during distillation when the solubility limit is crossed. The size of the active compound particle is thus proscribed by the concentration of the active compound solution and the size of the emulsion droplet. The particle size distribution can be adjusted within wide limits by the droplet size distribution of the o/w emulsion through the choice of homogenizer (colloid mill, high pressure homogenizer, ultrasound disperser).[101, 103, 106] A solid nanoparticle which is protected against agglomeration by the suitable choice of the protective colloid is obtained from each emulsion droplet of a well-stabilized emulsion upon removal of the solvent.[116] The particle morphology is usually polycrystalline in the thermodynamically stable crystal structure since the solid formation takes place by evaporation crystallization at low supersaturation.[117] Numerous recipe variants can be realized by the addition of further lipophilic additives to the emulsification formulation. A fundamental difficulty in this process lies in the removal of as much solvent as possible from the final product.

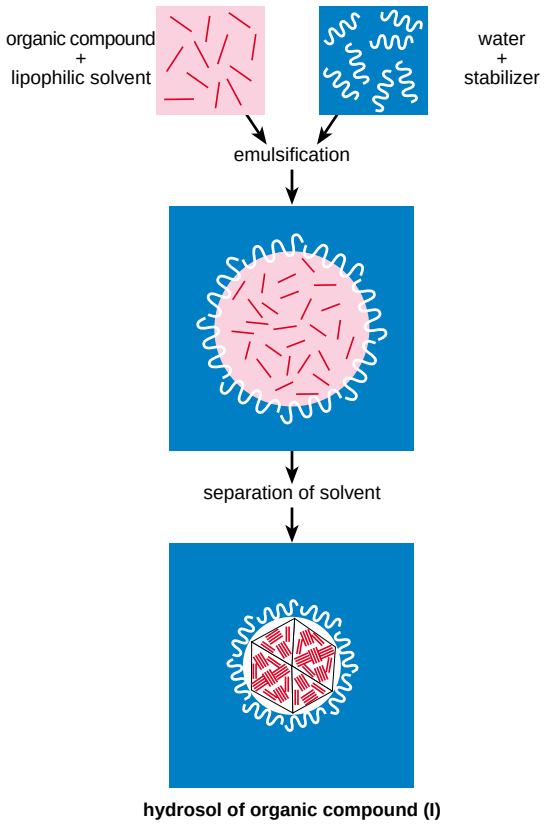

Figure 11. Principle of the preparation of active-compound hydrosol nanoparticles by the emulsification – evaporation process.

3.1.2. Pseudolatex Systems (Process II)

If lipophilic polymers, such as biodegradable polylactides (PLA), poly-β-hydroxybutyrates (PHB), polylactide-co-glycolides (PLGA), polycaprolactones (PCL), or polyalkylcyanoacrylates, are used with the active compound[110, 118–122] nanoparticulate polymer dispersions are obtained which contain the lipophilic active compound either adsorbed or embedded as molecular dispersions or microcrystals (Figure 12).[110, 119, 123] The formulations thus prepared are of increasing interest as parenteral dosage forms (see Section 4.2). A special version of this method for the preparation of protein-loaded polylactide nanoparticles was recently published.[120] In a double emulsion process an aqueous solution of the active protein (protein C plasma inhibitor) was emulsified in a methylene chloride/acetone polylactide solution and this o/w emulsion was then emulsified in an aqueous solution of polyvinyl alcohol (PVA) as protective colloid. After removal of the solvent PLA nanoparticles (200 – 250 nm) were obtained. The protein activity in the nanodispersed formulation could be controlled by the preparation conditions.

On account of the excellent solubilizing properties of chlorohydrocarbons for lipophilic active compounds and galenically interesting additives.[101, 106, 124] The procedure described is in principle widely applicable to the formulation of lipophilic active compounds. However, there is one intrinsic disadvantage in that these toxicologically dubious solvents

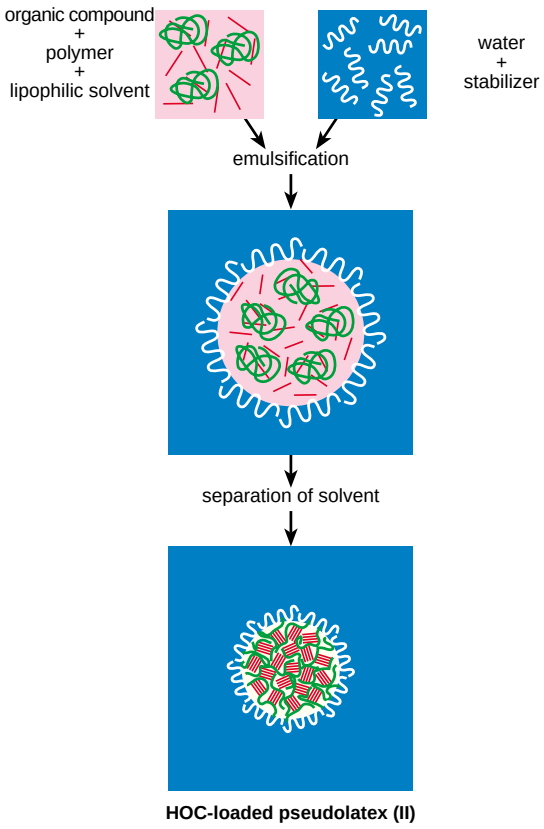

Figure 12. Principle of the preparation of active-compound pseudolatex nanoparticles by the emulsification–evaporation process. HOC = hydrophobic organic compound.

must be fully removed during the workup of the formulation.[106] Therefore, more recently attempts are being made to change the emulsification–evaporation process to the use of more acceptable solvents such as cyclohexane. With the example of cholesteryl acetate as a model compound Sjöström and Bergenstahl were able to show that with an optimized emulsification system it was possible to obtain stable nanodispersed formulations with particle sizes of about 25 nm.[117] Where it is possible in individual cases to find an acceptable solvent the emulsification–evaporation procedure has the advantage in that it should be possible to select suitable protective colloids and emulsifiers on the basis of semi-empirical concepts and thus allow targeted process optimization.[101, 125]

3.2. Nanoparticles by the Emulsification–Diffusion Procedure from Amphiphilic Solution (Process III)

The different process variants are all based upon the use of solvents which are of limited water miscibility and capable of spontaneous emulsion formation (e.g. propylene carbonate, benzyl alcohol, ethyl acetate). This method thus offers the advantage of the use of pharmaceutically acceptable solvents and does not require the use of high-pressure homogenizers for the formation of the o/w emulsion as the preliminary stage of nanoparticle formation.[110, 126, 127] The trick of this process, the physico-chemical principles of which are still not fully clarified, is that the water-saturated solvent phase (+polymer and active compound) and the solvent-saturated aqueous phase (+protective colloid), that is, in thermodynamic equilibrium, are first emulsified by intensive stirring. With the subsequent addition of water to the merely microdispersed o/w emulsion the diffusion equilibrium is disturbed. This induces solvent diffusion into the homogeneous aqueous phase at which point the solubility limits of polymer and active compound are crossed and particle formation commences (Figure 13). Mechanistic investigations with variation in preparation conditions and the use of PLA as polymer and polyvinyl alcohol (PVAL) as protective colloid have shown that each emulsion droplet gives a multiplicity of nanoparticles.[127]

This remarkable finding was explained by the development of concentration fluctuations in the boundary layer region caused by solvent diffusion, when the solubility limit is narrowly exceeded locally and the precipitation of polymer and active material is induced. The interaction of the

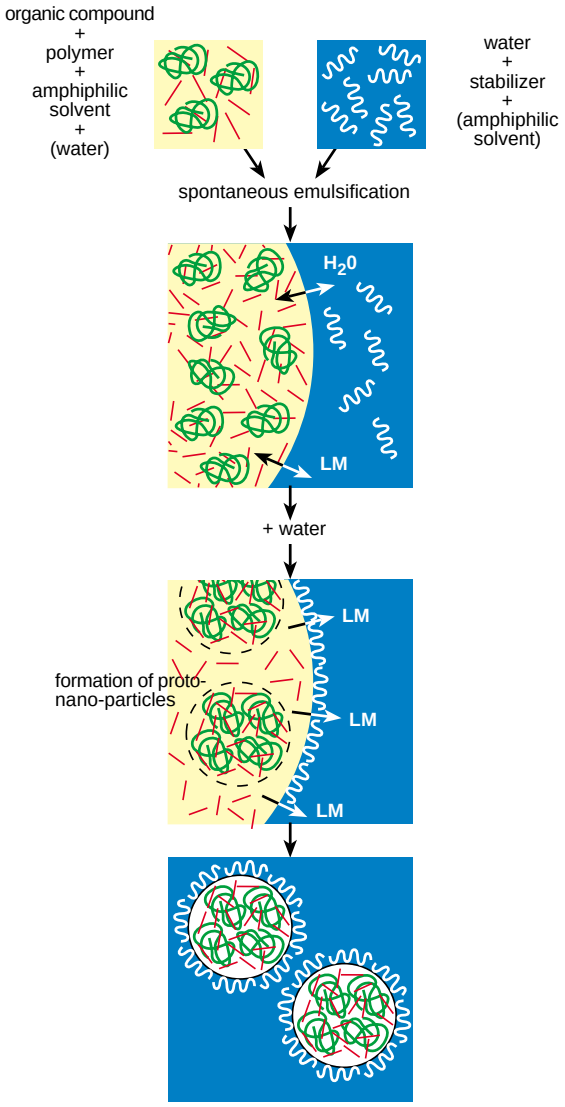

Figure 13. Principle of the preparation of active-compound pseudolatex nanoparticles by the emulsification–diffusion process.

protective colloid molecules present in the aqueous phase with these "proto-nanoparticles" suppresses their further agglomeration and thus determines the achievable particle size distribution of the nanodispersion, which is obtained after removal of the solvent by distillation. According to the mechanism described in Section 2.6 it would also be conceivable that when the solubility limit is crossed during interdiffusion of the two phases, active compound particles which are inhibited in growth by adsorbing protective colloids, and thus colloidally stabilized, form continuously. A process variant which allows a further simplification of the procedure was reported recently.[128] Here the dilution step with water and the separation of the solvent is combined by steam distillation. The process was tested with a series of biodegradable and non-degradable polymers.

