NANOTECHNOLOGIES *for* SOLUBILIZATION and DELIVERY *in* FOODS and COSMETICS PHARMACEUTICALS

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Preface

Surfactants are involved in almost every facet of life, from cell membranes to products such as pharmaceuticals and cosmetics. Surfactants are studied by a range of experts, in colloid chemistry, interfacial science, and interfacial technology, and specialists from all these areas must be active in the design and development of drugs, foods and cosmetics, whose functionality depends on surfactants.

The present volume presents the fundamentals of colloid science, as well as emerging knowledge of functional mesophases, with a stress on applications. The first chapter provides definitions and general analyses of surfactants in solution and explains numerous manifestations of surfactant aggregations. Chapter 2 offers an overview of micelle formation, representing the simplest class of association by self-assembling amphiphilic molecules. The thermodynamics and kinetics of micellization described in this chapter are crucial concepts for the control of the dissociation and reconstruction of micelles, such as in the solublization, stabilization and delivery of active ingredients in cosmetic, food, detergent and pharmaceutical formulation. In Chapter 3 nanoemulsions are examined in comparison to microemulsions, in terms of thermodynamics, stability and potential applications.

The next section of the book concentrates more on advanced technologies. Given the interest in natural alternatives to synthetic emulsifiers, many proteins and polysaccharides are used as functional ingredients to form colloidal systems for commercial products. Thus, Chapter 4 focuses on the synthesis and properties of sub-micron to micron-sized colloidal particles formed by controlled aggregation of mixed biopolymer systems. In addition, the physical and chemical conditions of fabrication processes are discussed, as well as applications of biopolymer colloidal particles. Chapter 5 investigates the physicochemical and structural properties of protein and lipoprotein assemblies naturally present in foods or induced by thermomechanical treatments. The chapter presents new material on food nutrient carriers, nano- and fine-lipid droplets as food matrix carriers, and a natural nanocapsule to vectorise micronutrients.

The following three chapters offer a comprehensive overview of modern liquid crystal mesophases, a subject areas enjoying a current revival as a result of the failure of lyotropic liquid crystals to find significant applications. Chapter 6 summarizes recent work using soft lipidic liquid crystalline systems as food and drug nanocarriers. The chapter also highlights recent technical developments in characterizing mesostructures. Lyotropic non-lamellar lyotropic mesophases and their nano-dispersions as topical delivery vehicles, are the subject of Chapter 7. Recent advances in transdermal and mucosal drug delivery via lyotropic liquid crystalline carriers are demonstrated in this chapter. The subsequent chapter, the 8th, deals with the modern contribution of these mesophases in nanotechnology, based on their ability to provide new synthesis procedures and self-assembly of nanoscale materials with controllable uniform sizes, shapes and dimensionality. The chapter explains synthesis and self-assembly of nanomaterials using mainly lyotropic liquid crystals and partially thermotropic systems as direct and reverse templates.

Chapter 9 explains the emerging area of organogels fabricated from oils and waxes. This chapter mainly addresses the gel state formed in organic phases through a number of techniques. The organogel technology reveals that scientists can engineer molecules to structure in the continuous phase, which provides new methods for controlled delivery.

Chapter 10 analyzes the subject of solid nanoparticles as applied to pharmaceuticals. It also describes the characterization and therapeutic applications of immunonanoparticles as targeted drug delivery systems, with a focus on cancer therapy.

In Chapter 11 nanoinorganic particles as delivery vehicles are explained. The chapter reviews the antibacterial functionalization of textiles by a sonochemical technique, which has proven effective for the synthesis of various kinds of nanoparticles. In addition, the unique properties made by ultrasound irradiation for adhering nanoparticles to a large variety of substrates is demonstrated.

Finally, Chapter 12 summarizes recent investigations on the characterization, structures and potential applications of dendrimers. The chapter focuses on dendrimers in anti-cancer therapies.

CHAPTER 1

Surfactants in Solution—Basic Concepts

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1. INTRODUCTION

Surfactants are involved in almost every facet of life, from the membranes of living cells to diverse industrial products (such as pharmaceuticals, agrochemicals, and cosmetics). A detailed and comprehensive discussion of the basic physicochemical principles pertinent to understanding surfactants and describing their exciting but intricate phase behavior in detail, is too great an undertaking for a relatively short and introductory chapter. Instead, we will restrict ourselves to defining fundamental concepts that are frequently mentioned in this book. Self-assembled aggregate structures of surfactants will also be described. The list of structures treated here makes no claim to completeness, but it is representative. Obviously, the subsequent chapters will go into some of these concepts in great detail. In writing this chapter we have kept in mind mainly two types of audiences—first, people just entering this fascinating branch of science, who may feel daunted by the large and rapidly growing volume of literature on surfactants; and second, scientists and engineers in industry, who encounter or use surfactants and desire a broader background in a subject that has often been treated inadequately during professional training.

Although no attempt is made to be highly rigorous in discussing the following concepts, the reader is assumed to possess a basic knowledge of physical chemistry.

It is hoped that after understanding the concepts reviewed, and realizing the wealth of manifestations of surfactant aggregation, the reader will be able to take advantage of the wide range of the authors' expertise and experience brought together in this book to produce an erudite and revealing snapshot of the current state of surfactant science.

2. INTERFACE

Formally, interface is the point of interconnection between two entities, such as systems or subsystems. In the context of surface chemistry, the ordinary definition conceives the interface as the common boundary between at least two distinct immiscible phases [1]. A more rigorous definition, based on characteristic features of the spatial variation of specific thermodynamic variables, has been suggested by Laughlin [2]. He defines a phase as "a volume element of a mixture within which smooth variations in space of the density variables exist [2]." Density variables (formerly known as extensive variables), depend on the system size or the amount of material in the system; for example mass, volume, or energy. An interface is then "a surface across which a spatial discontinuity in density variables exists [2]." Thus, the numerical value of the density variables that is characteristic of the phase on one side of the interface will be different from the numerical value of the density variables that is characteristic of the phase on the other side of the interface [2]. Whereas mathematically and intuitively this boundary between phases may be visualized as infinitely thin, interfacial effects may in fact extend over a considerable distance, say several molecules thick [1].

