

Amphiphilic Molecules in Drug Delivery Systems

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Abstract Numerous drug delivery colloidal systems are formulated using polymers or surfactants or a mixture of both, typically due to their self-assembly properties. Molecular self-assembly creates the possibility to dissolve and protect drugs from adverse external environments. Therefore, it is important to understand the interactions behind the self-assembly phenomena of surfactant and polymer molecules, polymer-polymer and polymer-surfactant mixtures. A number of colloidal structures used in drug delivery formulations such as micelles, vesicles, liquid crystalline phases, microemulsions, polymer gels, aerosols, polymer-polymer and polymer-surfactant complexes will be illustrated in this chapter and their main physicochemical properties will be highlighted, keeping in mind their relevance to the drug delivery research field.

Keywords Self-assembly • Amphiphilic • Nanoaggregates • Phase diagrams • Drug delivery systems • Personalized medicine

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1 Introduction

The states of matter extend well beyond atomic solids, liquids and gases. Matter organizes itself at many different length scales and in many distinct forms, each distinguished by its microscopic symmetries and dynamics. The properties of most materials result from disorder or heterogeneity at some length scale much larger than the atomic scale. More and more the details of the interactions at the atomic scale need to be understood in order to explain the properties of everyday materials.

The field of soft matter is broad and extremely interesting. There are, for instance, non-crystalline states with various degrees of order (liquid crystals) and there are some states (glasses and gels) that are disordered but which behave as solids. Polymers (biopolymers), surfactants, emulsions, microemulsions and biomembranes are some examples that belong to the complex field of soft matter. The organization within such soft structures, at a certain length scale, brings the potential for encapsulating drugs, turning the structure into a drug delivery vehicle. That is to say, for instance, that the hydrophobic core of a self-assembled structure can dissolve large amounts of water-insoluble drugs.

In applications such as drug therapy, soft systems are generally preferred due to their flexibility and biocompatibility. It is possible to tailor the properties of soft systems such as internal structure and surface activity, in a relative easy way. Furthermore, specific environments (within the body) play their role in tuning such properties and allowing the most desired effect. Also, drugs may be amphiphilic and surface active, altering the organization/structure and the stability of the drug delivery vehicle.

Together with the organization of matter at different length scales, the size of the drug vehicle is in many cases very important. On one hand, the small size of the vehicle creates the possibility for intravenous administration; on the other hand, nanoscale devices and/or nanoscale components of larger devices, of the same size as many biological entities (e.g. proteins and DNA) and structures (e.g. viruses and bacteria), create the potential for crossing many barriers within the body and engage with the cellular machinery.

The “internal” organization and the size of the drug vehicle need to be carefully chosen regarding some crucial aspects of the therapy. For instance, for an efficient and safe therapy, the concentration of the drug should be both sufficiently high at the site of action and constant within the therapeutic window over the period of action. Usually, drugs are randomly distributed to the entire body resulting in high drug concentration in non-target sites, leading to detrimental side effects. Also here, the use of sub-micron drug delivery vehicles is highly advantageous when compared to conventional drug formulations due to the possibility for drug targeting. Other important aspects to take into account when choosing the drug delivery vehicle are: shape, stability, susceptibility to breakdown/degradation, the tendency to undergo self-aggregation, drug selectivity, rate and extent of the drug release, drug adsorption

and solubilization capacity, preservation of drug activity and integrity, reduction of drug toxicity, site of action, sustainability and route of administration.

Many complex interactions are present in complex mixtures, such as pharmaceutical formulations. To create appropriate and efficient drug delivery vehicles, one needs to be aware and possess the knowledge on molecular biology and surface/colloid chemistry. The advances in these fields does not always correlate with the development in drug formulations. In part, this is due to the lack of knowledge on the physicochemical and surface properties of the formulation components. In many cases, one component may play more than one role in the system. The “deconstruction” of the formulation recipe and the understanding of the phase behavior of the mixture may be a crucial step to engineer novel ways to deliver drugs. Furthermore, understanding the physicochemical and surface behavior of polymers and, particularly, biopolymers, which play a crucial role in the regulation and integration of life processes and act with high specificity and effectiveness, is very important in the design of physical or chemical modifications that may increase the life-time of the biopolymer and improve its bioavailability.

Polymers and surfactants can be used individually or as mixtures bringing new and strong advantages into the field of drug delivery. The characteristics of these drug vehicles may be tuned varying different parameters such as size and type of the hydrophobic alkyl chain of the surfactant, the nature and size of the polar head group of the surfactant, concentration, salt content, temperature, pH and presence of co-solutes.

Polymers are used in drug delivery due to their efficiency as stabilizers, their capacity to form gels and to control the rheology, even at low concentrations, and also, in special cases, analogous to surfactants, their capacity to form self-assembled stable structures. In some cases, another advantage is their biodegradability potential.

Polymer-surfactant and polymer-polymer associative mixtures present several different properties from the individual behavior of polymer or surfactant systems. Of particular interest, it is the fact that polymer-surfactant and polymer-polymer associative mixtures are capable of forming concentrated complexes/nanoparticles upon dilution. If on one hand the degradation or disruption of surfactant and polymer systems has particular interest in some cases, on the other hand dilution or degradation of the drug vehicle in the body fluids is not desired before a particular site of action is reached, keeping particle integrity. The delay of vehicle degradation and drug release may be achieved by using polymer-surfactant or polymer-polymer complexes as drug vehicles. The polymer and/or the surfactant can be the active component (i.e. drug) and, in this case, the drug is said to be complexed.

The aim of this chapter is to go through the relevant physicochemical features of surfactants and polymers, both individually and in mixtures, to the field of drug delivery. First, surfactant and polymer systems will be analysed individually, followed by the discussion on their synergetic interactions. Finally, a brief practical overview on drug delivery systems/formulations and the *in vitro* and *in vivo* applications will be presented.

2 Surfactants

2.1 Introduction

Surfactant systems play an important role in modern drug delivery since they allow, for instance, the control of drug uptake and release rate and minimization of drug degradation and toxicity. An effective synergism between surfactant systems and drugs is nowadays recognized as a key issue to assure therapeutic efficiency. Thus, it becomes important to understand the physicochemical properties and behavior of surfactants in formulations.

2.2 Surfactant Properties

Surfactants, or surface active agents, are very exotic molecules due to their amphiphilic behavior. This means that a surfactant molecule contains both a hydrophobic part (lipophilic) and a hydrophilic part (lipophobic). The non-polar hydrophobic part is typically referred to as tail (composed by one or more hydrocarbon chains, although fluorocarbon and dimethylsiloxane chains can be used) and the polar hydrophilic part is referred to as head group which might be either charged or uncharged.

Surfactants exist in many different forms in nature [1–3]; typically, these molecules are classified according to the chemical nature of their polar head group, i.e. surfactants with a negatively charged head group are referred to as anionic, whereas cationic surfactants contain a positively charged head group. Uncharged surfactants are generally referred to as non-ionic, while zwitterionic surfactants contain both a negatively and a positively charged group. Zwitterionic phospholipids such as phosphatidylcholine and phosphatidylethanolamine are lipids (naturally occurring surfactants) extensively used in drug delivery since they can form a variety of interesting self-assembled structures (liposomes, in particular), frequently presenting low toxicity and good biocompatibility [4]. Lately, new surfactants of low toxicity and high biodegradability, particularly from renewable resources, have been developed. Among them, surfactants with carbohydrate or amino acid polar head groups have been found to be interesting in that respect [5]. Figure 1 shows the structure of surfactant molecules.

Surfactants are found everywhere [6]: in detergency and cleaning, cosmetics and personal care products, plant protection and pest control, paints, lacquers and other coating products, foods and packaging, paper and cellulose products, plastics and composite products, metal processing, textiles and fibbers, oilfield chemicals, leather and furs, mining and flotation, foams and finally in pharmaceuticals, medicine and biochemical research. Surfactants are also responsible for compartmentalization which is fundamental for all living forms.

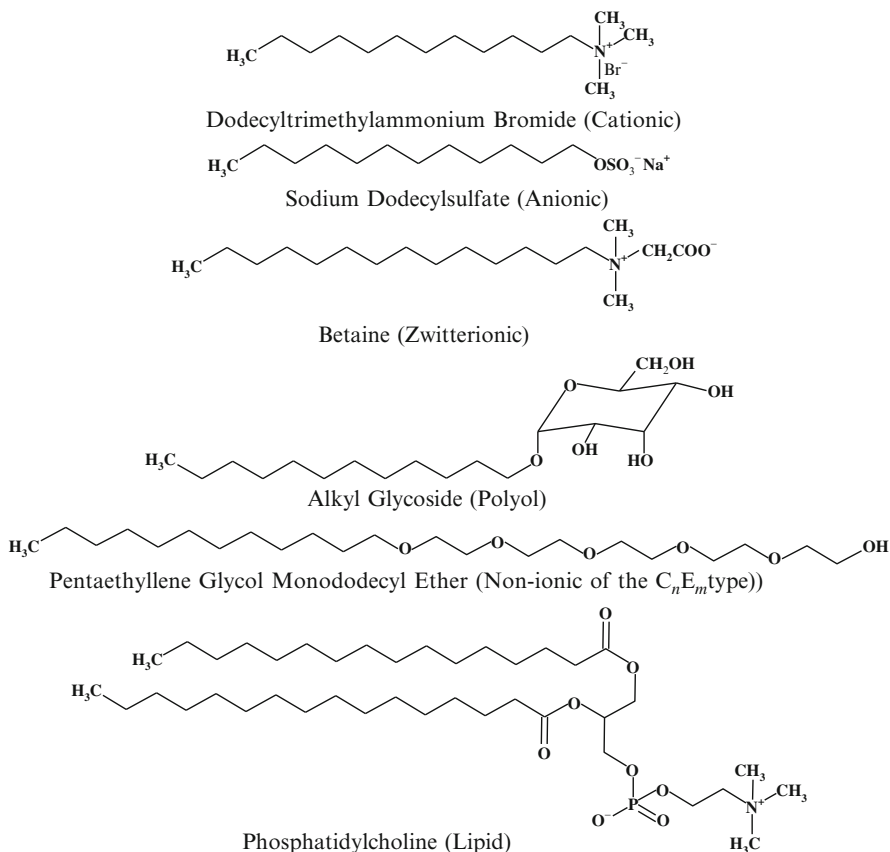


Fig. 1 Examples of surfactant molecules

2.3 Self-Assembly and Phase Behavior

Due to the amphiphilic nature, surfactants molecules display two very interesting and useful properties; they reduce the surface tension when adsorbing at a specific interface (i.e. air-water or oil-water) and they have the ability to self-associate and self-organize.

At low temperatures, the solubility is low with surfactant molecules in equilibrium with the surfactant solution. There is a critical point, known as the Krafft temperature, above which the solubility appears to increase rapidly and the solution consists of surfactant aggregates as well as single molecules. Below the Krafft temperature, surfactant aggregates are not formed. Many surfactant molecules aggregate spontaneously in aqueous media generally by starting to form normal micelles with aggregation numbers (number of molecules constituting the aggregate) ranging from 50–100. These micelles are in most cases spherical units, resulting in an isotropic solution with low viscosity.

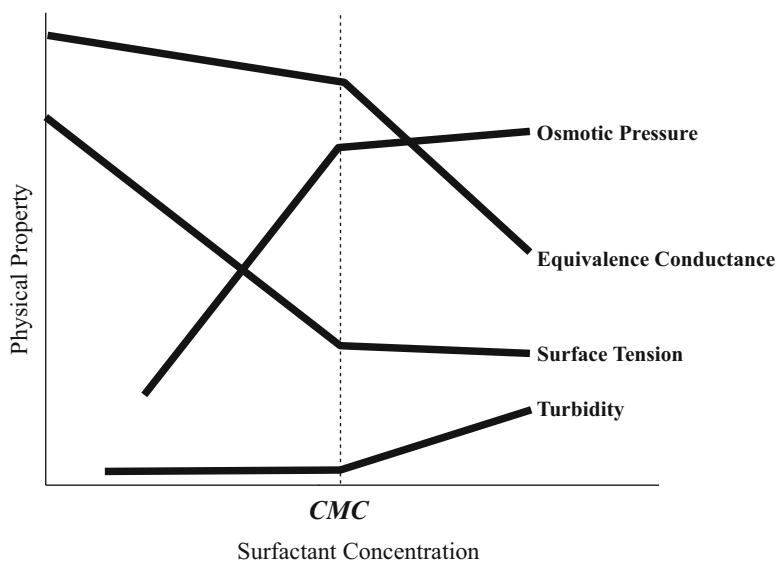


Fig. 2 Variation of different physical properties of a surfactant solution before and after CMC

Micellization is a strongly cooperative self-association process occurring at a particular narrow concentration, critical micellar concentration (CMC). CMC is thus an important parameter to characterize the self-association and may depend on the chemical nature of the surfactant and solvent as well as other factors such as the number and size of the hydrophobic tails. At CMC, the fraction of free monomers in bulk solution is the same as the fraction of molecules building up the aggregate. A further increase of the concentration results in an increase of the number of molecules in the aggregate, while the concentration of monomers in solution remains unchanged. The process is dynamic and, therefore, there is a constant exchange of molecules between the aggregates and the bulk solution [7].