A further variant has been used which also manages without a homogenization step, since emulsion formation again occurs spontaneously as a preliminary stage in nanoparticle formation. However, this so-called SESD (spontaneous emulsification solvent diffusion) process[122, 129] suffers from the disadvantage that amphiphilic solvent mixtures with methylene chloride are used as the hydrophobic component. Technically a polymer/active-compound solution, for example PLGA, in acetone/methylene chloride is added with stirring to an aqueous protective-colloid solution (polyvinyl alcohol). A coarse-particle o/w emulsion forms spontaneously the particle size of which is rapidly reduced by diffusive loss of the acetone in the dispersed phase. After evaporation of the solvent PVAL-stabilized polymer particles with incorporated active compound are formed in the nanometer range. The solvent mixture and the polymer-protective colloid combination are so adjusted that clear affinity differences between polymer and protective colloid for the solvent components guarantee phase separation and allow colloidal stabilization.[129] It is clear that in view of the complexity of the individual physico-chemical processes involved in each system a detailed optimization is required to regulate the desired particle size distribution. In this context a modified SESD process has been described by Murakami et al. that uses solvent mixtures without the undesired chlorohydrocarbons.[129] However, since water-miscible solvent mixtures are used the particle formation mechanism must be differentiated from that of the SESD process (Section 3.3.1).

A further variant for the formation of nanodispersed pseudolatex dispersions, possibly loaded with active compound, through an emulsion phase as intermediate stage is the so-called salting-out process.[110, 130] The process is based upon the ability of electrolytes (for example NaCl, $MgCl_2$, $CaCl_2$[110, 131]) or saccharose[132] to salt-out acetone from an aqueous solution. The active compound/polymer solution in acetone is initially emulsified in the aqueous electrolyte or sugar solution in the presence of a protective colloid and then diffusion of acetone into the aqueous phase—with simultaneous formation of nanoparticles—is induced by the addition of water. Here too boundary surface turbulence (initiated by acetone diffusion) or mechanisms as described in Section 2.6 (precipitation during the interdiffusion of the phases) can explain the nanoparticle formation. However, there have been no detailed investigations on the mechanism of particle formation to date. These relationships have proved to be extremely complex. Thus, incorporation of the active compound into the nanoparticle is influenced considerably by the salting-out components.[110, 133, 134] Hitherto only the protective colloids PVAL, polyvinylpyrrolidone (PVP), and hydroxyethylcellulose have demonstrated an adequate effectiveness.[110] The solvent and the salting-out components are separated by distillation or cross-current filtration

3.3. Nanoparticle Formation by Solvent-Displacement Processes from Hydrophilic Solution

The industrial advantages of the processes discussed at this point rest upon the use of water miscible, toxicologically acceptable solvents, (e.g. acetone, short-chain alcohols). The methods were described both for the preparation of nanodispersed pseudolatex transport forms of lipophilic active compounds and for the preparation of pure nanohydrosols of active compounds and effect materials which are poorly soluble or insoluble in water.

3.3.1. Pseudolatex Systems (Process IV)

The modified SESD process (described in Section 3.2) represents a special case of pseudolatex formation by way of a transient emulsion stage.[129] The solvent mixture consists of two water-miscible solvents (acetone/ethanol) with different affinities for the polymer and the protective colloid. In the example used the polymer PLGA has a higher affinity for acetone, whilst the protective colloid PVAL is more soluble in ethanol or methanol. A five-stage model was suggested for the mechanism of particle formation.[129] After mixing the PLGA solution with the aqueous PVAL solution rapid diffusion of the alcohol component first leads to particle size reduction of the transient emulsion intermediate which is formed according to the Marangoni Effect.[135] The preferred diffusion of the alcohol component is explained by the lower affinity of the alcohol for PLGA in the dispersed phase. The likewise occurring acetone diffusion leads to a collapse of the PVAL protective colloid in the boundary layer, accompanied by a PLGA condensation in the increasingly acetone-depleted dispersed phase. Even under mild stirring conditions this spontaneous particle formation process also leads to nanoparticulate pseudolatex dispersions (Figure 14). To what extent this novel process can be extended to other polymer/protective-colloid combinations cannot yet be assessed. Clearly, experience with polymer incorporation of lipophilic active compounds is currently not available.

Fessi et al. showed for the first time that instead of a solvent mixture the use of only one solvent with unlimited water miscibility can also lead to the spontaneous formation of nanoparticulate pseudolatex dispersions.[136] Normally acetone, ethanol,[110, 136–138] or THF[121] are used. In analogy to the modified SESD process the polymer solution, which possibly also contains active compound and other additives, is mixed with the aqueous protective colloid (PVAL) solution. The spontaneous particle formation is again explained by boun-

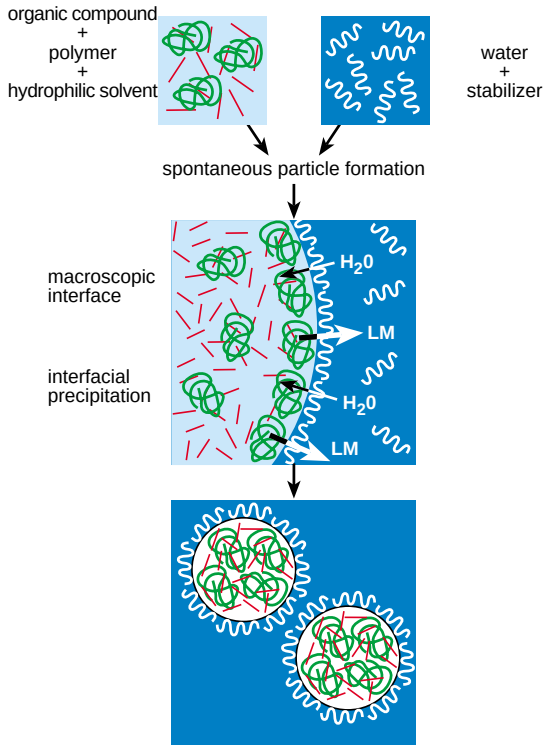

Figure 14. Principle of the preparation of active-compound pseudolatex nanoparticles by the solvent-displacement process.

dary layer turbulence which is initiated by solvent diffusion.[110, 127, 136] According to this model concept boundary layer turbulence leads to breakdown of the nanodimensional solution compartments in which, with increasing diffusive depletion of solvent, polymer and active compound are concomitantly precipitated. Occasionally the term nanoprecipitation is also used to characterize this process.[110, 138–140] The stabilization of the particle by adsorption of the protective colloid is still critical for the nanodimensioning of the particle to prevent a subsequent agglomeration. Here too the question arises whether the assumption of boundary layer turbulence is necessary to explain particle formation. For example, experiments by Lannibois-Drean[84] have shown that even with macroscopic boundary layers between the two educt phases the formation of small particles can be explained by interdiffusion (see also the discussion in Section 2.6).

Wu and co-workers have reported that if the polymer is an ionomer, for example sulfonated polystyrene, a protective colloid can be omitted as electrostatically stabilized nanoparticles are formed.[141, 142] The nanodispersions obtained by precipitation from a THF solution were extremely fine with mean particle sizes in the range 5–12 nm. The particle sizes could be deliberately made smaller with decreasing polymer concentration and increasing degree of sulfonation. The formation of charge-modified PLGA nanoparticles by condensation from an acetone/ethyl acetate solution has also been reported by Kissel and co-workers.[143]

The conversion of these attractive processes into practice is, however, fraught with difficulties as the model concepts on particle formation in the individual case can only supply approximate information as to the optimal composition of the active compound/polymer/solvent/stabilizer system.[110, 144] It appears plausible that the particle size falls with decreasing polymer concentration in the solution phase because of the material balance and the decreasing viscosity.[121, 137, 141, 145–147]

An important requirement for particle formation appears to be that the resulting water/solvent mixture is non-solubilizing for the polymer.[137] The yield of nanoparticles—in addition to the coarse and thus valueless agglomerate—as well as their particle sizes are determined within wide limits by the nature of the solvent and by the mixing ratio with water.[146] In model investigations with hydroxypropylmethylcellulose phthalate (HP55) as polymer and acetone/water mixtures as solvent it was found that the yield of nanoparticles could be increased with increasing water content (up to 30% (v/v)) from a fraction of about 30% to over 90%. This observation was explained by a change in the solvent character from a good to a poor solvent. With the thus associated reduction in chain interaction of the dissolved macromolecule the nanoparticle formation is already predetermined by the structure of the solvent phase (see also Section 2.2.6).[146, 148, 149] Further experimental details have been described recently by Fessi and co-workers.[137] In particular, it was shown in that the usual stirred vessel method for the precipitation of nanoparticles could be developed further by a controlled precipitation in a T-mixer. The objective is a continuous industrial production process. In analogy to the processes described previously a separation of the solvent by distillation is also carried out here, which presumes an adequate colloidal stability of the dispersed system.