It is also noteworthy that although there is no fundamental distinction between the terms surface and interface, the first term is generally used to describe the boundary between two phases, one of which is gaseous, whereas interface is maintained for the boundary between two condensed phases [1, 3].

3. INTERFACIAL (SURFACE) ENERGY

An interface will be stable when it possesses a positive interfacial free energy. Work must be done to extend this interface by overcoming the short-range attractive forces between its constituting molecules, otherwise random forces will continually change the interface until the phases become mixed [4]. The higher the interfacial energy, the smaller the interfacial area—and thus the phases will separate to the greatest extent possible within the system constraints [4].

4. SURFACE TENSION

The aforementioned attractive forces are relatively large. However, the molecules within the liquid are pulled equally in every direction. The time-averaged force exerted on any given molecule by its neighbors, therefore, is zero. It should be noted that although random intermolecular collisions may cause a certain molecule to undergo a diffusive displacement in a certain direction, this effect is momentary and is equally likely to operate in all directions. In contrast to these balanced forces in the bulk of the liquid, the forces experienced by the molecules in the surface region are unbalanced: these molecules are not surrounded by like

molecules on all sides, since there are no molecules of the liquid beyond the interface. Thus, the molecules in the surface region are drawn by a net attraction directed towards the interior of the liquid [1, 3]. This inward attraction is balanced only by repulsive collisional forces from the other molecules (i.e., the liquid's resistance to compression). The shrinkage of the surface stops then, when the surface area is a minimum.

It must be emphasized that the long accepted conception of the surface as a "contractible skin" or an "extremely thin, stretched elastic membrane (film)," utilized, for instance, to explain the spherical shape of water droplets, is too simplistic [5]. The above description of the behavior of molecules in the surface region seems to eliminate the need for such a skin. Moreover, the definition of surface tension of a liquid as the force acting normally to any line of unit length on the liquid surface is somewhat misleading (although appropriate for liquid films) [3], as it implies the existence of an elastic skin or a tangential force at the surface of the liquid [3]. Therefore, it is better to define surface tension and surface free energy as the work required to increase the area of a surface isothermally and reversibly by unit amount [3].

5. SURFACTANTS

5.1. Surface Activity

We have seen that the energy of surface molecules is higher than that of molecules in the bulk phase. This is because the interaction between the surface molecules and the adjacent phase is weaker than the interaction between them and molecules in the bulk. Therefore, the formation of a new surface by the transfer of molecules from the bulk phase to the surface requires work [1] and leads to an increase in the free energy of the surface molecules [4]. However, there are materials which, at relatively low concentrations, preferentially adsorb at interfaces, replace the higher energy bulk phase molecules, and significantly diminish the total free energy of the system [1] (namely, the amount of work required to expand those interfaces) [6]. Such materials are called surface-active agents, or more briefly, surfactants.

5.2. Molecular Structure of Amphiphiles

The surface activity of surfactants stems from their characteristic chemical amphiphilic (which means, literally, "loving both") structure [4]. Surfactant molecules have two opposing components:

1. A lyophobic (hydrophobic) group, i.e., having very little attraction for the solvent (water) or bulk phase.
2. A lyophilic (hydrophilic) group, i.e., having a strong attraction for the solvent (water) or bulk phase [1, 4].

The use of the terms lyophobic (hydrophobic) and lyophilic (hydrophilic) deserves further comment.

1. The more usual terms polar and nonpolar have apparently no exact definition in the context of surface activity [5]. We can only say that although such polar molecules possess no net dipole moment, they tend to be more soluble in polar solvents (for example, water), whereas nonpolar molecules tend to be more soluble in nonpolar organic solvents (for example, benzene) [5].
2. Hydrophobic means "water-fearing," although there is a (weak) attractive interaction between a hydrophobic molecule and water arising from the dispersion force [7]. Hydrophobic molecules are expelled out of the water due to the cooperative strong interactions of the water molecules that are based on dispersion forces and hydrogen bonding [8]. On the other hand, hydrophilic (i.e., water-loving) molecules interact strongly with the water *via* dipole-dipole or ion-dipole interactions [8] and are believed to disrupt the local water structure [7], in contrast to hydrophobic molecules, which tend to increase the ordering of water molecules around them [7], as explained in the following section.
3. Nonpolar molecules that have the right geometry and contain electronegative atoms (such as the nitrogen atoms in amines or the oxygen atoms in alcohols and polyethylene oxide) capable of associating with the hydrogen-bond network in water—can be hydrophilic [7].
4. In addition to ordinary surfactants that have one hydrophilic head and one hydrophobic tail, surfactant molecules can also have the following structures [9]:
   (a) One head with two tails.
   (b) In bolaform surfactants (also known as α,ω), one tail is terminated at both ends by hydrophilic groups.
   (c) In Gemini surfactants hydrophilic heads of two surfactants are attached to a linear (or ring), rigid spacer.
   (d) In polymeric surfactants more than two hydrophobic groups are linked in the same molecule by covalent bonds.

5.3. Hydrophobic Hydration

Upon dissolving a surfactant in a solvent, the lyophobic group (the tail) causes an unfavorable distortion of the solvent liquid structure, thereby increasing the overall free energy of the system [1, 6]. This distortion means, then, that less work will be needed to bring surfactant molecules than solvent molecules to the available interfaces and the energy of the system will be reduced [1, 4]. Taking, for instance, an aqueous surfactant solution, it is well known that water forms hydrogen bonds using the hydrogen atoms on one molecule and the oxygen lone

pairs of electrons on another, leading to a loose network of tetrahedral bonds at the corners [10]. As the hydrocarbon groups of the dissolved surfactant do not form hydrogen bonds with the water, we could *prima facie* assume that these alkyl groups occupy cavities in the liquid water structure formed necessarily by the breaking of hydrogen bonds with an attendant positive enthalpy contribution [10]. In fact, these alkyl chains function as nucleation sites for network formation [10], and the water is induced to structure around them (hydrophobic hydration) [11]. This stems from the ability of tetrahedrally coordinated molecules to pack around almost any inert solute molecule, regardless of its size or shape, without giving up any of their hydrogen-bonding sites [7]. Actually, the new reoriented water structure around nonpolar solutes (forming highly organized cavity walls) [10] is more ordered than that in the bulk liquid [7].