Surface tension measurements are commonly used for CMC determinations. Figure 2 shows that as the surfactant concentration in solution increases the surface tension steadily decreases. This happens due to an increasing adsorption of surfactant molecules at the air/water interface disrupting the local water hydrogen bonding. At CMC, the slope of the surface tension curve decreases to almost zero. There are other physical properties that can be used to monitor micelle formation and CMC determination as represented in Fig. 2. For instance, at CMC, the rate of increase in osmotic pressure falls into a plateau. A sharp increase in turbidity is also observed by light scattering techniques. In conductance measurements, a marked decrease in the slope is observed after crossing CMC indicating that there are much less mobile charged units than expected from the individual surfactant molecules.

The so-called hydrophobic effect is believed to be the main driving force in self-association [8, 9]. It is an entropic driven process; the free energy of a process is

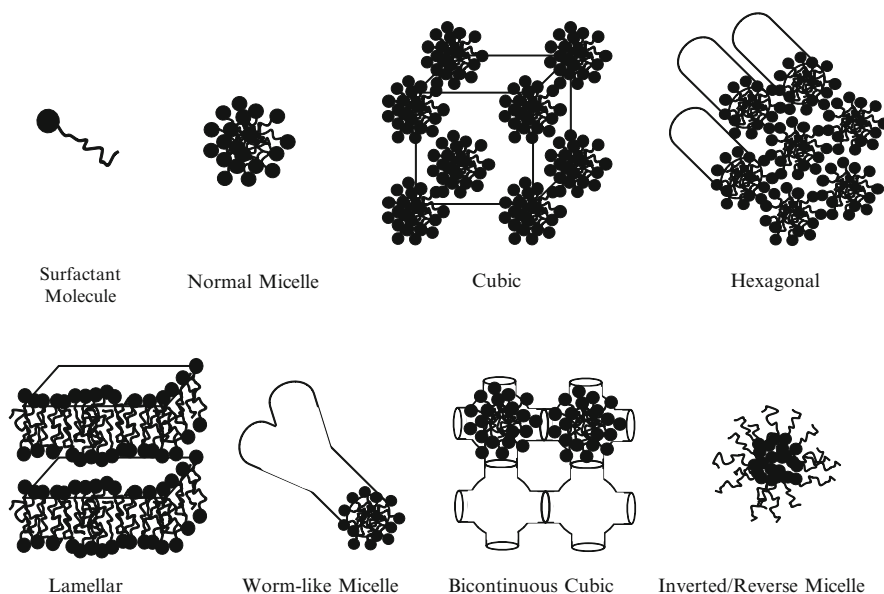


Fig. 3 Examples of different self-assembled surfactant structures

composed both by enthalpic and entropic terms. At room temperature, the enthalpy associated with the transfer of a hydrophobic molecule to an aqueous environment is negligible since the interaction enthalpies are practically the same in both cases. The main contribution comes from the loss of entropy associated with the formation of ordered water cages around the hydrophobe since it implies the disruption of hydrogen bonds between water molecules. As a consequence, non-polar molecules, which decrease the entropy of water, tend to be expelled from the aqueous media triggering the self-aggregation phenomena.

A delicate balance between opposing forces is the key aspect in surfactant self-assembly. It is affected by a range of factors, such as the size of the hydrophobic moiety, surfactant concentration, nature of the polar head group and counterion, salt concentration, pH, temperature and presence of co-solutes [5, 6].

A simple spherical micelle may grow forming cylindrical structures that are anisotropic and show features of macroscopic scale, e.g. flow birefringence. Even in this case, the solution appears as a single phase. Increasing concentration, linear growth can also lead to branched structures that may lead to interconnected structures (normally referred to as bicontinuous), since the solutions are not continuous only in the solvent but also in the surfactant. As concentration increases further amphiphiles can self-assemble to form a great variety of structures as the ones represented in Fig. 3.

Tuning some of the above mentioned parameters may allow the transition of one structure into another, offering interesting opportunities and strategies for drug delivery [4].

Micelles and bilayers are said to be the building blocks of most of the self-assembled structures. Surfactant aggregates (some presented in Fig. 3) can be divided into two main groups: those that are built of limited or discrete self-assemblies, which may be characterized roughly as spherical, prolate or cylindrical; and infinite or unlimited self-assemblies whereby the aggregates are connected over macroscopic distances in one, two or three dimensions. The hexagonal structure is an example of one-dimensional continuity. This phase is built up of (infinitely) long cylindrical micelles arranged in a hexagonal pattern, with each micelle surrounded by six other micelles (Fig. 3). The radius of the circular cross-section (which may be somewhat deformed) is close to the surfactant molecule length. On the other hand, planar lamellae show two-dimensional continuity. This structure is built of layers of surfactant molecules alternating with water layers. The thickness of the bilayers is somewhat lower than twice the surfactant molecule length. The thickness of the water layer can vary considerably depending on the nature of the surfactant. The surfactant bilayer can be stiff and planar or very flexible and undulating whereas the bicontinuous cubic and the sponge structures are examples of three-dimensional continuity.

These supramolecular surfactant structures are considered soft since they are fluid-like, flexible and easily affected by weak external forces. This is due to the nature of the self-assembly where molecules are not held together by covalent bonds but rather by physical forces, such as Van der Waals, hydrogen bonding and hydrophobic associations.

At this point, it becomes important to mention the role of the surfactant molecular geometry in predicting the surfactant structure that is formed [10]. This is of special relevance since physical properties can be quantitatively understood without the need of detailed knowledge of, for instance, the complex short-range forces between surfactant molecules.

2.4 Critical Packing Parameter and Mean Curvature

The driving force for all processes occurring in a non-specific system is the minimization of free energy. Self-association is no exception. As stated above, the balance between favorable and unfavorable interactions between solvent molecules and the particular sites of the surfactant molecule, i.e. minimization of energy penalty by exposing the hydrophobic moiety to water, is crucial for self-assembly. However, two other contributions to the total free energy have to be taken into account; the opposing force to self-assembly, due to head group steric repulsions and the geometric term which requires exclusion of water and head groups from the hydrophobic region occupied by the hydrocarbon tails [11]. These terms can be conveniently expressed by the surfactant critical packing parameter, CPP, which describes how the amphiphile geometry determines the aggregate structure (see Eq. 1).

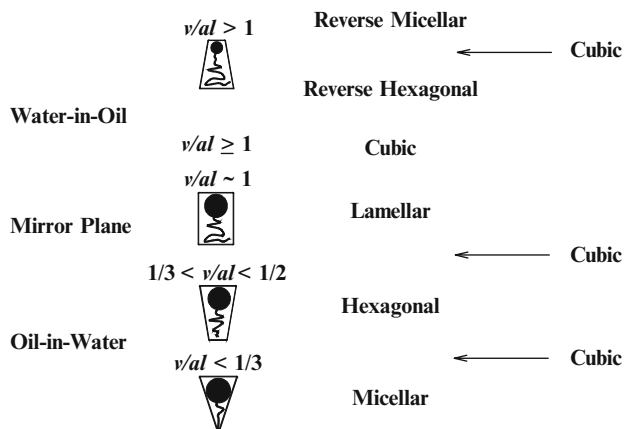


Fig. 4 CPP and preferred surfactant aggregate structures

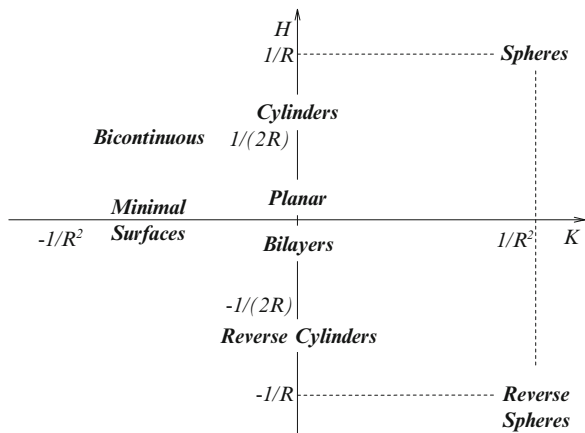
$$CPP = \frac{a_s}{vl_c} \quad (1)$$

In Eq. 1, a_s is the effective area per head group, v the volume of the hydrocarbon chain and l_c is the maximum effective length that the hydrocarbon chain can assume.

There is a direct correlation between the value of the CPP and the type of aggregate formed [2, 5]. Spherical micelles are formed when $CPP < 1/3$. The spherical aggregates are extremely small and their radius is approximately equal to the maximum stretched out length of the surfactant molecule. Cylindrical micelles are formed when $1/3 < CPP < 1/2$ (single chain surfactants with small head group areas such as non-ionic surfactants and ionic surfactants in high salt concentration). Vesicles, liposomes and flexible bilayers are formed when $1/2 < CPP < 1$ (double chain surfactants with large head group areas such as phospholipids, surfactants with bulky or branched tail groups and the mixture of anionic and cationic surfactants with single chain at nearly equimolar concentration). These types of surfactants cannot pack themselves into a micelle and they form bilayers (lamellar structure). Finally, inverted or reverse structures are formed when $CPP > 1$ (surfactants with small head groups or large tail groups such as double tailed anionic surfactants). These structures are normally formed in non-polar solvents. In these inverted structures, polar head groups are clustered together and hydrophobic tails are extended towards the solvent, as exemplified in Fig. 3. As one will see later, this makes possible the formation of microemulsions, special water-in-oil “containers”. Fig. 4 summarizes the expected sequence of surfactant structures regarding the direct dependence on the CPP.

CPP increases by addition of salt to ionic surfactants, since electrostatic repulsion among the head groups is screened. This effect can also be achieved by increasing the surfactant alkyl chain or by adding a co-surfactant (e.g. a long chain alcohol). Other parameters such as concentration, pH or temperature may play a role and contribute to tune CPP and, thus, the structure [3].

Fig. 5 The various membrane topologies modelled in terms of mean (H) and Gaussian (K) curvature



A related approach to the geometric surfactant parameter, CPP, is the concept of mean curvature, H , of the aggregate which is defined as follows

$$H = \frac{1}{2} \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (2)$$

R_1 and R_2 are the radii of curvature (reciprocal of the principle radii of curvature, c_1 and c_2 , respectively) in two perpendicular directions. For a sphere $R_1 = R_2$ and thus $H = 1/R_1$. For a cylinder $R_1 = R$ and $R_2 = \infty$ making $H = 1/2R$. On the other hand, for a planar bilayer $R_1 = R_2 = \infty$, $H = 0$. By convention, the curvature is positive when curved towards the hydrophobic part of the aggregate.

The Gaussian curvature, K , is another useful parameter defined by

$$K = \frac{1}{R_1} \cdot \frac{1}{R_2} = c_1 \cdot c_2 \quad (3)$$

Figure 5 shows a schematic map of the predicted membrane geometries modelled in terms of mean (H) and Gaussian (K) curvature.

The abovementioned observations can be summarized as follows: the more the surfactant aggregate curves towards the oil, the smaller the value of CPP is, i.e. the larger the head group area in relation to the surfactant volume is. For reverse structures, the CPP increases following the order lamellar → hexagonal → micellar.