The nanoprecipitation process is also suitable for the preparation of nanocapsules. In this case a suitable oil in which the active compound is highly soluble is added to the solution phase. Core-shell particles are then formed with the active compound–oil solution as core.[110, 149, 150]

The motivation for the extensive work on the preparation of nanodispersed pseudolatex systems comes from the demand for aqueous peroral and parenteral (that is, administered by mouth, and for non-intestinal administration) transport forms of lipophilic active compounds. The choice of the hitherto investigated polymer systems is therefore aimed primarily at pharmaceutical-technical acceptance and in individual cases at their biodegradability and tolerance. The particles serve as absorptive or adsorptive active compound supports.[118, 151] This property explains why work is orientated preferentially towards active compound and application-specific areas and a systematic treatment of the complex problem of particle formation based on polymer-physics principles is still essentially absent.[145]

3.3.2. *Active Compound Hydrosols (Process V)*

To date little has been reported on precipitation techniques for the preparation of nanodispersed hydrosols of active compounds (Figure 15),[152–157] in contrast much has been written on precipitation techniques for the preparation of

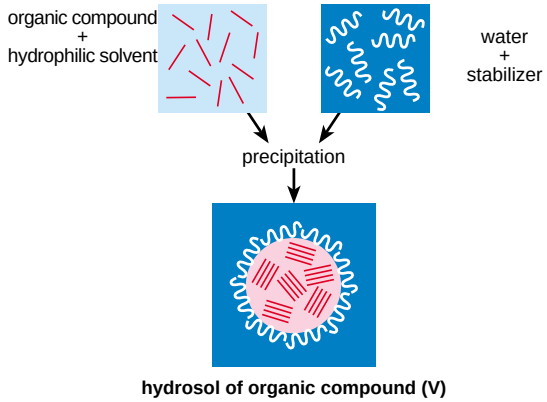

Figure 15. Principle of the preparation of active-compound hydrosol nanoparticles by the solvent-displacement process.

inorganic nanoparticles.[2, 103, 158–162] In particular Matijević and co-workers developed the latter further with the aim of preparing nanodispersed systems with uniform particle size and shape.[2, 161, 162] In contrast, the emphasis of work on the bulk crystallization of organic molecule crystals lay mainly on the production of readily filterable crystal suspensions with particle sizes in the middle micrometer range as the final stage of a synthesis procedure,[163–168] or in a defined crystal modification.[169–171] With organic dyes and pigments and poorly soluble active components further process steps are usually performed, for example, to reduce the particle size by milling or homogenization techniques and thus to modify the color or photoelectric properties or to improve the bioavailability of the active compound.[101] It therefore appears attractive to replace the technically cumbersome mechanical formulation methods by precipitation methods which directly give the active compounds or effect materials with the desired particle size in the nanometer range.

According to the discussion in Section 2 the following conditions must be fulfilled for the preparation of nanodispersed hydrosols by precipitation reactions:

1. nucleation must take place at the highest level of supersaturation possible to maximize the nucleation rate.
2. The limitation of particle growth and the setting of a narrow size distribution require a controlled and rapid reduction in supersaturation subsequent to the nucleation phase, possibly under the influence of specific growth inhibitors.
3. The agglomeration of the primary particles should be prevented or controlled by the presence of viscosity-increasing additives and/or by low molecular weight protective colloids with high surface activity.[172]

If the precipitation of the active material from molecular solutions takes place by the addition of an antisolvent, for example, water, and not by the formation of poorly soluble salts or as result of a chemical reaction, the first condition can be fulfilled by a suitable choice of a solvent which is fully miscible with water and by a highly efficient mixing technique.[164] If it is possible to set high values for supersaturation ($>10^5$) then, according to the most recent investigations of Botet et al., particle formation can also occur by a diffusion-limited aggregation mechanism with active participation of suitable additive molecules, in contrast to the classical model concepts of nucleation and growth.[173] Detailed thermodynamic considerations should show whether or not this is a spinodal separation process (see Section 2.2.3).

The choice of suitable solvents for setting the highest supersaturation possible is naturally limited since in addition to a good solubilizing ability for the active compound, complete miscibility with the antisolvent water, and a lowest possible boiling point for the distillative separation of the solvent from the nanodispersion are necessary. For the formulation of pharmaceutically active compounds toxicological safety is a further limiting parameter. Consequently small-chain alcohols, acetone, or THF are used.

In the mixing methods for the rapid establishment of high supersaturation (Section 2.6) there is need to differentiate between processes with and without back mixing. For preparative work on laboratory scale, processes have been described in which the solution phase is added to the stirred, aqueous protective-colloid solution, either dropwise or by spraying.[154, 165, 174, 175] Precipitation conditions are more readily controlled by the so-called double-jet technique in which the reaction solutions exit the jet pair immersed in protective colloid solution and are mixed at a defined flow angle.[156, 161, 176] In all cases there are hydrodynamically poorly controllable batch processes involved with possible back mixing, so that nanoparticulate systems are only obtained if the precipitation reaction is carried out at sufficiently low active-compound concentration so that a secondary crystallization or particle agglomeration can be held within limits.

A further group of procedures is one in which the active-compound solution and the aqueous protective-colloid solution are vortexed vigorously in a mixing chamber,[152, 153, 163, 164] a static mixer,[154] or a Y- or T-mixer[163, 165, 177] with mixing times down to the millisecond range and particle formation occurring in a subsequent flow tube. Although the hydrodynamic and reactive processes in each of the method-specific mixing zones have in no way been explained (see Section 2.6), the techniques, in particular the non-pulsate delivery of the component flows, allow controllable particle formation with a narrow size distribution by means of varying the mixing conditions and temperature control. Kinetic data on the course of the precipitation reaction can be obtained in certain cases with suitable sensors situated along the flow reactor (see Section 2.7). Furthermore, a particular advantage of this process is that a scale-up from the laboratory apparatus to continuous-production processes is possible.

The method developed by Horn and co-workers for the production of nanodispersed carotenoid hydrosols by precipitation from hydrophilic solution with water uses a continuous mixing chamber process.[152, 153] Figure 16 shows the principle behind the process. Poorly soluble and temperature labile active compounds can also be converted into nanodispersed hydrosols by this method. For this purpose a second mixing chamber is attached in front of the actual precipitation chamber in which a molecularly dispersed solution is produced immediately before precipitation by shock heating of the microdispersed active-compound suspension by turbulent mixing with a hydrophilic solvent at high temperature

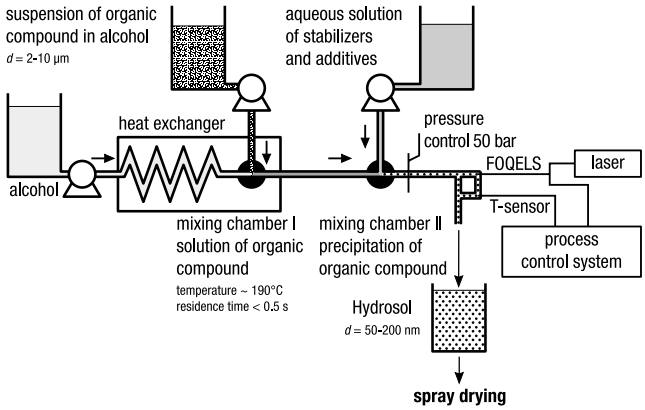

Figure 16. Process scheme of the mixing-chamber process for the preparation of active compound hydrosol nanoparticles by precipitation from hydrophilic solution. Further details in the text.

(ca. 200 °C) and extremely short residence time ($\ll 0.5$ sec). With short-chain alcohols or acetone this method requires a limitation of the system pressure to around 50 bar. The temperature course, residence time, and mixing relationships in the precipitation chamber can be varied within wide limits in a manner specific to the active compound. A laser optic device (FOQELS: fiber-optical quasielastic light scattering) allows online measuring of the mean particle size for process control.[92] As Figure 17 shows, X-ray amorphous, spherical

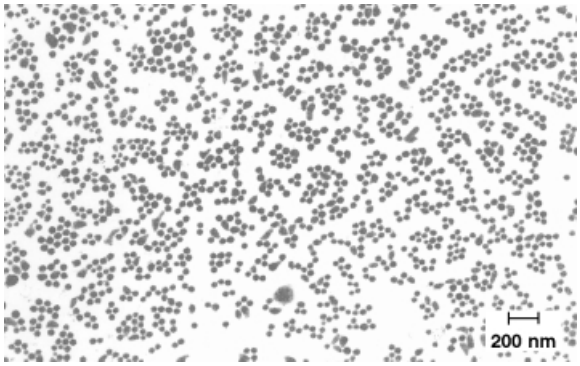

Figure 17. TEM micrograph of a β-carotene hydrosol prepared by the mixing chamber process.

particles of uniform size (in the range 50 – 100 nm) are usually obtained in this way with poorly water soluble or insoluble active materials—in accordance with the Ostwald law and the molecular dynamic model concepts on additive-controlled particle formation of Botet et al.[173]

In agreement with the respective industrial and physiological requirements demanded of hydrosols, biopolymers such as proteins and polysaccharides, or synthetic macromolecules are used as the protective colloids which stabilize the nanoparticles sterically and/or as electrostatically active adsorbate layers.[116, 178] Furthermore, the formulations can be complemented by other industrially or galenically acceptable additives which allow a subsequent conversion of the hydrosols into a redispersible granulate by spray drying or freeze drying (see Section 4). Such a process step can be coupled directly with the continuous hydrosol formation, if as a result of the solvent fractions initially remaining in the precipitation medium there is the risk of particle growth by Ostwald ripening, especially since in accordance with the Kelvin equation (Section 4.1) the apparent solubility of mesoscopic systems increases exponentially with decreasing particle size. This consideration applies particularly to particles with an amorphous solid structure.

Finally, the continuous process for hydrosol formation, that is described here in principle, has the methodological flexibility to make a wide range of nanoparticulate formulations of organic compounds accessible. Thus, for example, polymers and surfactants as well as fats and oils can be introduced by means of the solution phase and in this way novel nanodispersed transport forms for active compounds and effect materials can be prepared (see also Section 4).