Thus, the overall entropy of the system is decreased. However, when the surfactant molecules are transported (more accurately, expelled) [10] to the interface, the associated water molecules will be released [4], and the cavity will revert to the less organized structure of pure liquid water with an increase in entropy [10].

5.4. Features of Amphiphilic Molecules

The presence of a lyophilic (head) group on the surfactant molecule prevents (or retards) [1] the complete removal of the solute molecules from the solvent as a separate phase [1, 6] (at least at low concentrations), since desolvation of the lyophilic group would be needed [6].

Thus, the situation is a compromise between a complete phase separation, where the lyophilic groups would be removed from the solvent, and the formation of a molecular disperse solution, where unfavorable lyophilic-lyophobic interactions would be expected [11].

This amphiphilic structure of the surfactant leads then to the following conclusions [6]:

1. The surfactant concentrates at the interface.
2. The surface tension of the solvent is reduced.
3. The orientation of the adsorbed surfactant molecules is such that the lyophobic groups are directed away from the bulk solvent phase, whereas the lyophilic groups are located in this phase [4].

Before proceeding any further it should be emphasized that on the molecular scale, surface activity is a dynamic phenomenon in which the final state of the interface represents a balance between the above-mentioned tendency of surfactants to concentrate at the interface and the tendency towards complete mixing resulting from the thermal motion of the molecules [3].

5. 5. Effect of Solvent

We have shown that the common structural feature of surfactants is the presence of both lyophobic and lyophilic groups in the same molecule. Obviously, the chemical structures of lyophobic and lyophilic groups having suitable solubility properties for surface activity depend on the solvent system to be employed and the conditions of use [1, 4, 6]. Two examples will suffice.

1. In water (a highly polar solvent), the lyophobic group may be a hydrocarbon, fluorocarbon, or siloxane chain of proper length for achieving the desired solubility characteristics [4, 6], whereas in a less polar solvent, such as polypropylene glycol, only fluorocarbon or siloxane chains may be suitable [6].
2. Ions have a strong affinity for water due to their electrostatic attraction to the water dipoles [3], and thus they are capable of pulling fairly long hydrocarbon chains into solution with them [3]. Therefore, they may act as lyophilic groups in water [6]. However, in a nonpolar solvent, for instance, heptane or hexane, these ionic groups may function as lyophobic entities [4, 6].

The classification of surfactants according to their hydrophilic groups (anionic, cationic, etc.) will not be discussed here. The interested reader may consult references [1] or [6] of this chapter.

6. MICELLES AND MICELLIZATION

6.1. Formation of Micelles

It has been demonstrated that the adsorption of surfactants at interfaces lowers the free energy of the system within which they interact [1]. When all available interfaces are saturated, however, surfactant monomers start accumulating in the solution [9], and the overall energy and the distortion of the solvent structure may be diminished *via* other mechanisms—such as crystallization (or precipitation) of the solute from solution, namely bulk phase separation [1]. Alternatively, the amphiphilic molecules spontaneously self-organize into colloidal-sized clusters or aggregates, such as micelles [1, 6].

When the surfactant concentration in solution is low, its properties are similar to those of an ordinary solute [3, 11], except that the surface tension decreases sharply as the surfactant concentration increases [9]. However, at a fairly well defined concentration, called the critical micelle concentration (usually abbreviated to cmc), a sharp and sudden change—characteristic of a given surfactant [4, 9]—in some physical properties such as surface tension, electrical conductivity, specific heat [12], electromotive force [12], osmotic pressure, or turbidity [3, 9], is observed. The value of the cmc is most commonly determined by utilizing the

breaks in the surface tension, light scattering (turbidity), electrical conductivity, or fluorescence spectroscopy [12]—concentration curves [6]. This behavior could be interpreted *via* the formation of thermodynamically stable [1] surfactant aggregates, the micelles [3, 6], in which the lyophobic groups are associated and shielded from extensive contact with the bulk of the water phase [4, 9] and the lyophilic groups are arranged in a shell directed towards the solvent [10, 11]. This entropy-driven aggregation process [10], called micellization, is the result—as in the case of surfactant adsorption—of two competing factors [11]:

1. Removal of the hydrocarbon chains from contact with the solvent (say, water) and transferring them to the hydrophobic interior of the micelle [11]. At the same time, strong water-water interactions can proceed uninterrupted as the alkyl chains are kept away from the water molecules [3]. The hydrophobic water-hydrocarbon interaction is then not repulsive, but rather the result of the combination of this water-water attractive interaction and a weak nonpolar attractive interaction between the hydrocarbon groups [1].
2. Micellization is opposed by the repulsion between headgroups as they come close together [11] and by thermal fluctuations [3].

Consequently, when the structure of the solvent is only a little distorted by the lyophobic group, as in the case of a surfactant with a short hydrophobic chain dissolved in water, there is little tendency for micellization [6]. When the ambient conditions such as solvent, temperature, pressure are identical, the balance between these two opposing tendencies is dictated by the chemical constitution of the amphiphilic molecules. Thus, the micellization characteristics can be determined [1].