For non-ionic surfactants, the surface curvature properties are even more relevant in the sense that they are strongly temperature dependent [12–16]. When the temperature is increased, the water becomes a poor solvent for the ethyleneoxide head group. In this case, the surfactant-surfactant interactions are more attractive and stronger than the surfactant-solvent ones, leading to a release of water molecules from the head group and to a change of the surfactant shape. At low temperatures, water is a good solvent for the ethyleneoxide head group, leading the aggregate to adopt a curvature towards the hydrophobic core. These opposite

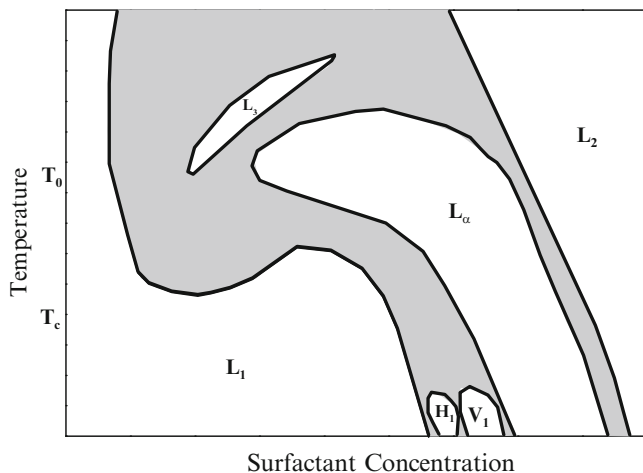


Fig. 6 A general phase diagram for non-ionic surfactants of the C_nE_m type. L_1 – normal micellar; L_α – lamellar; L_2 – reverse micellar; L_3 – sponge; H_1 – hexagonal; and V_1 – cubic. The grey area represents multi-phase samples

effects are explained by a change in conformation of the ethyleneoxide; while at low temperatures the lowest energy conformation for the $-OCH_2CH_2O-$ segment (see Fig. 1) corresponds to a gauche conformation around the C–C bond and anti around the C–O bond, which has a low statistical weight but highest dipole moment, at higher temperatures the conformations with low and zero dipole moments become more populated (trans conformations). This leads to an increase of the ordering in the ethyleneoxide chains making the solute–solute interactions more favorable than the solute–solvent ones. Note that the spontaneous curvature, H_0 , temperature dependence can be described by

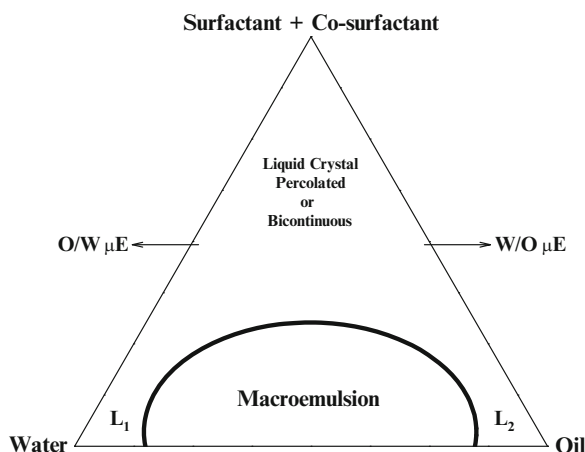
$$H_0 \approx -\beta (T - T_0) \quad (4)$$

where β is the system specific temperature independent constant and T_0 is the phase inversion temperature where the surfactant film neither prefers to curve towards water nor towards oil.

2.5 Phase Diagrams

Phase diagrams are extremely useful in the soft matter field. Often, a binary phase diagram shows the temperature along the y axis and surfactant composition along the x axis. Typically, the phase diagrams of aqueous non-ionic surfactant systems present a great variety of structures [17, 18]. Figure 6 shows a schematic and rather general phase diagram for non-ionic surfactants of the C_nE_m type.

Fig. 7 A hypothetical pseudo-ternary phase diagram of an oil/surfactant/co-surfactant/water system. L_1 – normal micelles; L_2 – reverse micelles; W/O μ E – water-in-oil microemulsions; O/W μ E – oil-in-water microemulsions



C_n represents the number of carbons in the alkyl chain and E_m represents the number of ethyleneoxide units of the surfactant head group. It is possible to change systematically the hydrophilic head group, m , or the hydrophobic tail, n . For low concentrations and low temperatures, the micellar phase, L_1 , dominates. Further increase in concentration leads to bilayer-like phases. As mentioned above, two topologically different organizations of the bilayers are observed: the anisotropic lamellar, L_α , phase characterized by stacks of bilayers with long range order and the sponge, L_3 phase that consists of a network of randomly multi-connected membranes and is isotropic [19, 20]. The latter tends to be more stable at reasonably higher temperatures than the former. Lamellar phases often occupy larger areas in phase diagrams compared to the neighbouring hexagonal, H_1 , or cubic, V_1 , phases. The reason for this is that three- and two-dimensionally, ordered cubic and hexagonal phases respectively, do not allow so many structural variations before losing their symmetry. On the other hand, the one-dimensionally ordered lamellar phase may sustain larger fluctuations without losing its one-dimensional order. To emphasise this, the melting points of the different phases can be compared; for a collection of phase diagrams see, for instance, Mitchell et al. [17].

Typically, systems are more complex than simple binary compositions. Not only surfactant and water, but also oil can be found in such systems. In such cases, the phase diagram used is generally referred to as ternary [5, 6]. A schematic illustration of a ternary phase diagram is given in Fig. 7. At constant temperature, each surfactant/oil/water system displays a unique ternary phase diagram. The composition of the different phases in a two- or three-phase sample is given by the points where the tie-lines intersect the corresponding one-phase areas. It is important to note that ternary phase diagrams are strictly valid only when the system contains three components (e.g. surfactant, oil, and water). There are situations where

multi-dimensional phase diagrams might be needed to fully describe a system, as it will be presented later on in this chapter. The construction and interpretation of multi-dimensional phase diagrams is not an easy task and, thus, one usually fixes one or several variables, and changes systematically the other parameters. One can now note that the richness of surfactant phase behavior is due to the combination of packing constraints and free energy changes associated with hydrophobe-water interactions, the oil-water interface and head group interactions.

2.6 *Theoretical Description of Bilayer Characteristics: Simplified Framework of the Elastic Curvature Energy Model*

The surfactant bilayer may be described as a thin plate whose shape and behavior depends only on the competition between its elastic properties, any applied constraint and the entropy. The stability of a phase made up of membranes is governed by, among other factors, the competition between the elastic energy of the membrane and the thermal energy, $k_B T$ [21–23].

When describing membrane structures and the change on the free energy associated to the transformation from one topology to the other, the concept of curvature free energy has proven to be very useful. The Helfrich flexible surface model basically consists in an interfacial description of the curvature free energy density, g_c , of the polar-apolar interface [24]. For each structure or configuration of the surface, the lowest order in curvature is very often expressed as

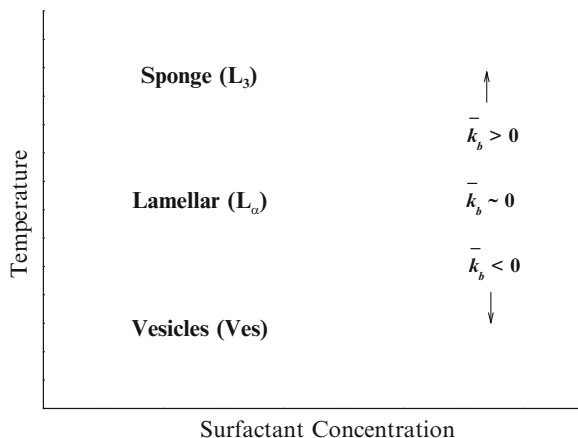
$$g_c = 2k(H - H_0)^2 + \bar{k}K \quad (5)$$

where k is the bending rigidity modulus and $\bar{k}$ is the saddle-splay modulus. For a given membrane configuration, the total curvature free energy, G_c , is obtained by integration of the energy over the interfacial area

$$G_c = \int g_c dA \quad (6)$$

The two elastic constants, k and $\bar{k}$, describe the elastic properties of the membrane playing different roles. The bending rigidity modulus, k , can be understood as the energy that has to be provided to bend the membrane around its equilibrium position. When k is comparable to the thermal energy, $k_B T$, thermal fluctuations give rise to significant displacement fluctuations of the membranes around their equilibrium position. These fluctuations have important consequences on the formation of different phases and on their static and dynamic properties. On the other hand, the saddle-splay modulus, $\bar{k}$, is the driving parameter for the membrane topology. Theoretically, if $-2k < \bar{k} < 0$ (assuming k positive), the membrane prefers to be a plane and this has a tendency to favor the lamellar phase. If $\bar{k} < -2k$, the

Fig. 8 Saddle-splay modulus changes for different membrane topologies



membrane prefers to curve into a spherical shape and the vesicle phase is then favored. If $k > 0$ the membrane adopts a saddle-like form, tending to favor cubic or a multi-connected sponge phases. Therefore $\bar{k}$ plays a role each time a structural transformation involves a topological change for the membrane, but it has no effect as long as the curvature fluctuations take place at constant topology or degree of connectivity. Figure 8 shows how the saddle-splay modulus changes for different membrane topologies.

2.7 Surfactant Structures and their Use in Drug Delivery

Surfactant systems, such as micellar solutions, liquid crystalline phases or microemulsions are often used in drug delivery [4]. When using these systems in pharmaceutical formulations, generally the goal is to obtain an optimized drug loading and release properties, long shelf-life and low toxicity. Some important features regarding the use of surfactant structures in drug delivery formulations will be mentioned next.

2.7.1 Micelles

Micellar systems are of particular interest in drug delivery [25]. Hydrophobic solutes, which are essentially insoluble in water but readily soluble in oil, can be solubilized in the hydrophobic core of micelles. Such solubilization of hydrophobic compounds is, obviously, achieved when the surfactant concentration is above CMC. Drugs can be physically entrapped in the core of surfactant micelles at concentrations that can exceed their intrinsic water-solubility. Moreover, since the hydrophilic part of the surfactant can interact favorably with the aqueous surroundings, a tight shell around the micellar core is formed. As a consequence, the

contents of the hydrophobic core are effectively protected against any adverse effect from the external environment, such as hydrolysis or enzymatic degradation. Thus, the use of micellar systems can effectively reduce the hydrolytic breakdown and degradation rate of a certain compound. This is important because several molecules used for therapeutic ends are moderately stable in aqueous environment. Esters and anhydrides are compounds particularly sensitive to aqueous medium which undergo hydrolysis at high water content, high or low pH and moderately high temperatures [4]. Such hydrolytic degradation may be problematic regarding the storage stability of the formulation. In addition, the degradation products may not be well tolerated or be even toxic.

Another important feature is related to the control of the release rate. The release rate of a drug in a micellar solution depends on its state of solubilization and, hence, on the properties of both the drug and the surfactant system. Since solubilization is nothing more than the partitioning of the drug between the aqueous phase and the micellar microcontainers, both the equilibrium concentration in the aqueous surrounding and the release rate of the solubilized drug is reduced on solubilization. Thus, solubilization may offer an opportunity to control the release rate of certain drugs. In addition, a sustained release over a prolonged time can be obtained. This is particular important in situations where, for instance, the compound is rapidly metabolized.

2.7.2 Liquid Crystalline Phases

Comparable advantages to those obtained using micellar solutions are achieved when solubilizing drugs in liquid crystalline structures [4, 26]. In this case, the release rate is strongly dependent on the localization of the compound in the surfactant self-assembled structure. If the drug contains hydrophobic modifications, an increased partitioning to the hydrophobic domain is observed. This is the case when liquid crystalline phases curve towards the oil (e.g., discrete cubic or hexagonal phases). In principle, liquid crystalline structures are more versatile than micellar solutions since they are able to incorporate rather large amounts of molecules spanning from very hydrophilic to very hydrophobic, and from very small to very large. Apart from the normal hexagonal, reverse hexagonal and lamellar phases, cubic phases are also quite interesting for drug delivery. Liquid crystalline cubic systems can consist of either discrete micelles or reverse micelles close-packed in a cubic symmetry or of a bicontinuous structure. Depending on the structure, the mean curvature can vary considerably and, hence, cubic structures can be found over an entire surfactant concentration range, as shown in Fig. 4. Bicontinuous cubic phases offer the possibility of solubilization of large amounts of both hydrophilic and hydrophobic drugs. In addition, due to their normal high stiffness, these structures allow, for instance, to locate the drug at a desired site. Cubic structures have been shown to deliver small molecule drugs and large proteins by oral and parenteral routes [27]. An interesting feature is the fact that incorporation of a drug in a cubic phase can eventually cause a phase transformation to lamellar or to an inverted

hexagonal phase depending on the polarity and concentration of the drug, which can also be used as extra parameter to tune the delivery. The drawback of these systems is very often related to shorter release duration and the extremely high viscosity which may limit their use in certain applications.

2.7.3 Vesicles

Vesicles (sometimes referred to as liposomes when vesicles are made of lipid bilayers) have attracted considerable attention due to their capacity to solubilize oil-soluble substances and to encapsulate water-soluble drugs. Different preparation techniques yield different vesicle type and size, and depending on the requirements or the administration route, a particular preparation method may therefore be necessary or preferred [4]. Once prepared, there are several important properties to be considered. In particular, both the stability of vesicles towards aggregation or fusion, and the leakage rate of solubilized or encapsulated drugs are of major importance.

Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes acting as size-selective filters, allowing only passive diffusion of small solutes such as ions, nutrients or antibiotics. Thus, drugs that are encapsulated in a nanocage-functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes. Multilamellar vesicles, MLVs, are multi-layered liposome-like structures which share similar properties with the planar lamellar state (e.g., interlamellar spacing). Among other advantages, MLVs, show very high encapsulation efficiency for water-soluble and poorly water-soluble molecules [28]. Furthermore, the encapsulation process is devoid of organic solvents and might be less severe when compared to the process for encapsulation into classical liposomes. MLVs have also been used to specifically encapsulate enzymes, small oligonucleotides, and anticancer agents [29]. Since MLVs are only kinetically stable, they can revert to the equilibrium planar lamellar structure with time, working as a multi-shield time-release liposome. Accordingly, this time dependent stability features offer interesting opportunities in drug release.