3.4. Nanoparticle Formation with Supercritical Fluids

Supercritical fluids (SF) are used in a number of different circumstances for the preparation of micro- to nanodispersed organic systems.[168, 179, 180] Of industrial use are their special solvent properties and the technical feasibility of large temperature gradients.[181] The generation of a sufficiently high supersaturation for the initiation of a precipitation reaction with conventional solvents is on the one hand limited by the generally low dependency of the solubility on temperature, on the other by the difficulty to realize technically the necessary rapid heat exchange. With this in mind one process in which liquid CO_2 is used as cooling agent deserves attention.[182] In this so-called contact cooling process the active compound solution at $-78\,^{\circ}C$ is sprayed into a CO_2 stream and particle formation is induced in the spray droplets by crystallization. Experimental results with an abecarnil/isopropyl acetate system process showed that it was possible to devise systems which also appear to make the formation of nanodispersed precipitates possible.

The use of liquid CO_2 as solvent and precipitating medium for active compounds[183] has been investigated by Larson, King, and other authors.[184] From the viewpoint of toxicological acceptability, the non-combustibility, and the favorable critical data of CO_2 ($p_c = 74$ bar, $T_c = 31\,^{\circ}C$) the so-called RESS process (rapid expansion of supercritical solutions) appears particularly attractive, especially because extra process steps to remove residual solvent may no longer be required. However, SF-CO_2 can act as an oxidizing agent with oxidation-sensitive compounds such as β-carotene and is thus ruled out as a precipitation medium. Consequently, ethane and ethylene were investigated by Chang and Randolph as inert SF precipitation media for β-carotene.[180] In supercritical ethylene ($T_c = 9\,^{\circ}C$, $p_c = 50$ bar) β-carotene shows a change in solubility of about three orders of magnitude to a molar fraction of 10^{-7} in the expansion from a pressure level of 400 bar and a temperature of 50 °C.[180, 181] By immersion of the expansion nozzle into a 10% aqueous gelatin solution stable crystalline solutions could be obtained the mean particle size of which lay at 1 µm, with a fine core fraction

up to 300 nm. In spite of these findings it must be noted that the achievable solubilities of polymers and organic active compounds and effect materials in SF-CO_2, SF-C_2H_4, SF-C_2H_6 are so low that the development of a technically feasible process only appears promising in exceptional cases.

In contrast, in three further process variants, the low SF solubilizing ability for active compounds is used industrially. Both in the GAS process (gas antisolvent process)[185] and in the PCA process (precipitation with compressed fluid antisolvent)[186] SF-CO_2 instead of water serves as the precipitation medium from organic solvents. In the physically related SEDS process (solution enhanced dispersion by supercritical fluid)[183] the organic active-compound solution and the SF-CO_2 are brought into contact in a coaxial mixing nozzle, and thus a rapid extraction of the solvent (e.g. acetone) is possible.[187] However, to date there have only been reports of particle formation in the micrometer range.

Finally, a suggestion from Nakanishi and co-workers deserves attention, namely the preparation of nanoparticles of poorly soluble active compounds and effect materials by precipitation from water-miscible solvents in the supercritical state, if the thermal stability of the compounds permits.[188]. Thus the precipitation of spherical titanylphthalocyanin particles (see also Section 4.3) in the size range of 30–50 nm from SF-acetone at temperatures of 230–330 °C and water as antisolvent was reported.

3.5. Nanoparticles from Polyelectrolyte Complexes

The complex formation of polyanions with polycations in aqueous solution can equally be used for the preparation of nanoparticles under special reaction conditions. They usually take place stoichiometrically by charge compensation,[189] even with structurally very different reaction partners such as globular proteins and linear polyelectrolytes.[190] If the reaction is carried out at sufficiently high dilution, electrically neutral nanoparticles are obtained primarily[189, 191] which, however, because of their hydrophobic nature coagulate to microdispersed agglomerates. The known precipitation reactions for the isolation of proteins from aqueous solution are based upon this secondary reaction.[191, 192]. Electrosterically stabilized primary particles can be obtained when the reaction takes place with a charge-stoichiometric excess of one reaction partner.[189, 193]

Initial experience with nanodimensioned polyelectrolyte complexes in which the reaction partner DNA itself functions as the effect material has been reported recently.[194]

Finally attention is drawn to two more recent developments which represent interesting variants of particle formation with the involvement of polyelectrolytes. In analogy to naturally occurring transport forms of vitamin A by its bonding to specific transport proteins, Thünemann and co-workers[195] have prepared charge stoichiometric vitamin A acid complexes with the polycations poly(L-lysine), poly(L-arginine), and poly(L-histidine) in the form of lamellarly structured nanoparticles (ca. 300 nm) by a special precipitation/redispersion process.[196] The nanoparticles, stabilized with polaxomer 188 poly(ethylene oxide)-poly(propylene oxide) triblock copolymer), could represent an interesting transdermal administration form for vitamin A acid.

Following a procedure described by Decher,[197] Möhwald and co-workers[198] initially formed core–shell hybrid particles by alternating adsorption of polyanions and polycations onto nanosized template particles which were converted into polyelectrolyte capsules after dissolving out the template core. These semipermeable hollow spheres could be used as nanosized precipitation reactors for the preparation of dyestuffs and active compound hydrosols should it prove possible to modify the boundary surface of the hollow space in a substrate-specific manner such that a precipitation reaction takes place preferentially within the hollow spheres.

4. Properties and Areas of Application of Suspensions of Organic Nanoparticles

The emphasis in the application of nanodispersed organic active compounds and effect materials as well as current research and development targets is concerned mainly with the optimization of the bioavailability of active compounds (pharmaceuticals, cosmetics, plant protection) and the control of the optical properties, especially color attributes, by particle size and the supramolecular structure of the particles. In addition, the particle size dependency of the photoelectric properties of organic xerographic photoreceptors is currently the subject of increasing interest in the further technical development of copying processes, in particular laser printers.[199–201] With similar objectives, investigations on nanosized ultrathin layers of such materials have been carried out, but which will not be discussed more closely at this point.[202] In contrast, colloidal-physical aspects of the use of the photovoltaic, photoelectrochemical, and catalytic effects of organic materials have been paid little attention in comparison to inorganic semiconductor materials,[203] in spite of all the hopes which have been placed on this class of materials.[204]

In the next Section the current work and the ensuing products will be reviewed, in which the biological activity and the optical properties of active compounds and effect materials have been optimized by the adjustment of both the particle sizes within the nanometer range and of the supramolecular particle structure.

4.1. Strategies for Increasing the Bioavailability of Poorly Soluble, Organic Active Compounds

By definition, the equilibrium solubility of a substance is understood to be the solubility of the most stable crystal modification in a solvent.[205] This modification is the one with the highest melting point. If, in addition to the melting point T_m (in °C), the octanol/water distribution coefficient, $\log K_{ow}$, is known, then, according to Yalkowsky the molar solubility S_u of an uncharged organic compound, for example, in water, can be estimated with a semi-empirical solubility equation [Eq. (7)].[205] With poorly soluble active compounds a ther-

$$\log S_u = 0.8 - \log K_{ow} - 0.01\,(T_m - 25) \quad (7)$$

modynamically stable increase in solubility by modification of solvent character can be achieved by four alternative methods:[106, 205] 1) Where the active compound is a weak electrolyte the targeted setting of an optimal pH value is obvious. In addition the solubility can be increased by, 2) a change in the solvent polarity (cosolvent), 3) by micelle formation (surfactants), or 4) by active compound complexing (e.g. cyclodextrins).

These measures do not always lead to the objective, since the achievable increase in solubility with acceptable additive concentrations is often insufficient, or, especially for parental administration, the extent of adverse effects does not appear tolerable. Moreover, administration-dependent dilution effects can also lead to unwanted in situ precipitation.[205]

Because of these limitations, methods which are based upon an increase in the apparent solubility and in the rate of dissolution by means of nanoparticulate formulation of the active material, possibly with a simultaneous change in solid structure (metastable crystal modifications) are increasing in importance. According to the Kelvin equation [Eq. (8)][104, 205, 206] where γ represents the boundary surface energy, V the molar

$$\ln(S_{app}/S_0) = (RT)^{-1}2V/r \quad (8)$$

volume, and r the particle radius, at a given temperature T the apparent solubility S_{app} of spherical particles increases exponentially in comparison to the equilibrium solubility S_0 with decreasing particle radius. With values for γ and V which are typical for organic active compounds and effect materials the solubility exhibits a dramatic increase in solubility below a threshold value of $r < 1$ μm. Thus if it is possible to prepare and stabilize a poorly soluble substance in nanoparticulate form an apparently high active material concentration S_{app} can be made available in the dynamic equilibrium.

According to the Noyes – Whitney equation [Eq. (9)][104, 205] a corresponding increased rate of dissolution dM/dt is also correlated as a result where dM/dt stands for the transfer of mass from the solid to the solute state. The high values

$$dM/dt = kA(S_{app} - C(t)) \quad (9)$$

of the specific surface A in a nanoparticulate system also contributes to an increase in the rate of dissolution. The system-specific hydrodynamic relationships of the dissolution process are taken into account in the constant k. In individual cases the solution kinetics are also determined by the temporal change in A and by transient concentration of the solute $C(t)$, as determined by, for example, the resorption kinetics. A further increase in the level of solubility can be achieved by an increase in the boundary surface energy γ if it is possible to prepare the nanoparticles in a metastable crystal modification or as an amorphous particle.[205]

It must be noted, however, that the strategies described for increasing the apparent solubility and the rate of dissolution by nanoparticle formation lead principally to thermodynamically unstable systems which, under the conditions of storage or administration, tend towards recrystallization by Ostwald ripening if the preparation process produces systems with a broad particle size distribution. As already mentioned in Section 3, the mechanical methods of milling or homogenization usually lead to broad particle size distributions the mean particle size of which can lie below 1000 nm. In the area of industrial pharmaceutical research especially, increased activity has been observed recently to improve further the mechanical dispersing procedures with the objective of lowering still further the values of mean particle size and breadth of distribution.[104] As described in Section 3.2.2, significant technical advances have also been made in precipitation processes, which have already led to a broad palette of nanodispersed formulations with improved application properties, particularly in the area of active compounds and foodstuffs colorants.