It should be stressed that, in contrast to solid particles or rigid molecules that are held together by strong covalent or ionic bonds, association colloids, such as micelles, are associated physically by van der Waals, hydrophobic, hydrogen-bonding, and screened electrostatic interactions [7, 11]. These forces are relatively weak: typically, on the order of *ca.* 10 k_BT per molecule, where k_B is the Boltzmann constant and T is the absolute temperature [13]. Therefore, small changes in ambient conditions such as concentration, pH, temperature, or ionic strength not only will affect the inter-aggregate interactions, but will also affect the intermolecular forces within each aggregate, causing the microstructures of these aggregates to respond to these changes and to modify their size or shape and even their aggregation geometry [13]. The aggregation is a start-stop process [11], which proceeds very fast: surfactant monomers constantly join and leave the microstructure on a timescale of microseconds [9]. Above the cmc, adding more surfactant molecules to the solution will simply increase the number of micelles over a significant concentration range rather than cause further growth of existing micelles [11]. These new micelles all have the same size [13].

6.2. Shapes of Micelles

The shapes of micelles have been studied intensively, but elaborating on all the details of this issue does not serve the purpose of this tutorial chapter, so we will only give the relevant background and data. The classical picture of a micelle describes a roughly spherical aggregate, having an aggregation number (the number of surfactant monomers within the micelle) of about 50–100. Old models portrayed the surfactant tails within such micelles as being straight and arranged radially, towards the center of the micelle, as in the spokes of a wheel [14] or a symmetrical asterisk [15]. This depiction, too simplistic and schematic, is still surprisingly prevalent [14], and used widely for illustrative purposes [16]. It would imply a high degree of organization and different densities in the micellar center (high), and near the circumference (low). But this is without physical basis [15], because, in fact, a uniform, fluid-like density is maintained in the aggregate core [17]. This misconceived and misleading picture should therefore be rejected [16], and the hydrocarbon core envisaged as compact and liquid-like [13]. The extent of water penetration into this hydrophobic core is somewhat unclear, although the notion of "an abundance of hydrocarbon-water contact" [14] may be rather exaggerated if it implies extensive penetration of water far into the micellar core [15]. In fact, the driving force for micelle formation minimizes such hydrocarbon-water contacts [1, 4], an argument amply corroborated by several experimental techniques such as X-ray scattering, small-angle neutron scattering (SANS), and measurements of microviscosities and order parameters, all showing that the hydrophobic core is disordered and similar to liquid paraffins [16].

The penetration of water into micelles is then very restricted. Even so, extensive contacts occur between methylene and methyl groups and water [16]. These seemingly inconsistent statements are explained by the following facts:

1. The conformational freedom of the hydrophobic chains [16].
2. The constant movement of surfactant molecules in and out of micelles roughens the surface of the micellar core [9], and this dynamic protrusion further favors water-hydrocarbon contacts without the need for water penetration [16].
3. The methylene groups attached to the polar headgroups are constrained to remain at the aggregate-water interface, thereby enabling some water-hydrocarbon interaction [17]. Even the first few methylene groups of the alkyl chain adjacent to the surfactant headgroup (and not just the attached CH_2 group) are frequently considered to be in the hydration sphere [6].
4. The surfactant headgroups do not cover all the surface area available per surfactant at the micelle surface. Contacts between chain segments and water can occur on the exposed surface even without penetration [16].

The division of the core into two distinct regions—a water-free inner core and a palisade layer, which is a hydrated shell [9], is then plausible and useful [6].

The radius of a spherical micelle with minimal contact between the alkyl chains and water is thought to be slightly less [7] than the molecular length of the hydrophobic chain of the surfactant when extended to its fullest [3, 4, 6]. Otherwise, the hydrophobic chains would have in the center either a void or hydrophilic groups [3]. This limitation is, of course, also valid for other aggregates. For example, a planar bilayer can grow laterally along two dimensions; but its third dimension, i.e., the distance between the two hydrocarbon-water interfaces, cannot exceed a distance that equals twice the length of the fully extended hydrocarbon tail [13]. The interaction between molecules in water is largely isotropic or non-directional [7], so that they are expected to coalesce and grow as small spherical droplets, leading eventually to phase separation at the solubility limit [7]. As has been mentioned, the classical models of micelles also assume a spherical shape of the aggregate, which is the most thermodynamically favorable micellar shape [1]. In fact, other structures, such as prolate ellipsoids (elongated, cylindrical, rod-like micelles, ending with hemispherical caps), disc-like extended oblate spheroids (large, flat lamellar micelles), and vesicles (roughly spherical structures, consisting of bilayer lamellar micelles arranged in one or more concentric spheres) [6] are more commonly encountered [4].

These more complex structures are determined by anisotropic binding forces acting between the lyophobic and lyophilic groups of the amphiphilic molecules [7]. For instance, we will analyze the sphere-to-rod transition. This is not the formation of a "pearl necklace" by linear association of spherical micelles that do not lose their shape, but, rather, the merging of these micelles to form a uniform, elongated cylindrical aggregate [18]. This transition may be induced by increasing the surfactant concentration, which leads to the formation of rods *via* one-dimensional uniaxial growth of the micelles [19]. The sphere-to-rod transition is entropically unfavorable [20], since the entropy of many spherical micelles is greater than that of fewer longer ones [20]. Therefore, the micellar growth is driven by the free energy difference between the surfactant molecules present in the cylindrical main body of the micelle and the molecules residing at the two roughly hemispherical caps, located at the ends of the micellar cylinder, that prevent direct hydrocarbon-water contact [13]. As the energy of the end-caps is higher than that of the cylindrical body, free energy is gained when short cylinders are joined linearly to form a longer cylindrical micelle, because the number of end-caps is reduced [21]. This free energy gain is then the thermodynamic incentive for the elongation of micelles, even to the size of giant, worm-like, entities.

6.3. The Critical Packing Parameter

The equilibrium structures of amphiphilic molecules are determined by the thermodynamics of self-assembly and both intra- and inter-aggregate forces.