2.7.4 Microemulsions

As explained above, microemulsions are systems consisting of water, oil and surfactant, which constitute a single optically isotropic, low viscous and thermodynamically stable liquid solution. The stability allows self-emulsification of the system whose properties are not dependent on the preparation process [5]. Microemulsions are easy to prepare and some of them are very versatile for drug delivery since they offer the capacity to solubilize both water-soluble and oil-soluble compounds, frequently in high amounts [30]. Obviously, this is due to the existence of microdomains of different polarity within the same single-phase solution. One can find several administration-specific advantages for these systems.

For instance, the small size of microemulsions droplets, below 100 nm, yields a very large interfacial area, from which the drug can quickly be released into the external medium when absorption (in vitro or in vivo) takes place, maintaining the concentration in the external medium close to initial levels. Microemulsions can improve the efficacy of a drug, allowing the reduction of the initial dose and, consequently, minimizing eventual side effects. The formation of microemulsions is a reversible process, thus these objects may become unstable at low or high temperature, reforming again when the temperature returns to the stability range. This property might be advantageous in some specific situations.

3 Polymers

3.1 Introduction

Polymers are assumed to be giant molecules; they can be more than 1 m long, although most of the investigated polymers are considerable smaller than this. Polymers are common plastics, synthetic fibbers, as well as cellulose, DNA and proteins. They are everywhere and can show different responses. Polymers can adapt to different stimuli. Often, in nature, this stimuli-response is behind many of bioprocesses.

New polymers are being designed to be applied in different fields, from molecular imprinting, artificial tissues, smart clothes, tumor treatment to controlled drug release. Many chemists, physicists, biologists, among others are dedicated to the understanding of different scientific challenges that are behind the design of new efficient materials for specific purposes. In addition, the understanding of polymer features helps to explain many phenomena in nature.

Many applications require natural occurring polymers, which may be chemically modified to reach a specific function. Chemical and physical versatility, and the potential for a broad range of applications, are good reasons to continue the research on the polymer field.

Natural polymers often possess good biocompatibility, making them popular choices for many bio-applications such as tissue engineering scaffolding. There is a lot of research on the drug delivery systems using polymers but there is still a lot to explore and to improve [31].

3.2 Polymer Properties

Polymers are large molecules composed of small chemical repeating units (monomers) covalently bound together. The number of repeating units in a polymer chain is called the degree of polymerization, DP. Synthetic polymers are synthesized by the polymerization of the monomers in any conceivable pattern.

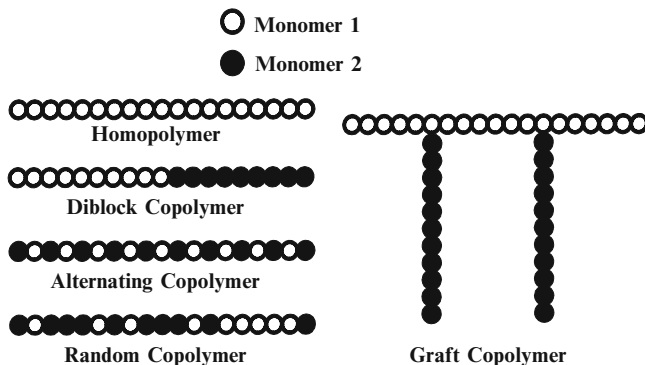


Fig. 9 Different polymers and their monomer organization

Polymers can show a variety of forms. They can be linear, branched or cross-linked and may be composed by monomers of the same type, and consequently, the polymer is referred to as homopolymer, or of different types, and, thus, designated as heteropolymer or copolymer. The latter type of polymers can be further classified by the arrangement of the different monomers along the chain. Therefore, copolymers can be random (randomly distribution of different types of chemical units), block (long segments of the same chemical unit) or graft copolymers (a chain of a type of chemical unit is grafted onto a linear chain composed by another type of chemical unit). Figure 9 shows schematically the different types of monomer organization.

Polymers may also be classified with respect to the charge. When the monomers carry charges the polymer is referred to as a polyelectrolyte. When the monomers are uncharged, the polymer is non-ionic. Figure 10 shows some examples of polymers.

The molecular weight of a polymer is a very important parameter in certain applications. Some physical properties are sensitive to the molecular weight. With the exception of proteins it is virtually impossible to find a polymer batch where all the polymer molecules have the same molecular weight. Since polymers are polydisperse, they are better characterized by the molar mass distribution and the associated molar mass averages, rather than by a unique molar mass. Conceptually, the number average molar mass is defined as

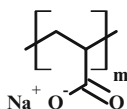
$$M_n = \frac{\sum N_i M_i}{\sum N_i} \quad (7)$$

where N_i is the number of molecules with molecular weight M_i .

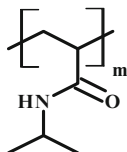
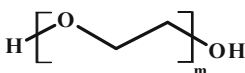
Another useful definition of molecular weight average is the weight average molar mass, M_w ,

$$M_w = \frac{\sum w_i M_i}{\sum w_i} \quad (8)$$

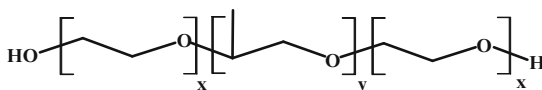
The ratio of the M_w to the M_n is a measure of the polydispersity of the sample.



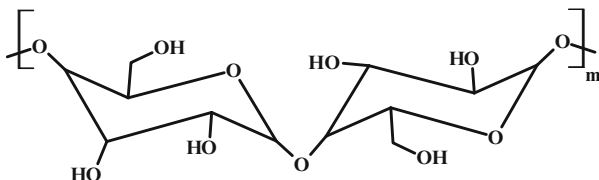
Sodium Polyacrylate (Polyelectrolyte)

Poly-*N*-Isopropylacrylamide (Non-ionic)

Polyethylene Glycol PEG (Non-ionic)



Poloxamer (Pluronic®) (Non-ionic)



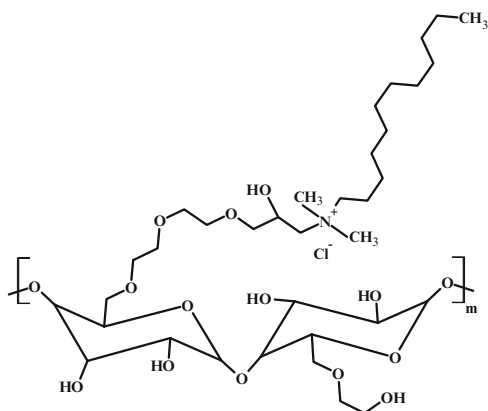
Cellulose (Natural Polymer)

Fig. 10 Examples of different polymer molecules

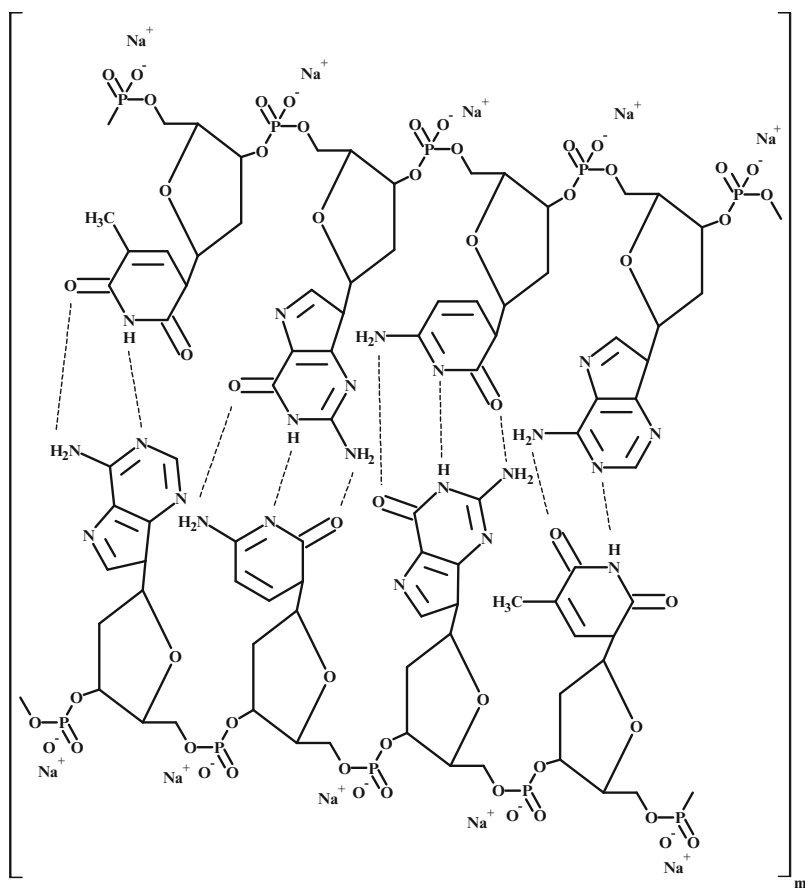
3.3 Polymers in Solution

Water-soluble uncharged polymers dissolve in water due to the gain in conformational entropy of the individual flexible polymer chains when these are diluted by the solvent.

Polymers can present an expanded or compacted conformation; they may be complexed with other substances or be used independently; they may bind active substances and/or release them. Depending on the polymer structure, solution and surface, the properties may differ considerably. To understand these different behaviors, a few considerations about polymers in aqueous solutions will be made. Some of the features are quite general and will be considered independently of the type of polymer. However, some other properties are specific of charged or amphiphilic polymers, and will be analyzed individually.



Derivative of Cellulose (Cationic Hydrophobically Modified Polymer - HMP)



DNA (Natural Polymer)

Fig. 10 (continued)

Polymer coils can adopt different configurations in solution. The general interest is usually on the extension of the polymer chain and its scaling with the change of internal or external parameters. The polymer coil dimension is commonly expressed as the radius of gyration, R_g , which represents the polymer coil radius. R_g is proportional both to the number of polymer segments and the length of the individual segment. The simplest way to express polymer chain dimensions is through the freely jointed chain model, illustrated as:

$$R_g^2 = N \frac{l^2}{6} \quad (9)$$

where R_g is the radius of gyration, N is the number of polymer segments and l is the length of the individual segment. In this model, the interactions among segments are neglected and the polymer chain is allowed to take any bond angle, independently of the positions of the neighbouring segments. This is, however, not seen in real chains, where chain rigidity is present. Flory included this additional parameter for a better representation of real polymer chains: the characteristic ratio, C_∞ , seen in Eq. 10 [32]:

$$R_g^2 = C_\infty N \frac{l^2}{6} \quad (10)$$

For flexible polymers, C_∞ is in the range 5–10 [33] while for semiflexible polymers, as the cellulose based, it reaches much higher values [34]. The rigidity of a polymer backbone influences the rheological properties of a polymer solution. The intrinsic viscosity, which is a measure of the capability of a polymer in solution to enhance the viscosity of the solution, is related to the size and molecular weight of a polymer [5, 35]. Flory also corrected the reported model by taking into account the excluded volume, which considers the impossibility of chain segments to overlap. When this effect is present, the number of segments, N , has an exponent of 1.2.

The size of a polymer chain is also strongly affected by the interaction among different molecules in solution. These intermolecular interactions are represented in the Flory-Huggins theory [32, 36, 37]. Here, the interactions between two molecules are quantified by the dimensionless interaction parameter, χ . For $\chi < 0.5$ the solvent is considered to be good and the polymer chain will be extended due to the dominating configurational entropy. It attains a stretched conformation as a rod. When $\chi > 0.5$, the solvent is bad and the monomer-monomer interactions dominate leading to a contracted polymer coil (globule). For $\chi = 0.5$, the steric interactions and the solvent repulsion balance, leading to the formation of a freely jointed chain; this is called the theta condition.

Polymer chains can self-associate in solution. If one chain is composed by different blocks, the general behavior may be extremely rich. When both units of a diblock copolymer are in a good solvent, the copolymer behaves like a homopolymer; however, when placed in a solvent which is good for one of the blocks and bad for the other, there will be segregation between the different blocks and the formation of self-assembled structures, similar to that found in surfactant systems [38–41].

The solubility of some of the polymer blocks can be tuned by temperature. Some non-ionic polymers show a remarkable temperature dependent solubility in water as well as in some other solvents. This behavior is shared with some other substances like cellulose derivatives, poly-N-isopropylacrylamide, poly(ethylene glycol) and poloxamers, commercially known as Pluronics® [16, 42–45]. The temperature effects are associated with conformational changes upon temperature change, as explained previously for the non-ionic surfactants.

3.4 *Concentration Regimes*

Polymer solutions belong to the dilute, semi-dilute or concentrated regime. For highly soluble polymers, in the dilute regime, the interactions between different polymer molecules are insignificant since the distance between individual polymer coils is considerably longer than the radius of a polymer coil. Thus, the polymer chains move independently from each other. The semi-dilute regime starts at the overlap concentration, c^* , i.e. the concentration where coils start to make entanglements with each other due to the proximity of the polymer coils. Typically the c^* is 0.1–10 wt.% depending on molecular weight and polymer configuration. R_g is larger than the average distance between polymeric chains and they are forced to overlap, resulting in a transient polymeric network, i.e. a network whose structure is based on transient junction points or cross-links arising from interactions between polymer chains; in such networks, as described in Green-Tobolsky model [46], when the old junctions break, new ones are established. Entanglements are topological rather than due to intermolecular forces and the easiest escape for sufficiently long chains will be achieved by sliding along the contorted contour (reptation) [47].