4.1.1 Active Compounds with Poor Water Solubility

In the search for new active compounds and principles of action, the number of interesting compounds whose potential may be overlooked in standardized screening procedures because of their low solubility or rate of dissolution in water or whose administration in the end fails because of inadequate bioavailability in the normal commercial form of administration is growing. As an alternative to the methods described for the improvement in bioavailability by the addition of cosolvents, micellar and complexing agents, the exploration of the particle size effects has recently become the preferred focus of formulation research and development. The further development of classical milling processes by grinding or homogenizing processes are currently in the foreground of interest—with the development of nanodispersed medical formulations for peroral or parental administration as the objective. R. H. Müller and Liverside et al. have recently reported the preparation and properties of corresponding preparations.[104, 106, 108, 207] In the procedure used by R. H. Müller so-called nanosuspensions were obtained from different active materials with a high-pressure homogenizer; by increasing the number of homogenizer cycles the mean particle size could be reduced to 600 nm in the case of the active material RMKP22, and to around 300 nm with Paclitaxel. The particles obtained were usually X-ray amorphous.

The second procedure is a milling process with ball mills.[108] Because of the use of mechanical milling aids (milling balls of glass, zirconium oxide, etc.) it is unavoidable that with increasing milling time the active compound dispersion is contaminated with milling body fines, which can be a disadvantage for administration, particularly in the area of parenterals.[104, 207] As far as is known the milling products obtained in this way are crystalline nanoparticles, so-called nanocrystals, also with a broad particle size distribution.

The biopharmaceutical aspects of drug formulations accessible by these methods as well as questions of registering have been extensively discussed by R. H, Müller.[104, 207, 208] In addition to the already discussed improvement in bioavailability by increases in apparent solubility and rate of dissolution the improved absorption of poorly soluble active compounds could also be explained by an increased adhesion tendency of the nanoparticles to the wall of the gastrointestinal tract, particularly in formulations for peroral

administration.[207] Furthermore, there are experimental indications that nanoparticles can be absorbed through the GALT system (gut-associated lymphoid tissue.)[209] A further advantage of nanodispersed active compound formulations (< 200 nm) is that sterile filtration techniques can be used without the preparations being subjected to thermal stress.[130] Reference is made here to the extensive literature regarding further details on the status of development and the biopharmaceutical properties of nanodispersed formulations based upon milling and homogenization procedures.[106]

In contrast, there have to date been only isolated reports on the preparation and properties of nanodispersed formulations of poorly soluble active compounds by precipitation reactions. Thus, after extensive investigations Sucker and co-workers[154, 175] were able to prepare X-ray amorphous nanodispersed hydrosols with poorly soluble active materials ($S <$ 10 μg mL^{-1} in water) by controlled precipitation from alcohol solution with water in a static mixer (see Section 3.2). To stabilize the nanoparticles in situ the precipitation reaction was carried out in aqueous solutions of different gelatin types (electrosteric stabilization) or poloxamers (steric stabilization). The hydrosols obtained were transformed by spray drying into storable, redispersible dry powders to prevent particle growth (Ostwald ripening). For this purpose lactose or mannitol were added as spraying aids. With the active compounds israpidin, beclomethasone dipropionate, and cyclosporin, nanodispersed formulations with particle sizes in the range of 200 nm were obtained. In detailed investigations with redispersed cyclosporin hydrosols in animals, concentrations of the active compound similar to those found after the injection of micellar solutions were detected in different tissue types after intravenous injection.[154, 175]

With the active steroid budesonide Matijević and Ruch recently drew attention to the rarely explored industrial potential of the preparation of micro- to nanodispersed active-compound hydrosols by a precipitation reaction from hydrophilic solution.[157]

4.1.2. Water Insoluble Active Materials

The most extensive experience on the improvement of peroral bioavailability of insoluble active compounds by hydrosol formation currently lies with synthetic carotenoids. The carotenoids constitute a class of color pigments widespread in nature with yellow to red color tone nuances.[113, 210, 211] All these compounds are characterized by a structural element consisting of a polyene chain built up from four isoprenyl units the different substitution patterns of which at the chain termini allow an extraordinarily large breadth of variation within the compound class. Today about 600 carotenoids are known.[212] The best known representative, β-carotene, was first isolated from carrots in 1831 and has been produced industrially since 1954.[213] Since then synthetic routes for a whole series of carotenoids have been developed and converted into industrial production processes;[214] a selection is illustrated in Figure 18.

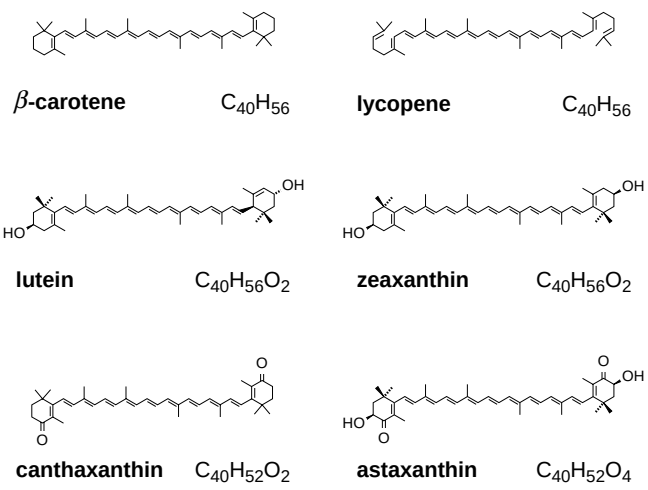

Figure 18. Selection of carotenoids of physiological importance and of interest as foodstuff colorings and for animal nutrition. All the compounds are insoluble in water. Aqueous applications therefore require nanodispersed formulations for optimization of the bioavailability and the coloristic properties.

In addition to the described color the physiological function of carotenoids is of considerable interest. Best known is the action of β-carotene as provitamin A.[211] The practical use of nature-identical synthesis products as active compounds in pharmaceuticals and cosmetics or as coloring agents in the foodstuffs and animal feeds is complicated, however, by the insolubility in water and poor solubility in fats and oils typical of the compound class as a whole.[114, 115, 210, 211] Therefore the conversion of the crystalline synthesis product into nanodispersed formulations is an important requirement, especially for the plethora of forms of administration of the carotenoids in aqueous media. Currently different recipe variants of the mixed chamber process described in Section 3.3.2 are used widely for the production of diverse carotenoid preparations with high color intensity and high bioavailability.[152, 153] Figure 19 gives an overview.

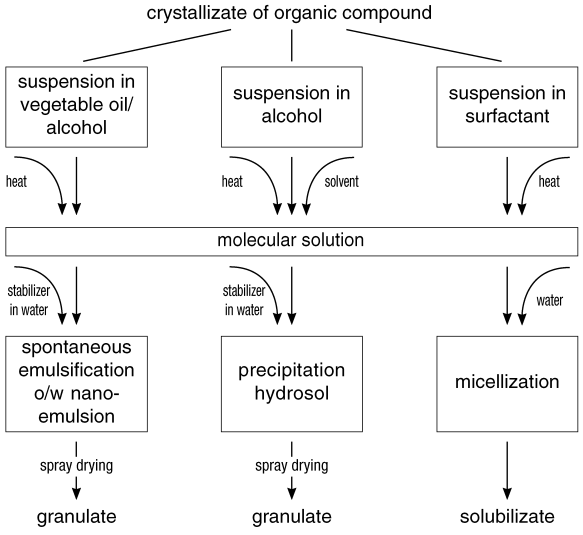

Figure 19. Preparative variants of the mixing-chamber process for the preparation of nanodispersed, water-compatible carotenoid formulations.

Depending on the composition of the active-compound solution produced by temperature shock, nanodispersed hydrosols, emulsions, and micellar solutions are produced for different uses.[152] The effect of particle size and the equally technically controllable supramolecular structure of the dispersed phase on the color are discussed in detail in Section 4.3. Moreover, there are increasing indications that biological absorption is influenced equally extensively by the supramolecular structure of the particles. In feeding experiments with calves and rats it was possible to demonstrate by measurement of the blood-level values or the vitamin A liver-storage values that the biological absorption of β-carotene hydrosols increases significantly with decreasing particle size (see Figure 20).[153, 215] Under comparable experimental conditions milled crystalline products in the micrometer particle size range were practically not absorbed (Figure 21). In other areas of animal nutrition, carotenoids in nanodispersed formulations show an increased bioavailability. Thus nanoparticulate hydrosols from canthaxanthin are widely used in poultry breeding for a natural egg-yolk pigmentation.[115] Analogously astaxanthin, the coloring agent which gives fish and sea food a reddish color, is used as a nanodispersed, amorphous hydrosol formulation in trout and salmon farming.[115]

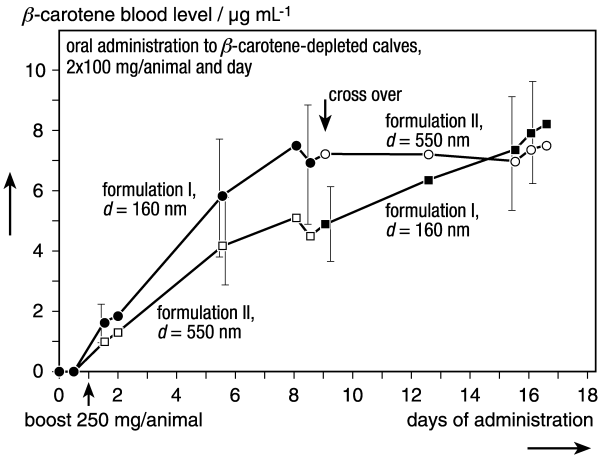

Figure 20. Influence of particle size on the bioavailability of β-carotene hydrosol during oral uptake by calves with minimal β-carotene status. At the cross-over point the feeding to the two calf cohorts was continued after exchange of formulations I and II.