Molecular packing considerations are obviously involved when the effect of the intra-aggregate forces on the formation of amphiphilic structures is examined [7]. Here, the notion of a critical packing parameter, or shape factor [1], $v/a_o l_c$, is very useful in predicting the preferred aggregation geometry in dilute solutions. The optimal headgroup area, a_o, at which the total interaction energy per molecule is a minimum [7], is dictated by the balance of two opposing forces operating at the hydrocarbon-water interface [7]:

1. The repulsion between the headgroups (which may be based on steric and hydration force contributions—and, in the case of charged headgroups, also on an electrostatic double-layer contribution) [7], which tends to maximize the interfacial area per molecule exposed to the aqueous phase.
2. Hydrophobic attraction, which induces the surfactant molecules to associate, and tends to minimize that interfacial area.

The geometric properties also depend on the volume of the hydrocarbon chain(s)—v, and the effective (or critical) length of the chains, l_c, namely the limit determining how far the chains can extend. Beyond this distance hydrocarbon chains can no longer be considered fluid [7]. Numerically, l_c is somewhat less than the fully extended molecular length of the chains.

The use of the packing parameter can be illustrated by the following example [7]: in a spherical micelle, the optimal surface area, a_o, must be sufficiently large and the hydrocarbon volume, v, sufficiently small so that the micellar radius, R, will not exceed the critical chain length, l_c. Simple geometric considerations lead to the following equation for a mean aggregation number, M: $M = 4\pi R^2/a_o = 4\pi R^3/3v$, so that $R = 3v/a_o$ [7]. Consequently, only for $v/a_o l_c < 1/3$ will the amphiphiles be able to pack into spherical micelles with their headgroup areas equal to a_o and $R \leq l_c$. This prediction has been experimentally attested, usually for simple surfactants with single chains and relatively large headgroups [1].

Relatively large cylindrical or rod-shaped micelles are expected to form when the value of the packing parameter is between 1/3 and 1/2. Appropriate surfactants would have relatively small headgroups, or be ionic in the presence of large amounts of electrolyte [1]. Vesicles and flexible bilayer structures will form when $v/a_o l_c = 1/2–1$. Here, double-chain surfactants with large headgroups and flexible chains are suitable candidates [1].

When the packing parameter equals 1, it is expected that double-chain surfactants with small headgroups or rigid, immobile chains will form planar extended bilayer structures [1].

When $v/a_o l_c > 1$, inverted, or reverse, micelles (i.e., micelles that have an outer hydrophobic layer surrounding the core in which the hydrophilic headgroups are held together *via* dipole-dipole interactions), [6] are formed, usually involving double-chain surfactants with small headgroups and very large bulky hydrophobic groups [1].

CHAPTER 12

Dendrimeric Polymers for Pharma Applications—Anti-Cancer Therapies

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Abstract *During the last twenty years dendrimers have attracted wide interest as potential therapeutics. These novel macromolecules differ in many ways from traditional polymers. Dendrimers are globular, possessing a core molecule to which layers of branched monomers are attached. The number of layers is described by so-called generations. The structure of dendrimers results in plenty of terminal surface groups and empty internal cavities. Both these features are important when considering dendrimers for biomedical applications. Moreover, precise methods of synthesis enable the tailoring of dendrimers to specific purposes. In this concise review, a few examples of the part dendrimers play in anti-cancer therapies will be presented. It is worth stressing that these examples of pharma applications of dendrimers do not include all areas of study that are presently being conducted, and that each year produce new ways to use dendrimers in medicine.*

1. INTRODUCTION

Delivery of drugs to tumors has been the area of constant research, because the development of safe and effective dosage is a necessity. One of the most common approaches in the fight against cancer is chemotherapy, which aims at complete elimination of tumor mass. Because of the great toxicity associated with most anti-cancer agents, however, there has been much effort devoted to the development of strategies for specifically and preferentially targeting tumors, while at the same time reducing the access of these drugs to healthy tissues. The disadvantages of conventional chemotherapy raised interest in other, innovative approaches, such as gene therapy, phototherapy and targeted radiotherapy.

The aim of this chapter is to summarize the achievements of the last few years in using dendrimers—a new group of polymers—to improve the treatment of can-

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Abbreviations

ALA	5-aminolevulinic acid
BNCT	boron neutron capture therapy
EGFR	epidermal growth factor receptor
G*n*	*n*-th generation
ODN	antisense oligonucleotide
PAMAM	polyamidoamine
PEG	polyethyleneglycol
PPI	polypropyleneimine

cer. Dendrimers offer several advantages over traditional polymers: they are regularly hyperbranched, monodispersive molecules that adopt a globular shape and provide multivalency by means of many functional groups on the surface. The second distinctive property of dendrimers is the presence of empty internal cavities. Both these feature make dendrimers excellent carriers of drugs (Figure 1).

Chemotherapeutics can be encapsulated inside dendrimers or attached to their surfaces. The main benefits are related to the enhancement of drug solubility, the improvement of drug transit across biological barriers, slower release, and direct targeting of the drug to diseased tissues.

When polyether-copolyester dendrimers were evaluated as methotrexate carriers for the treatment of gliomas, the amount of drug transported across blood-brain barrier was three to five times higher after loading in dendrimers [1]. To overcome the problem of poor solubility of paclitaxel, PAMAM dendrimers (G3

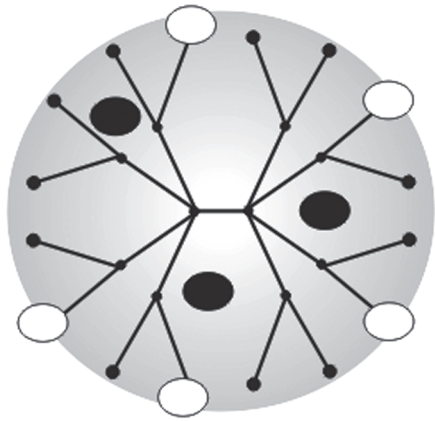

Figure. 1. The structure of the dendrimer and two strategies of carrying drugs—encapsulation (in black) and conjugation (in white).

Table 1
Examples of Using Dendrimers as Carriers of Anticancer Drugs.