The concentrated regime is reached well above the c^* and the molecules are strongly entangled, behaving more like a melt than like a network.

3.5 *Polyelectrolytes*

When some or all monomers are charged, the polymer is called polyion. Addition of charges to the polymer chain implies the presence of small mobile counterions to ensure the electroneutrality. The combination of polyion plus its counterions is called polyelectrolyte. Therefore, when dissolved in water, a polyelectrolyte dissociates into a polyion and its counterions. The entropy of mixing increases with the counterion dissociation, since the number of particles increases from 1 to $n + 1$ (being n the number of counterions). Thus, when compared with uncharged polymers, polyelectrolytes have higher solubility in water [48]. The distribution of the counterions is enhanced close to the polyion and levels off with increasing the distance from the chain.

The charge distribution along the chain increases R_g and I_p (and thus the rigidity) due to the repulsion between the charges and, for that reason, the polymer adopts a more extended conformation and can overlap at lower concentrations than the uncharged analogue. However, the increase in rigidity may be eliminated with addition of salt. Polyelectrolytes are very sensitive to salt because it screens the electrostatic repulsion among and along the polymer chains. Thus, miscibility can be decreased by the presence of salt since it eliminates the benefit of counterion entropy associated with miscibility. Some polyelectrolytes, as the cellulose derivative presented in Fig. 10, belong to the category of intrinsically insoluble polyelectrolytes because the origin of its solubility is the presence of the charges (these polymers do not dissolve if their charges are screened by the addition of salt).

In non-ionic polymer systems, the presence of salt may either increase or decrease the solubility depending on the type of salt [5]. Iodide and thiocyanate ions increase the solubility of non-ionic polymers while chloride or sulphate decreases the solubility. In some cases the presence of charges is induced by pH; pH can be regulated to obtain a strong polymer network and consequent drug entrapment, or to make the polymer chains collapse and release the drug. This has a tremendous effect when using polymers in drug delivery systems.

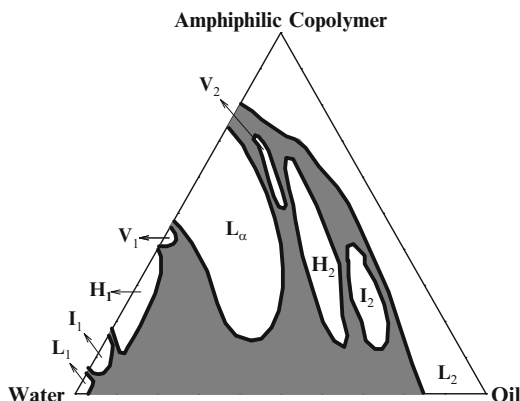
3.6 Amphiphilic Polymers

When polymers display a small number of hydrophobic side chains, or terminal groups, chemically attached to the backbone, they are called hydrophobically modified polymers (HMPs) [5]. While hydrophilic segments are responsible for the hydration and swelling, the hydrophobic groups tend to minimize the water contact, since their contact with water is energetically unfavorable. HMPs may associate hydrophobically with other amphiphilic or hydrophobic entities, forming aggregates. HMPs can form three-dimensional networks at polymer concentrations even below c^* , triggered by the hydrophobic association among different polymer chains, which largely enhances the viscosity. For this reason, HMPs typically behave as viscosity enhancers in a wide range of applications. The knowledge on the most efficient location, amount and composition of hydrophobes along the polymer chains is a crucial challenge and it has been continuously requested by, for instance, cosmetic and health care companies.

The amphiphilic properties of these polymers should increase compatibility with dispersions by adsorbing onto the surfaces of colloidal particles, at the same time as they give the possibility to form physically transient networks via the hydrophobic moieties of the HMP chains [49].

HMPs may be classified depending on the position of the hydrophobic substitution. They can have grafted modifications (where the hydrophobe is attached along the polymer backbone) or end modifications (hydrophobe at the chain ends) both exhibit intra- and intermolecular association. End-modified block copolymers

Fig. 11 Phase diagram of a ternary system consisting of an EO-PO-EO copolymer, p-xylene and water. L_1 – normal micellar; I_1 – micellar cubic; L_α – lamellar; L_2 – reverse micellar; L_3 – sponge; H_1 – hexagonal; H_2 – reverse hexagonal; V_1 – bicontinuous; and V_2 – reverse bicontinuous. The grey area represents multi-phase samples (reproduced from ref. [5])



have hydrophobic groups on each end. They inter-associate, drawing flower-like micelles in solution, with the hydrophobic groups inserted into the same micelle. At sufficiently high concentrations, the short inter-micelle distance favors polymer bridging between two micelles. The bridges provide connectivity in the system, which gives rise to an attraction between micelles [50, 51] and to strong increase in viscosity.

Poloxamers are also amphiphilic macromolecules. They are triblock copolymers, usually composed by a central hydrophobic chain of polypropyleneoxide and two hydrophilic chains of polyethyleneoxide, as shown in Fig. 10. The amphiphilic nature of these polymers gives rise to complex aggregation, depending on composition, temperature, etc. Each aggregated structure has specific delivery and encapsulation properties. Figure 11 shows the richness of structures of an amphiphilic block copolymer system. Amphiphilic polymers are thus extensively requested as nanoencapsulators in drug delivery systems [52].

3.7 Polymer Microgels

Cross-linked polymers, or microgels, are composed of polymer chemical networks, which have attracted much attention in fundamental studies of soft matter [53, 54] and in applied fields [55, 56]. In particular, they have rapidly gained importance in materials science owing to their applications in drug delivery [57–59] and sensing [60, 61].

The swelling of cross-linked polyelectrolytes can be extremely strong; some microgels may swell in water up to 1,000 times their dry volume [62]. The swelling of microgels has a remarkable effect on thickening, clearly much more pronounced than for linear polymers.

Table 1 Different stimuli used to activate drug release

Stimulus	Mechanism
pH/ionic strength	pH change causes swelling/deswelling and drug release Change in ion concentration inside the gel causes swelling/deswelling and drug release
Chemical	Formation of charge-transfer complex causes swelling/deswelling and drug release
Enzyme-substrate	Product of enzymatic conversion causes swelling/deswelling followed by drug release
Thermal	Change in polymer-polymer and polymer-water interactions cause swelling/deswelling and drug release
Electrical	Change in charge distribution causes swelling/deswelling and drug release
Ultrasound irradiation	Temperature change causes drug release

3.8 Polymers as Drug Delivery Systems

Living systems adapt to changes in the environmental conditions. Monomers may be designed to show changes in their behavior upon specific conditions of pH, temperature, ionic strength of the surrounding medium and quality of solvent [53, 63–66]. With a fair knowledge on polymer physical chemistry, it is possible to design versatile materials in the form of shrunk globules or swollen chains. Polyacrylate derivatives that work as efficient thickeners at pH above 7 may behave as liquids at lower pH, to be easily handled. The most commonly used stimuli to activate the release of a drug from the encapsulator are shown in Table 1 (adapted from reference [67]).

The temperature or pH at which the polymer behavior changes can be tuned either by monomer modification, addition of co-solutes (surfactants, second polymer or salt), addition of a second solvent with different polarity, change in concentration or mixture with a second “smart” polymer (synergistic effects were reported elsewhere [68]).

“Smart” polymers can be constituted by different “smart” blocks, covalently linked together. This type of multi-responsive systems used for multi-drug release at various specific conditions or for diagnosis, is currently attracting researchers. These systems can be simultaneously sensitive to temperature, pH, UV radiation and magnetic fields.

4 Polymer-Polymer and Polymer-Surfactant Mixtures

4.1 Introduction

The wide number of studies performed in polymer-polymer and polymer-surfactant aqueous mixtures is a consequence of their importance in many different fields from fundamental science to industrial applications. Mixtures of polymers or polymers and surfactants can be found in the human body, in cleaning products, in constructions materials, in paper, in food, in pharmaceutical products, etc. [69–74]. Early studies on polymer-surfactant systems were connected to the interest in understanding of the interactions between surfactant and proteins or other biological polyelectrolytes, with the aim of comprehending biological processes [75, 76]. In general, the mixtures can be made with several distinct aims. One purpose may be to introduce high viscosity through the polymer and/or to introduce the capacity of solubilizing hydrophobic molecules through the surfactant aggregates. In some other cases, the addition of surfactant to a polymer may intend to increase the polymer solubility [77]. In addition, the mixtures may form very stable nanoparticles [78–81].

Under some particular conditions, some polymers or polymers and surfactants attract each other and form complexes. These complexes can be soluble (homogeneous solution) or insoluble (separating out from the solution), depending on different features such as the chemistry of the molecules, the composition of the mixture and the ionic strength. The insoluble complexes can separate out from the solvent, forming a concentrated phase, which is in equilibrium with a dilute phase [5, 48, 82].

On the other hand, some other compound combinations may result in two separated phases each one enriched of in one of the components.

The type of phase behavior can be adjusted by changing the composition, salt content, etc. Each type may find use in different states of an application [5]. In addition, using mixtures of polymers or polymers and surfactants, different effects can be achieved such as colloidal stability, flocculation, emulsification, structuring or rheological control. In particular, polymers and surfactants are widely used as rheology modifiers or gelling agents and their mixtures can exhibit interesting (sometimes non-monotonic) rheological behavior, different from the individual polymer or surfactant solutions [70, 83–90].

Due to the extensive use of mixtures of polymers and surfactants in drug delivery formulations, it is very important to control and to understand the phase behavior of such mixtures.

4.2 Phase Behavior

The tendency for mixtures of polymers and surfactant self-assembled aggregates to separate into two-phases is much stronger than for mixtures with smaller molecules

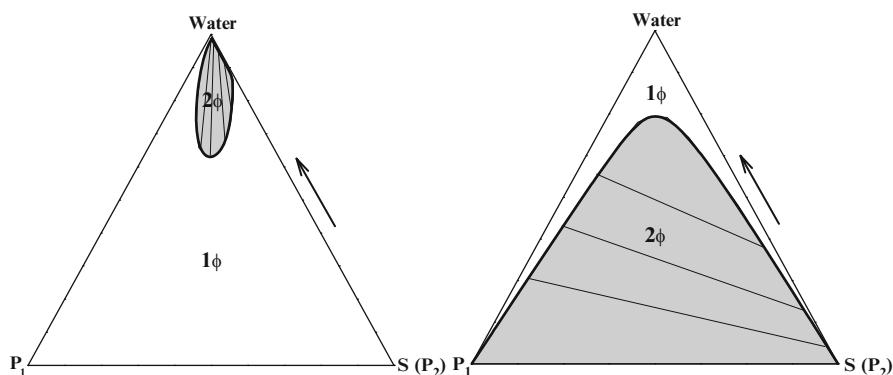


Fig. 12 Schematic phase diagrams for associative (*left*) and segregative (*right*) phase behavior. 1ϕ and 2ϕ stand for one- and two-phase, respectively

due to the lower entropy of mixing of the larger molecules. Thus, phase separation is very common for both polymer and surfactant mixtures. But the behavior of the mixtures is also dictated by the interactions among the macromolecules and between those and the solvent [91]. Two typical phase behaviors may be distinguished considering the distribution of the macromolecules: the associative and the segregative. In the former case, the macromolecules associate into a common concentrated phase in equilibrium with a dilute phase and, in the latter, the macromolecules segregate into different phases of similar total concentration. The two types of behavior were first observed for mixed polymer solutions, but, nevertheless, this is also observed for polymer-surfactant systems [5, 48]. Figure 12 shows schematic phase diagrams for both cases.

In the associative phase separation, the 2ϕ region is anchored in the water corner of the ternary phase diagram and has the shape of a drop, which can be more or less symmetric in relation to the bisector of the water corner. The tie-lines, connecting the equilibrium compositions of the co-existing phases in the 2ϕ region, run from the water corner to the opposite side of the loop, showing that one phase is very dilute and the other is mostly constituted of the complexed molecules. Outside the 2ϕ drop one finds a 1ϕ region, meaning that the mixture of the two associating components may lead to the formation of soluble or insoluble complexes depending on the composition or ratio between the mixed components [5, 77]. This association is quite general for oppositely charged mixtures, but can also be found for non-ionic mixtures [92–100]. The size of the phase separation region increases with polymer molecular weight, micellar size, surfactant alkyl chain length, and polyelectrolyte charge density, while it decreases, for instance, with addition of salt which screens the electrostatic interactions. For certain non-ionic systems, the extension of the phase separation region can also be strongly affected by temperature. Moreover, an associative phase separation is favored by a decreased hydrophilicity of one or both of the solutes.