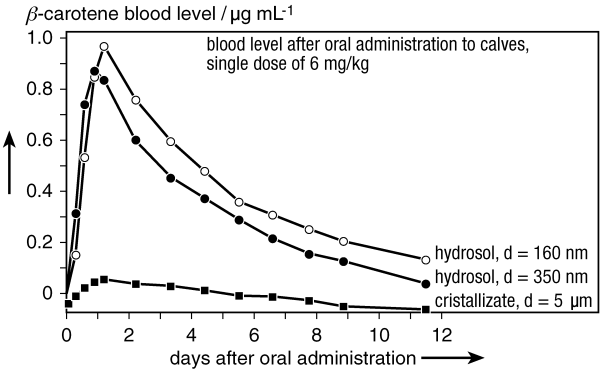

Figure 21. Time course of the β-carotene blood level after single oral administration of β-carotene to calves with minimal β-carotene status. Comparison of nanodispersed formulations with a microdispersed crystallizate.

In all the applications described the nanoprecipitates are used as granulates which are obtained by spray drying procedures. In these formulations the active compound is present in concentrations of 5–10%. The remaining constituents are composed of protective colloids (e.g. gelatin, polysaccharides) and other additives (emulsifiers, antioxidants) typical for precipitation processes as well as further additives (sugar, starch) which enable spray drying and which also contribute to storage stability and redispersibility in the administration medium.[152, 153] The mode of action of the proteins and polysaccharides used as protective colloids are described in detail in ref.[178]. Details on their function in nanodispersed carotenoid formulations are discussed by Horn et al.[152, 153, 216]

In addition to physiological properties of different carotenoid hydrosols described already—the coloring aspects, of interest to the foodstuffs industry, are discussed more closely in Section 4.3—the pointers for further, health-promoting actions of carotenoids are currently increasing. In analogy to the protective action in the plant kingdom, where in the photosynthesis system carotenoids protect chlorophyll from attack by oxygen radicals and singlet oxygen,[217] similar effects are also proposed in other organisms including humans. Thus in patients with low β-carotenoid status there are signs that the risk to health from prostate cancer can be reduced by β-carotene supplementation.[218] The protective action of β-carotene against UV-induced skin changes has been established by Biesalski et al.[219] Other carotenoids which are currently the subject of intense investigation for their health-promoting effects are lycopene,[220] the red colorant in tomatoes, and lutein and zeaxanthin.[221] Recent results suggest that a high lycopene intake by the consumption of tomatoes also reduces the risk of prostate cancer.[222] Lutein and zeaxanthin are the only carotenoids to be concentrated in the yellow spot of the retina and in other ocular tissues and could be involved in the prevention of macula degeneration associated with age[223] and a reduction in the risk of cataract formation.[224]

These are just a few examples of the function of carotenoids in the area of health and nutrition which in the future could extend considerably the use of the nature-identical active compounds. Nanodispersed formulated carotenoids could thus represent important examples for effect-optimized nutraceuticals, that is, nature-identical active materials which manifest their health-promoting, prophylactic action in the boundary between nutrients and pharmaceuticals.

In view of this experience with compounds of the carotenoid class it may also be possible to precipitate poorly soluble hydrophobic active compounds as nanodispersions from hydrophilic solution with appropriate temperature control, contrary to the occasionally expressed opinion.[104] However, it has to be presumed that for an understanding of particle formation in these systems the classical concepts on nucleation and growth as they have been used successfully for the understanding and process development of normal crystallization and precipitation reactions[163–166, 168, 225] play a rather subordinate role in view of the extremely high supersatura-

tions which are achievable, and spinodal separation phenomena possibly dominate in the particle formation process.

Nanoprecipitation processes in association with the exploration of suitable natural and synthetic additives with high active-compound affinity offer attractive prospects for the development of nanodispersed pharmaceutical formulations of lipophilic active compounds which could provide industrial-pharmaceutical research and development with new stimuli.

4.2. Nanodispersed Systems for the Targeted and Controlled Release of Pharmaceutically Active Compounds

In every day industrial-pharmaceutical parlance the term nanoparticle has come to be used to mean nanodispersed polymeric support systems for active compounds.[107, 109, 151, 226–228] They differ fundamentally from the nanosuspensions[106] and hydrosols[154, 175] discussed in Section 3 in which the polymers assume the function of surface-active protective colloids for the colloidal and possibly also the chemical stabilization of the nanodispersed active-compound phase.

Nanoparticles thus represent an alternative to other active-compound transport forms such as microemulsions,[229] niosomes,[230] liposomes,[227, 231] or other so-called SLNs (solid lipid nanoparticles).[208, 232, 233] One of the reasons for the search for alternative, polymer-based transport systems is that an increased stability of the nanodispersed formulation of the active material during storage and administration appears desirable.[151, 234] Moreover, the targeted functionalization of the polymer matrix or the solid-particle surface appears more readily realizable for the controlled and targeted release of the active compound.

Since the pioneering work of Birrenbach and Speiser[235] and Couvreur and co-workers[236] the search for biocompatible polymer materials and methods for their nanodispersed formulation has lead to a flood of publications (see Section 3). In particular, the biopharmaceutical, physiological, and therapeutic properties of such systems have been investigated in extensive studies, including clinical trials. Critical evaluation of the results have been presented recently in reviews[208, 228, 234] and monographs,[106, 151] and already belong to pharmaceutical-technology textbook knowledge.[107] Consequently, a detailed discussion is superfluous at this point especially since nanodimensioning only indirectly affects the properties of the active compound. In summary it must be pointed out, however, that in spite of the considerable research effort which has been expended and the encouraging results in certain areas, nanoparticles as support systems for active pharmaceutical compounds have as yet found no use in medical practice.[208, 228] The reasons given for this include unanswered questions on long-term stability and on the cytotoxicity of polymers and their degradation products—and thus obstacles to medical registration/legislation—as well as industrial production aspects such as the transfer of the laboratory methods described to competitive production processes.[151,208]

4.3. Optical and Electrooptical Properties of Nanodispersed Organic Pigments and Dyestuffs

Crystals of organic molecules are finding numerous technical applications as chromophoric dispersion colloids.[105] Amongst the most important applications are the coloring of lacquers, printing inks, color toners, and a large number of industrial plastics.[101] The most important class of pigments include the phthalocyanins (blue), perylenes (red), and azo dyes (yellow). Special demands are placed on pigments which are used in the coloring of drinks and foodstuffs; the carotenoids as nature-identical pigments are also mentioned in this context. The broad field of relevant technical aspects of respective pigment formulations cannot be undertaken here.[101, 105] Both the coloring properties (color strength, color shade, transparency) and the flow properties of pigmented systems important for processing are influenced quite significantly by the particle size and particle shape at a given pigment concentration.[237]

According to the current state of the art the technical demands placed on the fineness of the pigment and dispersion-dyestuff formulations used in practice are fulfilled by dry and wet milling processes.[101] Usually dispersion colloids with a mean particle size range of around 1 μm are obtained. Work with the aim of a further reduction in particle size down to the nanometer region is currently not of immediate interest from the practical viewpoint. Usually the milled products obtained are subsequently subjected to a so-called formulation or finishing process to obtain a product as uniform as possible in particle size and shape by controlled Ostwald ripening or modification transformation in organic solvents. More recently specific additives which by reason of their molecular structure preferentially occupy individual growth faces and thus enable a specific control of growth kinetics and thus particle habit have been used to control this recrystallization process.[238] Empirical methods of screening are being replaced to an increasing extent by strategic methods of crystal engineering with the use of more powerful computers. Thus with organic molecules it is in some cases already possible to predict their probable packing density in the solid, their orientation in the crystal lattice, and their interaction energies.[239, 240] However, it is to date still not possible to predict quantitatively the particle size dependency of the absorption band structure. The amount of pertinent experimental work is equally sparse, however. Thus hypsochromic shifts of the absorption bands have been observed with ultrathin epitactic layers (1 – 100 nm layer thickness) of perylenes, phthalocyanines, and other pigments, and interpreted as quantum size effects.[202]

Otherwise only isolated reports on investigations into particle size dependencies of optical constants of molecular crystals have appeared.[241–244] In the work of Nakanishi and co-workers the choice of systems investigated was aimed primarily at the potential fields of applications of organic nanocrystals in the area of microelectronics and photonics as alternatives to inorganic systems.[245, 246] Thus, on the basis of the methods described in Section 3.3.2 dispersed systems in the particle size range down to 20 nm were obtained by nanoprecipitation in water of different classes of compounds

(pseudoisocyanines, merocyanines, perylenes, polydiacetylenes) from hydrophilic solution (ethanol, acetone, THF).[174, 245] In the investigations on the perylene system a shift in the absorption band maximum of about 30 nm was found on a reduction in particle size from 200 nm to 50 nm.[242] Recently band shifts of up to 30 nm have also been observed with diphenyl-naphthyl-pyrazoline (DPNP) nanocrystals (400 nm to 20 nm).[244] In explaining these findings a confinement effect was excluded, however, because of the observed particle size range, and a reduction in intermolecular interaction with decreasing particle size through crystal-lattice deformation was discussed as the cause. In investigations on the polydiacetylene system a blue shift of the absorption maximum of about 15 nm was also found with decreasing particle size (150 nm to 70 nm) and similarly interpreted.[243, 247] For a complete explanation of the experimental results, possibly also within the context of confinement effects, an extension of the investigations to particle sizes to 10 nm and below appears necessary. A preparative access to this particle size range was recently demonstrated by the precipitation of organic molecular crystals in inorganic sol–gel templates.[248] The same applies in this respect to a possible increase in the third order non-linear optical susceptibility, $\chi^{(3)}$, by confinement effects in nanodispersed particulate systems.[246, 247, 249] In organic systems the expectations refer in particular to the polydiacetylene class of compounds.[243, 247] However, an experimental confirmation of corresponding theoretical predictions is currently unavailable.[246, 247, 249]