Drug	The Way of Carrying	References
5-fluorouracil	encapsulation	Tripathi et al. [4]; Bhandra et al. [5]
5-fluorouracil	conjugation	Zhou *et al*., 1999
adriamycin	conjugation	Kono *et al*. [6]
camptothecin	encapsulation	Morgan *et al*. [7]; Cheng *et al*. [8]
campothecin	conjugation	Fox *et al*. [9]
cisplatin	conjugation	Malik *et al*., 1997; Malik *et al*. [10]; Chen *et al*. [11]
dimethoxycurcumin	encapsulation	Markatou *et al*. [12]
doxorubicin	encapsulation	Kojima *et al*. [13]; Wang *et al*. [14]
doxorubicin	conjugation	Ihre *et al*. [15]; Ihre *et al*. [16]; Padilla De Jesús *et al*. [17]; Papagiannaros *et al*. [18]; Lee *et al*. [19]; Zhu *et al*. [20]
methotrexate	encapsulation	Kojima *et al*. [13]; Neerman *et al*. [21]; Patri *et al*., 2005; Pan *et al*. [22]
methotrexate	conjugation	Liu *et al*.[23]; Patri *et al*., 2005, Gurdag *et al*. [24]; Kaminskas *et al*. [25]
paclitaxel	encapsulation	Ooya *et al*. [26]; Devarakonda *et al*. [2]
paclitaxel	conjugation	Khandare *et al*. [27]; Majoros *et al*. [28]; Lim *et al*. [29]

and G5) were added. In the presence of lower and higher generations, the solubility increased 15 fold and 119 fold, respectively [2]. Examples of other drugs used and the way they were conjugated with dendrimers are presented in Table 1. More details can be found in the earlier work of the authors [3].

2. RECEPTOR-BASED TARGETED DELIVERY

Tumor cells are characterized by unusual genetic setups, and they differ from healthy cells in their high expression of various surface markers and proteins that can be utilized as receptors of ligands. The specific and selective binding of a ligand to its receptor can determine the biodistribution of anti-cancer drugs, and hence exert control over pharmacokinetic properties of the drug. The extraordinary advantage offered by nanotherapeutics using this approach was described in the review by Agarwal *et al.* [30]. Due to their multivalency, dendrimers provide a unique platform for targeted drug delivery: multiple copies of ligands can be attached to the dendrimer and facilitate targeting to the tumor surface.

Folic acid is a vitamin necessary for the synthesis of purines and pyrimidines. Due to the increased demand for folic acid by tumor cells, increased numbers of folate receptors appear on the tumor cell surface, in order to capture more folic acids. This fact was first utilized in methotrexate therapy—an anti-cancer drug which is an analogue of folic acid. Folate receptors serve as prominent sites for entry of methotrexate. Later it was found that folic acids can be ligands maneuvering the drug to enhance the access to tumor. Sometimes these approaches can be combined. Folic acid and fluorescein were conjugated to PAMAM G5 dendrimers through a thiourea and amide linkage, and methotrexate was conjugated through an ester linkage. By this means a trifunctional device was obtained. Fluorescein allowed the detection of the dendrimer and demonstrated cellular internalization of the conjugate. This trifunctional dendrimer induced a time- and dose-dependent inhibition of KB cells. The binding of dendrimer to folic acid receptors occurred because nontargeted dendrimer-drug conjugates failed to induce growth inhibition [31]. Multivalent enhancement of dissociation constants between dendrimers and folate-binding protein, not an enhanced rate of endocytosis, is the key factor resulting in the improved biological targeting [32]. The first study to demonstrate successful *in vivo* targeted drug delivery to cancer cells by intravenously administered dendrimers involved methotrexate-carrying dendrimers that could recognize cells expressing folate receptors. Targeted delivery of methotrexate via dendrimers was shown to be markedly more effective at delaying the growth of epithelial cancer xenografts in mice than the drug given alone [33]. Folate-PEG-PAMAM dendrimers were used to transport 5-fluorouracil in tumor-bearing mice. Tailoring of dendrimers via PEG-folic acid reduced hemolytic toxicity, which led to a sustained drug release pattern as well as higher accumulation in the tumor area [34].

Transferrin receptors have been shown to be over-expressed on rapidly growing and fast multiplying cells. Their expression on tumor surface is about 10-fold higher than in non-tumor cells. Because transferrin also represents a possible targeting moiety for brain endothelia, it was conjugated to PAMAM dendrimers via bifunctional polyethyleneglycol, to achieve a novel brain-targeting gene vector. This vector showed a concentration-dependent manner in a cellular uptake study, and a 2.25-fold increased brain uptake compared with PAMAM and PAMAM-PEG dendrimers. Transfection efficiency of complexes with transferrin in brain capillary endothelial cells was much higher than for non-complexed dendrimers [35].

Epidermal growth factor plays an important role in the disposition of neoplastic cells and transcription and proliferation of cells. The expression on tumors of epidermal growth factor receptor (EGFRs) is 100-fold greater than on normal cells, and hence they provide a potential target for immunotherapeutic agents. The monoclonal antibody, cetuximab, which binds to EGFRs, was covalently linked to PAMAM G5 dendrimer containing the anti-cancer drug methotrexate [36]. In

another study, cetuximab was conjugated with heavily boronated PAMAM dendrimers to create a delivery agent for boron neutron capture therapy for gliomas [37].

Atypical deposition of glucose and related monomers and abnormal glycosylation of cancer cells lead to the expression of various surface binding lectin-like receptors that have high affinity for carbohydrate molecules. Such glyco-coat changes on cancer cells can offer potential targets for immune recognition through lectin-like receptors present on immune cells. Octavalent PAMAM dendrimers were functionalized with N-acetyl-glucosamine residues. These glycodendrimers stimulated antitumor immune response in mice inoculated with melanoma cells [38].