The oppositely charged polyions interact mainly by electrostatic interactions but if, for instance, the polyions contain hydrophobic moieties, hydrophobic interactions might play a major role and can modulate the thermodynamic phase behavior that may be reflected in the extension of the phase separation region. When hydrophobic interactions are present, they are normally responsible for a decrease in extension of the phase separation [85]. In some cases, due to the hydrophobic interactions, insoluble complexes only form in a narrow composition region (close to charge equivalence), largely reducing phase separation. When both polyions are hydrophobically modified there is a tendency of the hydrophobic groups to associate and reside in one phase [84, 85, 90]. When one of the polyions is in excess (charge-wise) in the mixture, the complexes may acquire an excess charge due to hydrophobic association and, therefore, the mixtures go from two-phase to one-phase.

Segregative phase separation happens when there is no net attraction (hydrophobic or electrostatic) between the macromolecules. The extent of the phase separation is expected to depend on the short-range interactions between the various components of the system, including the solvent, and on the entropy of mixing. Differences in short range interactions generally favor phase separation and the entropy of mixing favors miscibility [77]. As explained earlier in this chapter, the entropy of mixing depends largely on the number of molecules and aggregates present in the mixture at a certain composition. For non-ionic mixtures, this is given by the number of the polymer molecules and the aggregation number of the surfactant aggregates. For charged macromolecules, numerous small ions, the counterions, are present and the entropy of mixing becomes larger when compared to non-ionic.

The segregative behavior may be strongly affected by the introduction of small fractions of ionic moieties; the miscibility increases owing to the fact that confinement of the counterions in one phase is less favorable [77]. This phase behavior can be useful when, in a mixture or formulation, certain components should not associate but kept separated (for instance, to prevent denaturation or precipitation of proteins in a mixture of those with surfactants).

For some systems, at very high salt concentration, the behavior can change from associative to segregative.

4.3 Asymmetry

It is indeed possible to observe that oppositely polyion-polyion (surfactant ion aggregates act as polyions) complexes do not separate out of the solution only at charge equivalence; the mixtures may show a pronounced asymmetry in relation to the charge equivalence. This asymmetry has its origin in some difference between the polyions other than the sign of the charge (a mixture of two polyions differing only in the sign of their charge must necessarily give rise to a symmetric phase diagram) [91]. For instance, a well-established cause of asymmetry in phase behavior in these systems is a pronounced difference in the number of charges per

polyion [101]. Also, hydrophobic association can be the cause of asymmetry in the phase separation region in relation to the charge equivalence as can be inferred for the abovementioned discussion [84, 85, 90].

4.4 CAC and Redissolution Phenomena

Uncharged slightly hydrophobic polymers and charged polymers display important similarities in their interaction with ionic surfactants [77]. In both cases, binding of the surfactant to the polymer chain can be observed, resulting in the formation of micelle-like clusters of surfactant molecules adsorbed to the polymer chain. The surfactant binding normally starts at a rather well-defined surfactant concentration, the so-called critical aggregation concentration (CAC) [102], which is always lower than the CMC of the surfactant. The CAC may simply be regarded as the surfactant CMC in the polymer solution. At the CAC there is a strongly cooperative binding. To clarify, the term cooperative binding illustrates a situation where the surfactants prefer to bind in a domain occupied by another surfactant molecule; non-cooperative binding indicates that the molecules have no preference for any domain; and anti-cooperative binding indicates preference by the unoccupied domain.

The binding of the surfactant is normally characterized by its marked cooperativity due to the hydrophobic interaction between the surfactant chains (the binding is actually a micelle formation at the surface of the polymer). But there are other behaviors, for instance, in the case of HMPs the scenario is quite different because the hydrophobic groups have the capacity to associate and the individual surfactant molecules are solubilized by the polymer micelles [77].

Just like the CMC, CAC decreases with increasing surfactant chain length, but in contrast to the CMC for charged systems, CAC increases with increasing polymer concentration or salt concentration, as the entropic gain in releasing the counterions bound to the polyion decreases at higher polymer concentration or salt content. The chemical nature of the surfactant head group may also affect the polymer-surfactant interaction strength. Anionic surfactants show a marked interaction with most homopolymers, while cationic surfactants show a weaker but still significant interaction [5, 77]. Non-ionic surfactants and zwitterionic surfactants only rarely show a noteworthy interaction [5, 77]. When CAC is reached a plateau in the surface tension is observed until all the polymers become saturated with surfactant molecules. Moreover, the plateau increases linearly with polymer concentration [5].

There are different scenarios for the micellization of the surfactant interacting with the polymer chain, involving different types of binding. For ionic surfactants and hydrophilic homopolymers the micellization occurs in the vicinity of the polymer chain. If the attractive interaction between the polymer and the surfactant is strong as for the case of oppositely charged systems, there will be a pronounced cooperative binding of the surfactant molecules to the polymer chain. The micelles sizes are similar with and without the polymer and the aggregation numbers are typically similar or slightly lower than those in the absence of polymer [5].

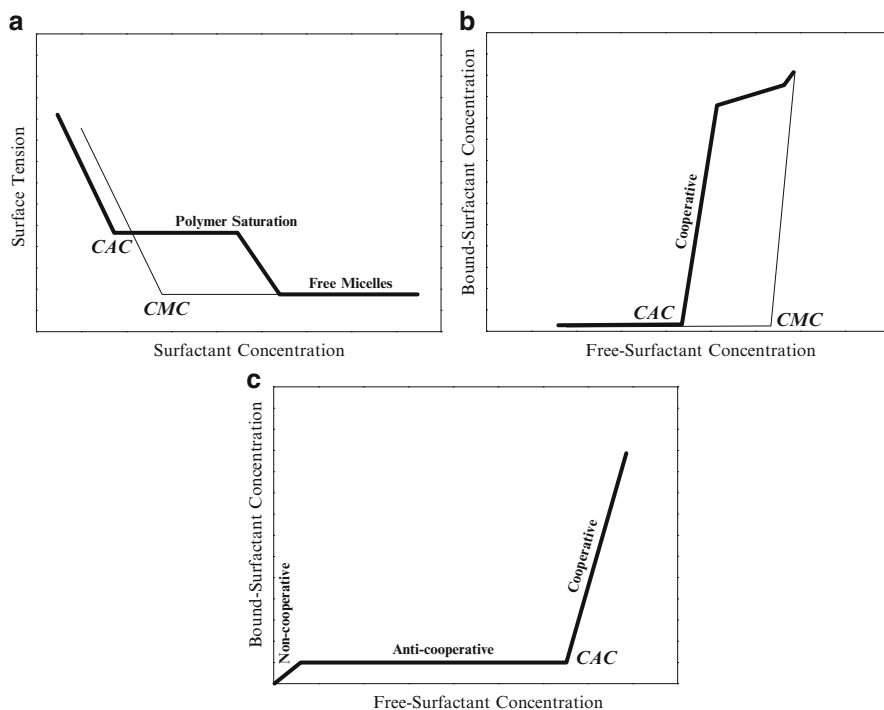


Fig. 13 Schematic trend of the surface tension as a function of the surfactant concentration in the presence and absence of a homopolymer (a) binding isotherm for the surfactant in the presence and absence of polymer (b) and binding isotherm for the association between an ionic surfactant and a hydrophobically modified polymer (c)

For hydrophobically modified polymers the scenario is more complex. There is a pronounced hydrophobic interaction between these polymers and the surfactant molecules. At low surfactant concentrations, there is a low amount of non-cooperative binding of individual surfactant molecules, at intermediate concentrations an anti-cooperative plateau appears and, finally, as the free surfactant concentration equals the *CAC*, the typically cooperative surfactant binding can be observed [5]. A schematic representation of these phenomena is presented in Fig. 13.

Polyions associate strongly with oppositely charged surfactant ions due to the strong electrostatic attraction between the two multi-charged species together with the large entropic gain (counterions release). Phase separation often occurs, resulting in a concentrated phase enriched in polyions and surfactant ion aggregates in equilibrium with a dilute phase [5, 48, 82]. Phase separation of polyion-surfactant ion complexes appear either at *CAC* or at concentrations slightly above the *CAC*, depending on the polyion concentration and the characteristics of the system [82, 103]; in other words, in some systems, it is possible to bind a considerable amount of surfactant ions to the polyion before phase separation occurs whereas in

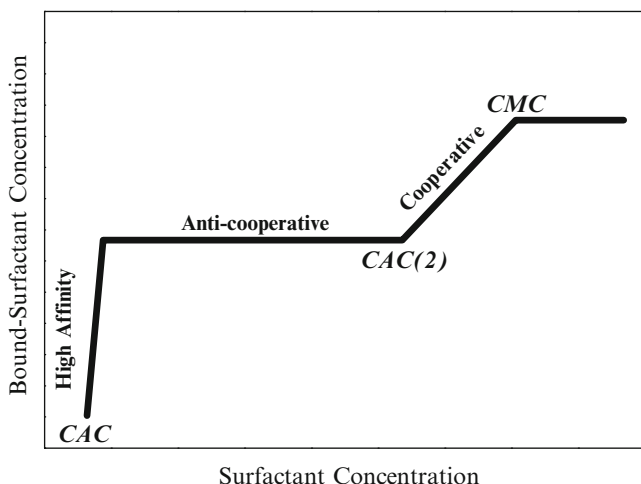


Fig. 14 Schematic trend of the binding isotherm when CAC(2) is present (adapted from Lynch et al. [107])

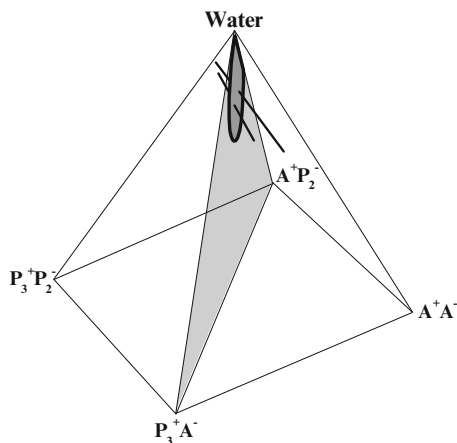
other mixtures phase separation is observed for very low surfactant concentrations [104–106]. On the other hand, redissolution of the concentrated phase might occur. It occurs when non-stoichiometric complexes are redissolved into a single phase. The redissolution can be accomplished by addition of excess surfactant or excess polyelectrolyte, but the mechanisms behind this phenomenon are not yet fully understood. One possible scenario is the following: when extra surfactant binds to the polyion (above the charge equivalence) the complexes become overcharged. In some systems, a second cooperative binding of surfactant ions can occur at the so-called CAC(2), which is sometimes referred to as the hydrophobic CAC, because cooperative binding happens due to a hydrophobic association of the excess surfactant ions to the neutral complex [107]. This may lead to redissolution. Figure 14 shows a hypothetical representation of CAC(2) type of binding isotherm.

In conclusion, in some systems efficient redissolution occurs; however, there are other systems where efficient redissolution is not achieved by adding excess of surfactant or polyelectrolyte. This is also valid for polymer-polymer systems.

4.5 More Complex Systems

Although some mixtures of oppositely charged systems have been represented using the Gibbs' triangle (ternary plot), as shown in Fig. 12, this is not entirely correct. When the system is composed of oppositely charged macromolecules, the most common type of associative mixtures is a 4-component system and the complete phase diagram would be described by a pyramid as exemplified in Fig. 15 [108]. For multi-component systems, the tie-lines do not generally fall in the mixing

Fig. 15 4-component system represented by a pyramidal phase diagram with an associative phase separation in the $A^+P_2^-/P_3^+A^-/W$ plane. The tie-lines are shown as straight lines passing through the grey mixing plane



plane and the full characterization of the separated phases involves a considerable amount of work [109]. The picture is as follows: when two neutral salts, for instance two polyions of opposite charges (P_2^- and P_3^+) with their respective counterions (A^+ and A^-), are mixed, the dissociation of the counterions and the different alternatives to combine the charged species into net neutral (mixed) salts turns the mixture into a 4-component system. Contrary to the non-ionic systems, presented earlier, for oppositely charged systems the classical triangular representation is generally not correct when five species (four neutral salts plus solvent—water) are mixed.

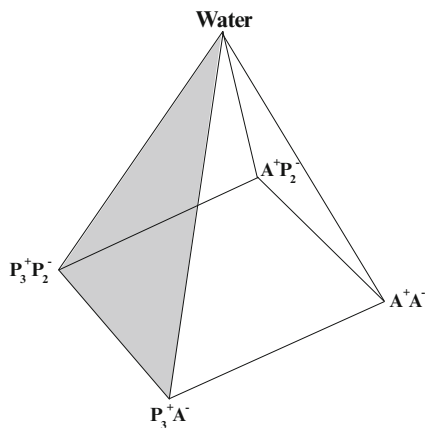
The four apices of the base of the pyramid represent the four different combinations of neutral species. The water content is given by the axis perpendicular to the pyramid base. The conventional triangular representation of polyelectrolyte/surfactant/water system refers to the plane in grey when the two-phase is presented. Despite this representation being not fully correct, it is useful to understand the general features of the system.