Particle size effects are also receiving increasing attention[250] in connection with the widespread use of organic photoconductors for xerographic copying processes.[201] In the optical production of a graphic electrostatic-charge pattern on a reversibly chargeable carrier, materials are required which at high quantum yield possess high photoconductivity in the visible spectral region—and more recently for use in diode laser printers in the near infrared region too. For this purpose organic semiconductor pigments such as phthalocyanines, perylenes, and azo pigments are converted into nanodispersed systems (20–500 nm) by complex milling processes and incorporated into polymeric support materials in high concentrations.[199, 201, 250] However, here too the relationships between the particle size distribution and the supramolecular particle structure and the technically relevant physical properties such as photoconductivity and charge transport have still been little researched—in spite of the high technical state of development—since the previously described morphological parameters are poorly controllable by milling processes.[199, 200, 201, 250, 251]

In this context particle size effects are significant for two reasons: on the one hand the production of the charge carriers at the boundary surface of the optically active pigment particles takes place by the interaction with the polymer matrix, with additive charge carriers, or also with adsorbed oxygen or water.[200, 201, 250] At a given pigment volume concentration the size of the photooptically active surface increases with decreasing particle size, which is important especially in field strengths of $< 10\ V\,\mu m^{-1}$.[250] On the other hand the photoconductivity of the material, especially with inert polymer supports is determined to a considerable extent by the percolation structure, that is, the mean particle distance and the connectivity of the particle configuration.[200, 250]

Whereas the important technological advances have hitherto been based upon empirical development work,[201] with the example of titanyl phthalocyanin (TiOPc), an especially efficient representative of organic photoconductors, a first step towards a systematic treatment of this problem has been made more recently.[199] The successful preparation of a TiOPc/BBL (BBL = poly(benzobisimidazobenzophenanthrone)) nanocomposite with spherical ($d = 100$ nm), X-ray amorphous TiOPc particles was achieved by in situ hydrolysis of a molecularly dispersed, solid solution of a Lewis acid complex of TiOPc in a polymer matrix (BBL). By subsequent shaping of the films in $CHCl_3$, vapor-phase transformation into the crystalline β-modification with rod-shaped pigment crystals took place.

In addition to the critical influence of crystal structure on the absorption process and thus on the frequency dependency of the photoconductivity with organic molecular crystals,[201] in the case of TiOPc a progressive short-wave shift of the characteristic Q bands (700–900 nm) of the β-modification was observed with controlled reduction in particle size.[199, 252] The photoconductivity found in this wavelength region with nanocomposites containing the equally photoconducting (450–650 nm) BBL support material with crystalline TiOPc lay more than two orders of magnitude above that of comparable materials with amorphous particles.[199, 251]

The process described for the preparation of nanodispersed composites of organic photoconductors with broad spectral sensitivity appears not only interesting from the point of view of basic research on xerography, but it could also open up new industrial methods for the preparation of photoactive materials with possible uses in the storage of solar energy on the basis of photovoltaics and photoconductivity.[199, 253]

The classical particle size effects which are quantitatively describable by the Mie theory in the case of isometric particles must be differentiated in principle from the previously described optical particle size effects, which are based upon a change in the optical constants in nanodimensional crystals with high intermolecular interaction.[254] Accordingly, at a given chemical and crystallographic structure and hence fixed optical constants the particle size determines to a large extent the optical purity and transparency of a pigmented system through the size-dependent balance of absorption and scattering. A special case arises with systems with X-ray amorphous particles recently prepared by the described precipitation reactions. As is shown below with the example of the carotenoids, different supramolecular short-range order structures can be obtained in a targeted manner by variation in the precipitation conditions, which at strong intermolecular dipole interaction leads to marked changes in the absorption spectrum and thus the coloristic properties. Figure 22 summarizes the effects of the different molecular, supramolecular, and solid-state structure on the coloristic properties of a nanodispersed system. In the case of β-carotene the change in particle size and the supramolecular structure, particularly the transition from the crystalline to the amorphous state, has a dramatic effect on the structure of the absorption spectrum.

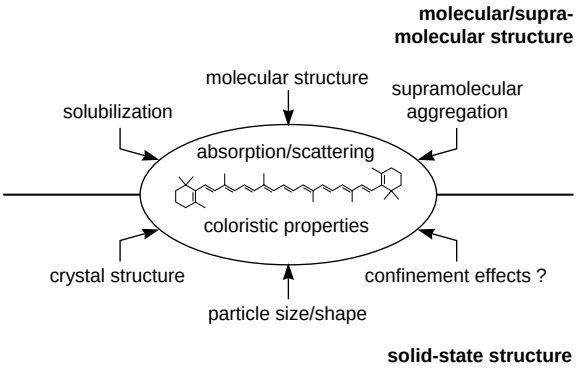

Figure 22. Control of the coloristic properties of nanodispersed carotenoid hydrosols by molecular, supramolecular, and solid-state physical factors.

Figure 23 shows a comparison of absorption spectra of different β-carotene formulations which allow color-tone nuancing from yellow, through orange, to red (see also Figure 24).[152] In comparison to the absorption spectrum of a molecular solution in *n*-hexane the spectra of the amorphous nanoprecipitates show an increasing blue shift with decreasing particle size, whereas a red shift with concomitant change in band structure characterizes the spectra of the crystalline dispersion colloids.[152] According to the most recent experimental and theoretical investigations supramolecular structure and particle size effects participate equally in the development of the complicated band structure.[255]

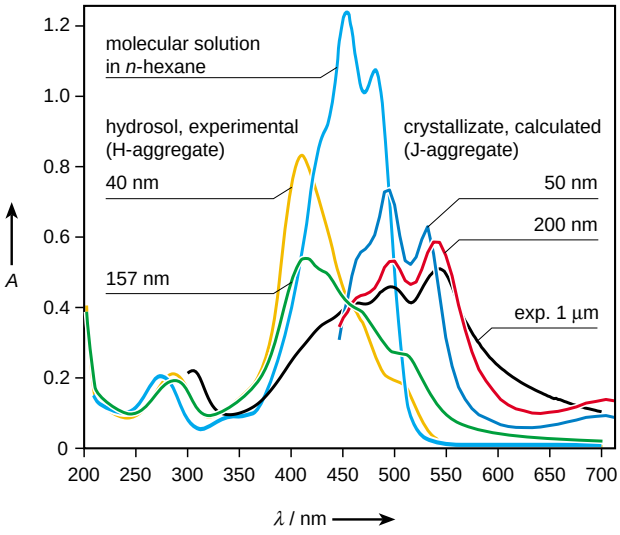

Figure 23. UV/Vis absorption spectra of 5 ppm β-carotene. Influence of aggregate structure and particle size compared with the molecular solution in *n*-hexane.

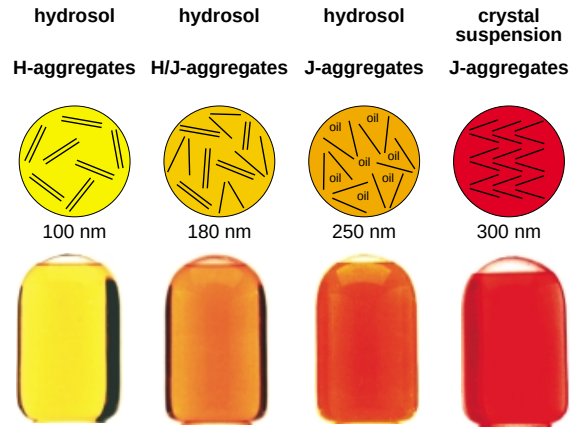

Figure 24. Influence of particle size and aggregation structure on the color tone nuance of nanodispersed β-carotene hydrosols.

In accord with the crystal structure of β-carotene[256] the red shift is explained by a so-called J-aggregate interaction (head-to-tail) of the two β-carotene molecules per unit cell.[152, 257, 258] As yet there has been no exact assignment of the superimposed vibrational coupling structure. The spectrum of a milled crystalline product (mean particle size ca. 1 µm) and the spectra of nanodispersed systems calculated by the Mie theory on the basis of single-crystal spectra[259] show no similarity with the spectra of the products of the same size class obtained by nanoprecipitation. As shown in Figure 25, these products are composed of particles with a core–shell structure which is visualized electron microscopically by the use of specific staining techniques.[152, 153] Details of the function of the gelatin used as protective colloid (type B100, adsorbate film thickness ca. 40 nm) and other protective colloids have been discussed thoroughly elsewhere.[152, 216]

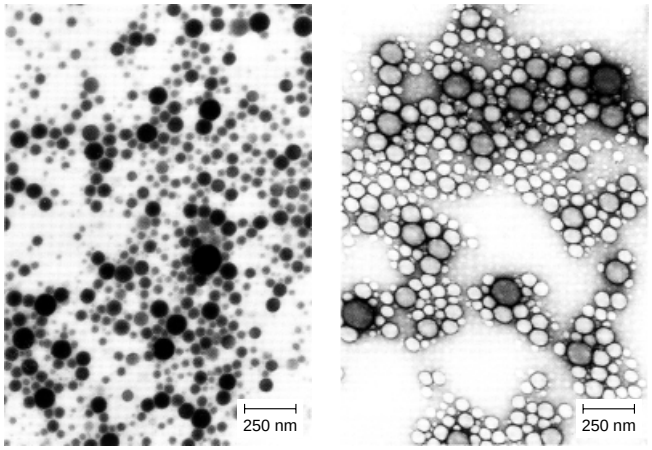

Figure 25. Electron microscopic representation of the core-shell structure of nanodispersed β-carotene hydrosols. The specific staining of the active-compound core (left) was carried out with OsO_4, the gelatin shell (right) with uranyl acetate.