3. ENHANCED PERMEATION AND RETENTION EFFECT

The rapidly expanding necrotic tumor mass needs an increased supply of nutrient, which leads to the altered morphology of blood vessels interpenetrating the tumor mass. They have several abnormalities compared to normal blood vessels, including a high proportion of proliferating endothelial cells with aberrant underlying basement membrane, increased tortuosity of blood vessels, and a deficiency in pericytes [39]. Size and structure of dendrimers favor their entry in the highly permeable tumor vasculature. The transport of dendrimers across tumor microvasculature may occur through open interendothelial junctions or transendothelial channels. The tumor lymphatic system is also abnormal, resulting in fluid retention in tumors and high interstitial pressure with an outward convective interstitial fluid flow [40]. The lack of an intact lymphatic system results in retention of dendrimers in the tumor interstitium since these macromolecules are not readily cleared from the interstitium. Dextran-conjugated PPI dendrimers have been found to be effective carriers of doxorubicin. They selectively entered highly porous masses of tumor cells, at the same time avoiding normal tissues. This selective location in the tumor mass increased the therapeutic margin of safety and reduced side effects associated with doxorubicin [41]. Monodispersive nature of dendrimers is crucial during drug delivery to tumor mass because the vessels can be accessible to particles of one size but not to others [42].

4. pH-TARGETED DELIVERY AND PHOTOCHEMICAL INTERNALIZATION

The microenvironment within tumors is a little more acidic compared with healthy tissues. This feature can be utilized for pH-targeted drug delivery. Drug release triggered by pH can be achieved for both PAMAM and PPI dendrimers. At the physiological pH, the tertiary amine groups of these dendrimers remain deprotonated. This prevents the release of drug in the environment. Once dendrimers

enter the tumor vasculature, decrease of pH causes protonation of amine groups that leads to conformational changes facilitating the release of drugs [43]. Alternatively, drugs can be conjugated to dendrimers via pH-sensitive linkers, such as doxorubicin to PAMAM dendrimers [16].

Photochemical internalization is a novel technology to release the endocytosed macromolecules into the cytosol. The mechanism involves breakdown of endosomal or lysosomal membranes by photoactivated photosensitizers that localize in the membranes of these organelles. Doxorubicin was conjugated to the PAMAM dendrimers through the amide (PAMAM-amide-DOX) or the hydrazone (PAMAM-hyd-DOX) bonds. The "light after" photochemical internalization treatment was efficient in releasing doxorubicin from the PAMAM-hyd-DOX conjugates, resulting in more nuclear accumulation of the drug and more cell death through synergistic effects [44]. The same strategy was applied to transport saporin by PAMAM dendrimers. The cellular uptake of saporin was increased after conjugation with the PAMAM dendrimer, and the cytotoxic effect improved by more than one order of magnitude. The cytotoxicity of free saporin and PAMAM-saporin was further enhanced by the photochemical internalization technology that changed the mechanism of cellular uptake of free saporin and caused more saporin to enter the cells—PAMAM-saporin was not only internalized into the cytosol, but also efficiently entered the nuclei [45].

5. CANCER GENE THERAPY

Gene therapy is based on a simple assumption: if there is insufficient expression of natural proteins, it can be compensated for by the delivery of exogenous gene into a cell in order to express the encoded protein. Special systems, called vectors, must be employed to achieve a successful delivery of DNA into cells. Vectors can be divided into two categories: viral and non-viral. Dendrimers belong to one of the most efficient non-viral carriers. The complex of DNA with dendrimers is called a dendriplex.

Tumor invasion can be inhibited by controlling angiogenesis [46]. PAMAM dendrimers were associated with 36-mer anionic oligomers for delivering angiostatin and tissue inhibitor of metalloproteinase (TIMP-2) genes [47]. First, the dendriplex capacity to promote gene transfer into breast cancer and endothelial cells was checked *in vitro* using plasmid coding for a green fluorescent protein. Next, the *in vitro* gene transfer efficiency of angiostatin and TIMP-2 to endothelial and cancer cells was analyzed. Gene transfer significantly reduced the proliferation of endothelial cells, the healing of endothelial and cancer cells, and the formation of capillary tubes. Finally, gene transfer was tested *in vivo* on mice. The results were very encouraging: primary tumor growth was inhibited dramatically and vascularization within tumors decreased. When PPI dendrimers were employed for gene delivery, gene expression occurred predominantly in the liver

but not in the lungs [48]. When anti-cancer apoptosis-causing genes are delivered, lung expression should be avoided [49].

Gene therapy not only allows the insertion of DNA into cells to express proteins, but also disrupts the expression of disease-related genes. There are two approaches: the translational level and the transcriptional level. In the first, designed antisense oligonucleotides (ODNs) specifically bind to a short complementary sequence of target mRNA to prevent the translation of the target gene [50]. In the second, transcription is disrupted by the binding of a triplex-forming oligonucleotide at the promoter region of a target gene [51]. The ODNs require an effective delivery system because naked ODNs are poorly transported across cell membranes and are rapidly destroyed by cellular nucleases. Dendrimers have been utilized as carriers for ODNs in both strategies.

Hollins *et al*. [52] evaluated the potential of low-generations (G2 and G3) of PPI dendrimers for cellular delivery of antisense oligonucleotides targeted to the epidermal growth factor receptor in epidermoid carcinoma cells. The receptor plays a central role in the initiation and development of breast, brain and lung tumors [53]. The tested dendrimers turned out to be good delivery systems for antisense ODNs. Targeted gene expression and cell growth were inhibited. For both generations of dendrimers, the antisense oligonucleotide uptake increased about ten times.

Santhakumaram *et al*. [54] investigated the efficiency of five generations of PPI dendrimers (G1-G5) in delivering a 31 nt triplex-forming oligonucleotide targeted to the c-*myc* oncogene in breast, prostate and ovarian cancer cell lines. C-*myc* is involved in cell proliferation. Their results showed that dendrimers were capable of facilitating the uptake of the ODNs in several cancer cell lines. In the case of breast cancer cells, dendrimers increased the uptake by 14-fold compared to control ODNs. Dendrimers did not have a significant effect on cell viability in a used concentration range. The efficiency of dendrimers depended on the generation number and was the highest for G4.