Apart from the charged species, there are other factors that may increase the complexity of the mixtures. As discussed, the formation of liquid crystalline phases for individual surfactant or self-assembling polymer systems in water originates multi-phase systems by itself. Furthermore, micellar growth into infinite structures increases the tendency for phase separation. Another factor that clearly increases the complexity of a mixture is the inherent polydispersity [77].

4.6 Alternative Phase Diagrams for Oppositely Charged Mixtures

A few number of works have presented the fully characterization of the separated phases including the tie-lines in multi-component polyelectrolyte-surfactant

Fig. 16 The pyramid represents all the possible components of a mixture of two charged macromolecules in water. The shadowed $P_3^+P_2^-/P_3^+A^-/H_2O$ area is one of the possible alternative mixing planes



systems [109]. To simplify the study of the complex phase behavior of oppositely charged mixtures, the number of components can be reduced by synthesizing the pure complex salt which results from the complexation of the polyions or polyion and surfactant ions ($P_3^+P_2^-$), and use it as one of the starting components [110–112]. The alternative mixing plane is perpendicular to that corresponding to the conventional mixing plane. This is exemplified in Fig. 16. The complex salt can be then mixed with any other of the components ($P_3^+A^-$, $A^+P_2^-$, A^+A^-). This mixture contains three components and thus represents a truly ternary mixture.

4.7 Rheology of Polymer-Polymer and Polymer-Surfactant Systems

Polymers have a broad range of applications in what concerns to rheological control. Viscosity depends on a number of parameters, such as molecular weight, concentration, solvency conditions and conformation of polymer molecules. The latter is also dependent on the salt concentration and polymer concentration. Associating amphiphilic polymers can give rise to very interesting rheological behavior. Polymers grafted with hydrophobic groups may associate with the hydrophobic groups from other polymers or surfactants. Therefore, for mixed systems, more pronounced changes may be seen.

For associating mixtures of ionic surfactant and non-ionic polymer, the viscosity depends on the binding of the surfactant. As the surfactant binds, the initial non-ionic polymer transforms into a polyelectrolyte and the viscosity rises by orders of magnitude.

The semidilute mixtures of surfactants and hydrophobically modified polymers have been used extensively to modify the rheology of a mixture. The mixed association of the hydrophobes brings physical cross-linking to the complexes and

a marked rise in viscosity. However, as the surfactant concentration increases the cross-linking may be lost due to the formation of a 1:1 stoichiometry (i.e. one surfactant micelle per polymer hydrophobe) and viscosity decreases. This non-monotonic viscosity behavior is a very useful phenomenon in many applications [71, 83–88]. Additionally, for non-ionic systems, viscosity can be also tuned using temperature changes, as previously alluded.

4.8 As Drug Delivery Systems

4.8.1 Polyelectrolyte-Surfactant Nanoparticles

In general, the structure of polyelectrolyte-oppositely charged surfactant complexes is dictated by the structure that the surfactant forms in the absence of polyelectrolyte, if the polyelectrolyte is sufficiently flexible to adapt. All the advantages of the self-assemblies formed by the surfactant molecules are present in the mixtures of polymers and surfactants. Many systems, including micelles and (micro)emulsions are very sensitive to dilution. Therefore, the polyelectrolyte-surfactant systems provide a viable alternative to these systems when rupture of the structure due to dilution is undesired.

In dilute solutions, discrete and compact polymer-surfactant nanoparticles form at various charge ratios, with long-range order internal structures [80, 81, 113]. These nanoparticles are regarded as potential drug and gene delivery vehicles. They may be used as carriers of nucleic acids and other biological components into living cells for therapeutic purposes. For instance, the non-viral vectors, formed by DNA and cationic species, may be used to treat cancer and genetic disorders [114, 115].

4.8.2 Polymer Gel-Surfactant Mixtures

Polyelectrolyte gels are swollen due to the osmotic pressure arising from the confinement of the counterions inside the network. When a cross-linked polyelectrolyte gel is exposed to a solution of oppositely charged surfactant, the surfactant molecules will diffuse into the gel, neutralizing the polyion, enabling the release of the counterions as in the case of linear polyions. As the polyion gel is neutralized, it collapses. The concentration of the counterions inside the gel decreases considerably and consequently the internal osmotic pressure decreases as well. The degree of collapsing may be tuned by the surfactant concentration. The collapse can start at the surface of the gel, forming a dense micelle-rich phase (“skin”) around the swollen core and as more surfactant is added to the gel, the surface phase grows at the expense of the core until, eventually, the gel fully collapses [116–120].

4.8.3 Protein-Surfactant Mixtures

Drug delivery technology holds several exciting challenges, among which the delivery of intact protein based drugs. Proteins are very susceptible to denaturation under unsuitable pH conditions or metabolizing enzymes. Hence, suitable ways of protecting proteins against denaturation, until the target site has been reached, are necessary.

Reports on interactions between proteins and surfactants comprise both phase separation and redissolution, including formation of physical gels [5, 77]. Systems containing proteins have an important advantage from the beginning; proteins are monodisperse. The differences found between the systems containing proteins and linear polymers are probably related to the exotic nature of the proteins. Proteins are amphiphilic polymers with variable charge density. Additionally, proteins differ very much from one to the other and general considerations are difficult to be drowned. Proteins can be globular or fibrous and the different structures strongly influence the phase behavior. Therefore, studies comprising mixtures of proteins with surfactants or other polymers are limited and scarce general models have been constructed [5, 77].

Proteins and surfactants interact not only because they may have opposite charges but also because they are both amphiphilic. In particular, there are some similarities between protein-surfactant systems and HMP-surfactant systems [5].

Surfactant binding to the protein may lead to irreversible denaturation of the protein and, consequently, the protein may be found in different conformational states in the mixtures. Protein-surfactant complexes involving denatured proteins find some analogies to complexes of surfactants and partially hydrophobic and flexible polymers [5].

4.8.4 Polymer Gels in Protein Delivery

Proteins can be delivered using gels. One possible way to achieve this is to use responsive gels where the drug is loaded through diffusion in the swollen state of the gel and trapped inside it on the collapsed state. Thereafter, the gels are triggered by an external stimuli and the drug released. Prototypes for gel vehicles with the ability to load and release proteins have been designed [121–124]. The interactions between gels and proteins are very complex and simpler models are usually useful to understand general interactions and behaviors. In this regard, the study of the interactions between gels and surfactants is very useful.

4.8.5 DNA-Surfactant Complexes

Many aspects on drug delivery are common to the gene delivery. For gene delivery, DNA, which is a large biopolymer, needs to be compacted in the cells by other

biological solutes. Compaction of DNA decreases its radius of gyration and offers protection from nuclease degradation. Positively charged proteins have the capacity to compact DNA in the nucleus of eukaryotic cells. Cationic lipids (natural surfactants) are also widely used to compact DNA. Moreover, the compaction needs to be reversible for transfection and gene expression. The control over the molecular mechanisms influencing the formation of complexes between DNA and lipids or between DNA and other biopolymers, which are very much similar to the synthetic polymers and surfactants, is crucial to obtain the desired formulation.

The strong binding of surfactants to DNA allows compaction and reduction of charges. However, the complexes of DNA and cationic surfactants do not show effective transfection. This is attributed partially to the cytotoxicity of the cationic surfactants and to the low stability of the complexes when the environment is changed [125]. Yet, cationic surfactants can be used in low amounts to charge the complexes of DNA with liposomes and, hence, increase the transfection efficiency [115]. The redissolution or decompaction of the complexes is necessary for DNA to be exposed to the enzymatic machinery in the cell. This can be achieved by the addition of anionic [126] and non-ionic surfactants [127] or even cyclodextrins [128].

DNA chemical gels have been prepared by crosslinking double stranded DNA with ethylene glycol diglycidyl ether (EGDE). The deswelling behavior of the DNA gels has been studied in the presence of different solutes. The deswelling of the gels is reversible when an oppositely charged surfactant is added to the collapsed gel [129].

4.8.6 DNA-Cationic Polymers

Cationic polymers exhibit an extreme efficiency in compacting DNA which may result in DNA-polycation complexes even in very dilute solutions. Phase diagrams of mixtures of DNA and cationic polymers, showing phase separation and redissolution regions, have been studied. The concentration of polycations to induce DNA compaction or phase separation depends on the polycation size and charge density. As expected, the presence of salt may also strongly alter the phase behavior. In general, the maximum precipitation is reached near the point of charge equivalence and typically insoluble complexes are formed already at very low polycation concentration. Often, increasing the concentration of polycation does not lead to redissolution of the complexes [130]. For long DNA molecules, the binding is strongly dependent on salt content and the extent of phase separation decreases with increase of salt concentration until phase separation is suppressed [131]. The size of DNA has also an influence on the phase behavior. While for long DNA molecules, phase separation may occur progressively starting at charge ratios below the charge equivalence, for short DNA molecules the phase separation region may be very narrow and near the charge equivalence [132]. The interaction between DNA and cationic polymers in semidilute regimes have also been studied and redissolution of the complexes was observed with formation of physical DNA-polycation gels [133].

As mentioned earlier for conventional polymer-surfactant systems, nanoparticles also form in DNA-surfactant or -polycation systems. There is a strong interest in controlling the size and shape of the nanoparticles for the development of efficient artificial non-viral gene delivery systems. Single DNA molecules collapse into toroids, rod-like shapes or globules. These morphologies are also found in the complexes with the cationic species.

4.9 Cytotoxicity

Cytotoxicity is an inherent property of many polycations. These cationic polycations can induce membrane damage and initiate apoptosis. In the case of polycations the cytotoxicity could be reduced by lowering the polycation molecular weight or branching [134]. Cytotoxicity can be further reduced by using appropriate type and/or number of side chains [135, 136].

5 Drug Delivery and Cells

5.1 Introduction

The entire concept of having a drug delivery system which allows the body to use the administered drug in the appropriate dosage and site is continuously spreading interest in the scientific community [137–139]. Typically, the drug delivery vehicle is introduced (the process of administration) into the body far from the site of action. The carrier must then move into the bloodstream (the process of absorption) and be transported to the target sites where the drug is needed (the process of distribution). Afterwards, some drugs are chemically altered (the process of metabolism) by the body before they perform their action; others are metabolized afterwards; and others are not metabolized at all. The final step is the removal of the drug and its metabolites from the body (the process of elimination). The main goals for the development of bio-nanotechnologies in drug delivery can be summarized as: specificity of drug targeting and delivery, reduction of toxicity while maintaining the therapeutic activity, biocompatibility and safety.

Currently, many substances are under investigation ranging from the more established systems, e.g. polymeric nanoparticles, surfactant based nanoparticles, solid lipid nanoparticles, liposomes, to the more cutting-edge systems, e.g. dendrimers, nanogels, nanoemulsions.

Nevertheless, the strategy of nanoparticle synthesis will always fade in critical prerequisites that need to be taken into consideration when the appropriate carrier/system is chosen, which includes (i) large carrier capacity (dose of drug to be solubilized), (ii) drug incorporation (increase circulation time) and sustained release

rate, (iii) long-term stability and shelf life, (iv) feasibility of incorporation of both hydrophilic and hydrophobic substance, (v) biocompatibility, (vi) bio-distribution and specific targeting, and (vii) functionality.

5.2 Nanoparticle Choice

Numerous factors need to be considered in the choice of the system. Therefore, the main advantages and disadvantages of the different polymer and surfactant systems are summarized in Table 2.

Depending on the preferred drug targeting, the physicochemical properties and stability of the drug, the required time of bioactivity and the patient acceptability, different drug delivery systems tend to have their primary application within a given route of administration, offering different types of advantages and limitations. Amongst all of them, oral route is still the most intensively investigated, which offers the greatest potential for more effective therapeutics and probably collects the highest preference from both the patient and the clinician. However, this type of administration has some critical limitations, due to the way in which a drug typically moves through the digestive tract. For orally administered drugs, during the pathway to the target site, absorption may begin in the mouth and/or stomach. The latter has a quite low pH, influencing drug stability and drug solubility, as well as the properties of the drug carrier system. However, usually most of the drugs are rapidly eliminated due to the hepatic metabolism and the passage through the enzymatic environment of other body tissues, such as those of the gastrointestinal track, where they are easily degraded. Therefore, because the way of drug administration will determine, to some extent, whether or not the patient gains any clinical benefit, and whether he/she suffers any adverse effect from the drug, a list of advantages and disadvantages of some of the main routes of administration are summarized in Table 3.