More recent X-ray and theoretical investigations are now providing the first clues on the supramolecular structure of the active compound core and on the significance of the characteristic blue shift of the absorption bands of β-carotene nanoprecipitates.[255] From a simple exciton model[257, 258, 260] and quantum-mechanical molecular-modeling calculations[261] it was possible to show[255] that intermolecular interactions within the context of an H-aggregate structure ("card-stack structure")[152, 257, 258, 262] are consistent with the spectroscopic findings. Hypsochromic band shifts of 44 nm and 40 nm were calculated for an ideal H-aggregate and a tetrameric (2_1_1) aggregate, respectively, which corresponds to a supercell formed from two H-aggregates. In contrast, a band splitting (exciton splitting) is expected for a J-aggregate: in addition to

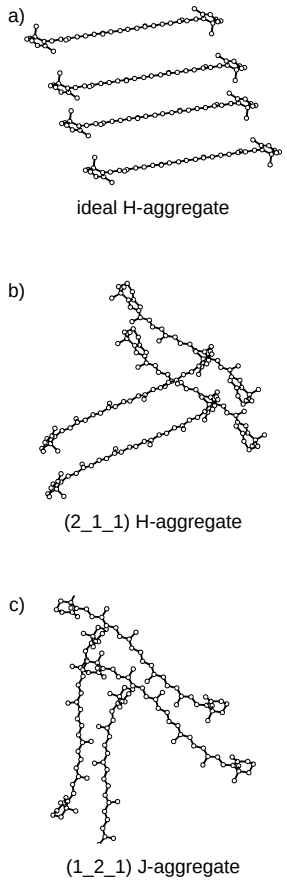

Figure 26. Spatial configuration of different β-carotene tetramers. a) ideal H-aggregate; b) superimposition of two H-aggregates in a (2_1_1) supercell; c) J-aggregate in a (1_2_1) supercell. Quantum chemical calculations give hypsochromically shifted absorption bands of 44 nm and 40 nm for the H-aggregates in (a) and (b), respectively. As expected, an exciton splitting with a bathochromic component (44 nm) and a hypsochromic component (7 nm) is calculated for the J-aggregate.

a bathochromic band shift of 44 nm calculations also gave a component with a 7 nm hypsochromic shift (see Figure 26).[255]

Process and recipe-specific influences on the optical properties of nanoprecipitates can thus be explained by the preferential formation of H- or J-aggregates.[255, 262] Apparently, with decreasing active-compound concentration the formation of H-aggregates is favored during precipitation, whereas small amounts of oil in the recipe induce the formation of J-aggregates. Information on the dimensions of the coherently aggregated supramolecular structures comes from the Bragg profile analysis of the X-ray wide-angle scattering data.[255] According to this the active-compound particles illustrated in Figure 25 are built up of crystalline structural units with dimensions down to about 30 nm in which the molecules of the active compounds are present mainly as H- or J-aggregates, depending upon the selected precipitation conditions. The aggregate sizes correspond to aggregation numbers of up to 10000 molecules. Figure 27 gives a conception of the supramolecular construction of the β-carotene hydrosol particle calculated from the spectroscopic and X-ray data. The thickness of the gelatin adsorbate layer was determined by dynamic light scattering.[216] Whether a morphological construction from crystalline structural units of the named order of magnitude can result in confinement effects is currently unknown

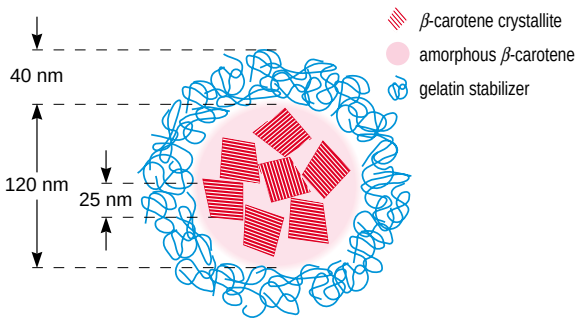

Figure 27. Core-shell structure of the β-carotene hydrosol particle. According to the spectroscopic data and WAXS measurements the active material core consists of H- and J-aggregates with dimensions of up to 30 nm, which corresponds to a maximum aggregation number of 10000 molecules.

5. Discussion and Outlook

A series of tasks may stem from the discussions here, which A) concern fundamental aspects of the nanoparticle formation, and B) which have as their objective the development of industrial processes for the targeted production of nanodispersed formulations of organic active compounds and effect materials. Closely associated with this is C) the evaluation of special physical properties associated with nanoscopic dimensioning with a view to new or improved physiological or industrial properties of organic nanoparticles.

On point A the following problems appear to be in need of special clarification:

1. Which structures are formed at high spontaneous supersaturation? On the one hand it remains to be explained whether—counter to textbook opinion—spinodal demixing phenomena do indeed play a certain role in low molecular weight systems. On the other hand investigations are needed to explain to what extent the structure of the particle exhibits similarity with the bulk structure or whether strongly hydrated, amorphous precursors, or aggregates of nuclei dominate.
2. Are long-lived precursor structures from aggregated molecules present prior to the actual nucleation? If this question is to be answered in the positive the significance that is attributed to these precursors in particle formation needs to be explained.
3. Which structure transformations occur during the transition from the initial nucleus to the observable nanoparticle? This question is of considerable relevance since as shown in Section 2 there are many examples for the particle formation processes which do not run according to the simple nucleation/crystallization scheme.
4. How is the surface of the intermediate stages composed in respect of structure, charge, etc? This point is of great significance in as much as these properties determine the homo- and heteroaggregation behavior.

On point B there are also a number of tasks which need to be addressed.

1. How, with knowledge of the molecular parameters, can the stability and aggregation behavior of nanoparticles be predicted? As was shown in Section 2, in this respect many questions remain unanswered.
2. Is the mean-field description of aggregation process, that is, without consideration of local structure formation and reconstruction processes, sufficient?
3. Can the process in which the production of supersaturated systems is carried out with turbulent or laminar mixing be theoretically described? This task is demanding as several length scales must be considered at the same time. Clearly this question is particularly relevant when the particle

formation processes take place on the same time scale as the mixing processes.

4. How do additives interfere with the particle formation process? This question includes a large number of subsidiary points, for example: nucleation by additives, adsorption/incorporation of the additive molecules into the initially formed nanoparticle, influencing the growth of the nanoparticle with additives, influencing the crystal structure with additives, control of aggregation, and colloidal stabilization with additives.

In view of the described complexity of the, in part competitively occurring, elementary process which take place during particle formation a strategic approach to the selection of suitable additives which, for example, limit particle growth to the nanometer range and stabilize the nanodispersed system against Ostwald ripening and agglomeration appears extremely complicated. Although much is known about the physico-chemical principles of colloidal stabilization of dispersed systems and the surface interactions of polymeric and surfactant additives, little is known of the molecular structure – activity relationships which in certain cases would allow a targeted approach. This explains the observation that the choice of additives has hitherto been made empirically and a mechanistic interpretation of their action is at best qualitative. In most instances technical development work is as a result extremely time consuming and perhaps could be somewhat accelerated by methods of statistical experimental planning.[263] Recent developments in the area of combinatorial materials research could bring advances here. After the successful adaptation of combinatorial synthesis techniques (initially developed for active-compound screening[264]) to inorganic function materials by P. G. Schultz and co-workers[265] the first reports on the combinatorial synthesis of polymer libraries[266] and suitable analytical methods for the rapid screening of such libraries in respect of interesting target parameters have been published.[267] Further development of these techniques would not only make the search for suitable additives more efficient, but there is the hope that with the further development of statistical procedures structure – activity relationships can be derived from analysis of the extensive data sets. Then in the next optimization cycle the discovery of substrate-specific additives could be accelerated still further with this knowledge by means of combinatorial methods.

In regard to the questions concerning the outlook for the innovative application of nanodispersed organic formulations arising from point C, the carotenoid class of compounds, the areas of application of which could be optimized and expanded by nanodispersed formulation, has already been discussed in Section 4. Similarly, new perspectives for the development of oral forms of administration with improved bioavailability and parental administration forms with increased tolerance arise for poorly soluble pharmaceutically active compounds. Moreover, nanodispersed formulations, particularly of thermolabile active compounds, open up the possibility of replacing thermal sterilization processes by a more gentle sterile filtration. In the area of cosmetics the high surface activity of nanodispersed formulations could make the development of new forms of application of active cosmetic and effect materials with improved dose – activity relationship a possibility.

In the area of plant protection as well, alongside the highly expensive search for new active compounds, the development of innovative formulations for the optimization of the effect is gaining in importance. The improvement in bioavailability and the related reduction in potential environmental contamination are therefore part of the crucial development activities of industrial formulation research. Nanodispersed active compound formulations in the form of water redispersible granulates could play an important role in this respect.[268] In addition to the previously described optimization of coloristic effects the improvement of processing and application properties by a nanodispersed formulation is also conceivable in the use of organic pigments, for example, in plastics processing or in ink-jet inks.[269]

Similarly, in regard to the previously discussed solid-state physical and catalytic properties of organic systems, which are of fundamental importance in the promising areas of photonics, electrooptics, and energy storage, theoretical considerations on particle size effects suggest that with nanodispersions of effect materials an improvement in technologically desirable properties may be expected. However, the development work is still in its early stages since until now there has been an absence of technologies which would allow the targeted production of organic effect materials with particle sizes in the desired lower nanometer range.

This review has attempted to present a summary of the numerous and thematically widespread activities in the preparation and use of organic nanoparticles. It is clear that the experimental work on the preparation of nanodispersed systems has been conceived purely empirically and without acknowledgment of the extensive theoretical state of the art. There is clearly an urgent need for action to develop the obviously interdisciplinary field of preparation, characterization, and use of organic nanoparticles more efficiently than before. In the light of the extensive activity in the area of inorganic systems the interest of university research institutes to work in the corresponding area of organic systems appears to be comparatively under developed. This under development is all the more surprising since the problems described deserve considerable attention, both from the viewpoint of basic research as well as in regard to the potential areas of use of nanodispersed active compounds and effect materials which range from biomedical to information technology fields of application.

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