5.3 Protein Corona

The understanding and the control of the fundamental interactions between the nanovehicles and the bio-systems (cells, tissues) plays a central role in the development and the safe implementation of nanomedicine and drug delivery. It is important to understand how nanoscaled objects interact with living matter, in order to better design drug delivery systems that will interact in pre-determined ways with living matter, increasing the efficiency in terms of targeting and delivering of the drug. Several characteristics of the nanovehicle determine a specific biological response, including size, shape and surface area, which will be considered later. It was found that the effective unit of interest in the cell-nanomaterial interaction is not the vehicle itself, but the associated proteins that are more or less strongly associated with the particle and its corona [140]. In the process of absorption, once

Table 2 Advantages and disadvantages of different structures in drug delivery

System	Advantages	Disadvantages
Micelles	Low surfactant concentrations required Small droplet size Easy preparation Good long-term stability Low viscosity	Sensitive to dilution Low solubilization Potential toxicity of surfactant
Liquid crystalline phases	Good long-term stability Generally viscous Good solubilization capacity Solubilization of both hydrophilic and hydrophobic drugs Solubilization of small and large molecules	In some cases difficult to prepare generally viscous Short release duration Sensitive to dilution
Vesicles and liposomes	Might be made of friendly compound Present in the body like lecithin and cholesterol Good solubilization capacity	High viscosity In some cases, difficult to prepare Often disintegrate once administrated
Microemulsions	Good long-term stability Good solubilization capacity Small droplet size Easy preparation Clarity	Requires high surfactant concentrations Drug solubility influenced by the medium conditions Potential toxicity of surfactant
Emulsions	High capacity of solubilization	Poor stability In some cases difficult to prepare Poor bioavailability
Polymers	Good stability Useful controlled release properties Increase stability of drugs/proteins	Low capacity of solubilization Low reduction in hydrolysis
Polymer-polymer/polymer-surfactant	Good stability Reduces molecule size on complexation Solubilization of both hydrophilic and hydrophobic drugs Solubilization of small and large molecules	Too stable Difficult to degrade/dissolve

the drug vehicle is dispersed in a biological fluid (e.g. plasma) it acts as a scaffold for biomolecules such as proteins and lipids, which rapidly adsorb onto the “naked” surface of the drug vehicle, conferring a biological identity and resulting in the so called “protein corona”. The biomolecular corona is a dynamic entity undergoing continuous exchange of proteins not only between the drug vehicle surface and the

Table 3 Advantages and disadvantages of some different routes of drug administration

Route	Administration	Advantages	Limitations
Oral	Mouth	Most common and convenient Safest Cheaper	Destruction of the drug Emesis Variable absorption Patient co-operation
Parenteral	Injection, infusion, or implantation	Active from of the drug is adsorbed Rapid adsorption Accurate selection of the effective dose	Painful Expensive Difficult to self-medicate Asepsis is important
Pulmonary	Inhaling orally for local or systemic effect	Low systemic side-effects Rapid clinical response Non-invasive needle-free delivery system Poor gastrointestinal absorption First-pass hepatic metabolism	Requires significant degree of coordination Variability in drug delivery Patients with lung disease may not be able to inhale adequately
Transmucosal	Throw mucous membranes	Rapid drug transport to the systemic circulation Poor gastrointestinal absorption First-pass hepatic metabolism	Nasal Local toxic effects of the drug Reduction of systemic effect due to the drug metabolism in the respiratory rack Buccal Prolonged exposure to the mucosal surface for significant drug absorption Absorption is only favored for drugs with a high pKa (pH of saliva is 6.5–6.9) Rectal Patient-to-patient irregular uptake Administered dose generally higher than intravenously or orally administration
Topical	Outer surface of the body	Effect in a localized area Less side effects	Irritant local effects Local and general allergies Can reach the blood circulation and have systemic effects

biological fluid, but also interacting with specific protein receptors that can be found on the cell surface [141]. Very important in this area is the determination of binding rates, affinities, and stoichiometries of protein association with, and dissociation from, drug vehicle in biological fluids, since during their internalization process

into the cell, the adsorbed “protein corona” is what the cell “sees”. Therefore, nanoparticle-protein complex composition and structure influence not only cell uptake and trafficking, but also particle bio-distribution within the cell [142].

One of the major challenges in the study of drug delivery is to be able to intravenously deliver and target drug vehicles, as much as possible, in cancer-related tissues or cells in order to generate a sufficient effect. This step can only be successful if the drug vehicle is not eliminated from the systemic circulation by blood monocytes and cells of the phagocyte system (associated with the reticuloendothelial system), specialized for uptake of larger particles. Macrophages are designed to eliminate pathogens from the circulation, by a mechanism in which they recognize and bind specific protein at their surface known as opsonins. Well-known opsonins include IgG (Immunoglobulin G) and IgA (Immunoglobulin A) or components of the complement system (innate immune system, especially C3b, C4b, and iC3b). This body defence mechanism obviously leads to a decrease in the bioavailability of the drug in the desired site. In addition, dose-limiting toxic side effects may occur in the reticuloendothelial related tissues/cells. In this context, researchers focused on the development of methods to reduce this undesired effect. One of the proposed methods is related to coating colloidal drug vehicles, e.g. micelles, O/W emulsions or liposomes, with molecules such as polyoxypropylene block copolymers (poloxamers) [143], polyvinyl alcohol [144], PEG [145] and surfactants like sodium oleate or dodecylamine [146], which showed to be quite attractive as carriers in intravenous drug delivery. The reason for this phenomenon is the reduction of non-specific protein binding, which results from a combination of steric hindrance related to the polymer flexibility and hydrophobicity, and also from the increase of surface compatibility with less disruption of protein structure at the interface, if an intermediate layer of a coating agent (e.g. PEG) is presented. Moreover, the coating of the carrier is also needed to prevent agglomeration, keeping the carrier stable in the colloidal suspension.

Significant efforts have been made to identify the most affine proteins of the “protein corona”, in order to understand how the protein binding can mediate their bio-interactions, which ultimately would lead to a better prediction on how the drug vehicle would interact with the target site (e.g. tumor cell, tissue) [147]. For example, as previously mentioned, adsorption of opsonins onto large carriers promote their removal from the systemic circulation, while binding of dysopsonins (human serum albumin, apolipoproteins) onto small size carriers leads to a prolonged circulation time. A variety of materials, including biodegradable polymers, polystyrene and silica, have been recently functionalized on the surface with a wide range of biological entities to specifically target cellular receptors [148–150].

5.4 Properties of Nanoparticles

Keeping in mind that nanoparticles enter the human body via various pathways, it is important to understand their interaction with the human body and their health

effects, in order to understand both the hazards associated with nanomaterials and the levels of exposure that are likely to occur. Thus, it is easy to understand how important it is for nanomedicine (and all consequences that entails in the strategy of nanoparticle synthesis) to have a broad nanoparticle-cell uptake study, in order to see the impact, and uptake efficiency that different types of nanoparticles have when they are exposed to different cell types. Even if all the details are not yet completely clear, it is evident that new nanoparticle properties will come into play when nanoparticles interact with the human body. For instance, as previously mentioned, it is important to understand the effect of nanoparticles on the immune system and how nanoparticles may contribute to suppress inflammatory responses.

Even with the “protein corona” having a major influence on the cell uptake, other properties of the carrier play a key role in the particle uptake scheme, such as the size, shape, dose and surface properties (hydrophobicity, charge, coating, etc.). Apart from these, also the cell type is of crucial importance in the particle uptake efficiency.

As a result of the variety and complexity in size and scale of materials and components that need to be transported into the cells, a wide range of different cellular uptake mechanisms have evolved. Until now, many distinct uptake pathways have been classified and characterized [151, 152]: (i) phagocytosis is characteristic of specialized mammalian cells such as neutrophils, monocytes and macrophages (particle-dependent); (ii) clathrin-mediated endocytosis (CME) is a mechanism involving specific receptors that recognize and internalize cargo into coated pits formed by the assembly of a cytosolic coat protein (clathrin) which constitutes the main assembly unit (mechanism generally used for nanoparticles of ~ 120 nm); (iii) caveolae-mediated endocytosis, which involves clustering of lipid “raft” components on the plasma membrane into the so-called caveolae (flask-shaped invaginations) formed due to the interaction of different proteins, mainly caveolin, with the cellular membrane (mechanism generally used for nanoparticles of ~ 60 nm); (iv) clathrin- and caveolae-independent endocytosis in which other types of cholesterol-rich microdomains (small structures of 40–50 nm in diameter) on the plasma membrane rather than caveolae, diffuse on the cell surface (mechanism generally used for nanoparticles of ~ 90 nm).

It is well established now that cell uptake leads to a particle-size-dependent biochemical response, with the levels of internalization decreasing when the size of the particle increases. In several studies, the maximum uptake by cells occurs for nanovehicles with 50 nm in diameter [153, 154]. Therefore, these may be the most suitable candidates to be used as carriers for further studies in biological applications. Smaller particles (i.e. less than 50 nm) enter less efficiently, proving the importance of the type of mechanism used by the cell in the process of internalization.

Cellular uptake efficiency is also correlated with the surface charge of the nanoparticles. It has been shown that a positive charge greatly promotes the efficiency of internalization. This occurs due to the electrostatic interactions that are established between the nanoparticle and the negatively charged plasma membrane which promotes their bio-adhesion resulting in an enhanced uptake. However,

Table 4 Toxicity and some adverse effects of different engineered nanoparticles

Adverse effects	Type of nanoparticle
Inhibition of macrophage phagocytosis	TiO ₂ Carbon black
Oxidative stress, (generation of surface radicals and inflammatory action)	Nanoparticle in general Carbon nanotubes
Platelet aggregation	Particulate matter Single walled carbon nanotubes Carboxylic modified nanoparticles
Effect of mitochondria activity	Cationic nanoparticles
Adversely affects cardiac activity	Particulate matter
Access to systemic circulation upon inhalation	Specific coating-nanoparticles

it has been reported that, in some cases, the uptake of positively charged nanoparticles leads to undesirable consequences to the cell. Therefore, one needs to be careful when addressing these questions. After internalization of positive charge nanoparticles, the cell has been shown to undergo distinct processes. The most reported one is cell death via apoptosis [155, 156], which starts with the destabilization of the lysosomal membrane and consequent release of both nanoparticles and proteolytic enzymes such as cathepsins into the cytoplasm [157]. These phenomena result in a series of events that ultimately lead to apoptosis. Other cellular responses have also been described such as the activation of the inflammasome with secretion of interleukin 1 β and production of an inflammatory response or activation of the endoplasmic reticulum stress response [158, 159]. Taking the abovementioned into consideration, careful analysis of deleterious effects to the cell needs to be considered when positively charged nanoparticles are to be used. Therefore, this access of nanoparticles to the cellular machinery can lead to unpredictable consequences and biological impacts, especially from those never intended for contact with humans. It is this concern regarding the safety of nanomaterials that opened the door to a careful nanosafety assessment. It is not immediately clear what the consequences of exposure to nanomaterials, even those of materials that are bio-inert in the bulk scale, such as gold, silica and polystyrene, will be for human health and the environment. Some of the toxicological effects caused by nanoparticles are described in Table 4, showing the great importance of the understanding of all the interactions and potential consequences of nanoparticles with living tissues.

In terms of other properties that may influence cell uptake, hydrophobic domains of the particle surface have an important role. Some studies have been performed showing that protein adsorption on the surface of nanoparticles decreases quite significantly the subsequent uptake of these by different cells [160]. In fact, when nanoparticles are exposed in the absence of fetal bovine serum to the culture medium, significantly higher uptake is obtained. The reason behind this phenomenon is explained by non-specific hydrophobic effects. When bare nanoparticles are administrated to cells, their high surface hydrophobicity will cause them to

adhere to the already hydrophobic plasma membrane. On the other hand, in the presence of serum, adsorption of proteins screens the non-specific hydrophobic binding of the nanoparticles to the cell surface leading to a decrease of the nanoparticle surface hydrophobicity and a consequently lower, but more specific, uptake of the nanoparticles via protein-corona mediated interactions with cellular receptors. In terms of specificity on the uptake, a balance between the administered particle dose and specific binding needs to be considered. In the case of drug targeting to a cancer cell, non-specific binding should be avoided, since also healthy cells would have higher chance to internalize drug-loaded particles, which would decrease the desired therapeutic effect. As previously mentioned, functionalization of the particle surface with different proteins can result in a more specific uptake. Other strategies should also be considered for this, such as coating the outer particle surface with anionic triblock copolymers, which would reduce non-specific interactions with the negatively charged cells.

Other property receiving increased attention is the effect of particle shape on cell response. Depending on the type of material used, different authors have shown significant different conclusions on how dissimilar shaped nanoparticles (spherical, long rod, short rod, etc.) affect the cell uptake [161, 162]. The conclusions show the importance of careful analysis and individual interpretation for each cell-nanoparticle system, although it is obvious that also the shape of nanoparticles should be considered when these are designed for therapeutic applications.

Studies across all different cell lines from different parts of the human body, representative of the main avenues of exposure to nanoparticles have been widely performed. Different cell types show very different uptake efficiencies for the same materials, with macrophages having the highest uptake rate for all nanoparticle sizes, while cells from protective barriers (epithelial and endothelial) show lower uptake [163]. Once more, this emphasizes the need for engineering nanoparticles that are able to evade the immune system, thereby increasing their circulation time, enhancing the efficiency of drug delivery, while being optimized for uptake in the target cells.

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Coelho, J. (Ed.)

2013, XVII, 421 p., Hardcover

ISBN: 978-94-007-6009-7