A sequence-specific gene-silencing process, RNA interference, can be triggered by small interfering RNA (siRNA), which exerts a biological effect by guiding the degradation of the cognate mRNA sequence, thereby shutting down production of the corresponding protein. Structurally flexible triethanolamine core PAMAM dendrimers are able to effectively deliver siRNA into prostate cancer cells (PC-3) by forming stable nanoparticles with Hsp27 siRNA, protecting the siRNA from enzymatic degradation, and enhancing cellular uptake of siRNA. The Hsp27 siRNA results in potent and specific gene silencing of heat-shock protein 27—an attractive therapeutic target in castrate-resistant prostate cancer. Silencing of the hsp27 gene led to induction of caspase-3/7-dependent apoptosis and inhibition of PC-3 cell growth *in vitro*. Moreover, the siRNA–dendrimer complexes were non-cytotoxic under the conditions used for siRNA delivery [55]. To enhance siRNA delivery to U87 malignant glioma cells, PAMAM dendrimers were modified by

the addition of cyclic RGD targeting peptides and then associated with siRNA [56]. Acetylation was another applied modification of PAMAM dendrimers. It turned out that acetylation of a modest fraction of primary amines of PAMAM dendrimers (approximately 20%) promoted the release of siRNA from dendrimer/siRNA complexes. However, higher degrees of amine neutralization reduced the gene silencing efficiency of PAMAM/siRNA delivery vectors in U87 malignant glioma cells [57].

It is possible to deliver, at the same time, chemotherapeutics and nucleic acids. Poly (L-lysine) dendrimers have been used for co-delivery of doxorubicin and siRNA. The complex showed higher cytotoxicity than free DOX in glioblastoma U87 cells and significantly higher gene silencing efficiency was observed [58]. Antisense inhibition of micro-RNA (miRNA) that is strongly overexpressed in breast cancer cells was combined with delivery of 5-fluorouracil by PAMAM dendrimers. The strategy significantly improved the chemosensitivity of 5-fluorouracil on breast cancer cells MCF-7 [59].

6. INTRINSIC ANTI-CANCER ACTIVITY

There are studies suggesting that the intrinsic anti-tumor activity of some dendrimers, together with their transfection capability, can be exploited to markedly improve the success of cancer gene therapy. PPI dendrimer vector not only has shown the ability to deliver genes to tumors but also has a modest anti-tumor activity of its own in xenografts of A431 epidermoid carcinoma or LS174T colorectal adenocarcinoma in nude mice [60]. The mechanism for the dendrimer-induced anti-tumor activity might be immune stimulation. The immune system is able to fight against cancers through the presence of several kinds of cells derived from stem cells in bone marrow, in particular natural killer (NK) cells, monocytes and dendritic cells (which are part of the innate immunity) and B and T lymphocytes (which are part of the adaptive immunity).

Phosphorus-containing dendrimers displayed the unexpected property of stimulating the immune system. They dramatically and selectively promoted the multiplication of human natural killer cells. Depending on the size of the dendrimers and on the type, number, and even the geometry of the end groups they bore, large differences in their bioactivity toward NK cell multiplication were observed—even up to 500-fold in certain cases, which was unprecedented. Furthermore, the bioactivity of the NK cells generated in the presence of dendrimers was not modified [61]. The mechanism of the action of phosphonate-capped dendrimers involves inhibition of $CD4^+$ T lymphocyte proliferation that leads to a rapid enrichment of NK cells [62].

In addition to the indirect effect of dendrimers, a direct mechanism cannot be excluded. A gene expression profiling study of human A431 cells treated with PPI dendrimers has shown that these dendrimers at low concentrations induced

global gene expression changes. This finding could (potentially) indicate that such dendrimers exert pleiotropic biological effects, including induction of apoptosis and some cytokine genes which could be important for their effects on tumor cells [63]. The extent and type of gene changes appeared to be dependent on the PPI dendrimer generation and cell type.

7. PHOTODYNAMIC THERAPY

Photodynamic therapy is mainly used in a topical treatment for cancer. It involves two procedures: the administration of a light-activated photosensitive drug, and illumination of the tumor to activate the drug. Activation of the photosensitizer leads to the generation of reactive oxygen species that damage intracellular species such as lipids and amino acid residues through oxidation, ultimately leading to cell death. Efficacy of this therapy is high for small superficial tumors and, except for temporary skin photosensitization, no long-term side effects are observed. The procedure can be repeated without cumulative toxicity [64–66]. The most commonly used photosensitizers possess a porphyrin structure. Protoporphyrin IX and Rose Bengal belong to this class of agents. They were encapsulated in PEGylated PAMAM and PPI dendrimers. PEG-PPI held photosensitizers in a more stable manner than PEG-PAMAM because of their inner hydrophobicity. The complex of protoporphyrin IX with PEG-PPI exhibited efficient cytotoxicity, compared with free protoporphyrin IX [67].

A natural precursor of protoporphyrin IX is 5-aminolevulinic acid (ALA): the cellular concentrations of protoporphyrin IX can be increased by the administration of ALA [68]. A major limitation in using ALA is its low intracellular availability due to its hydrophilic nature, leading to poor penetration through the tumor. To increase cellular uptake, ALA-containing dendrimers were synthesized [69]. ALA residues were attached to the periphery by ester linkages, with amide bonds connecting the dendrons. An increased production of protoporphyrin IX and higher toxic effect after irradiation were observed for dendrimers than for free ALA in a transformed PAM 212 keratinocyte cell line and skin explants [70]. When the dendron that is a building block of these dendrimers was administered (both systemically and topically) to tumor-bearing mice, it induced higher porphyrin levels in most studied tissues than did the widely investigated hexyl ester derivative of ALA [71]. Dendrimer bearing eighteen ALA residues has been studied in the PAM 212 keratinocyte and A431 human epidermoid carcinoma cell lines. The ALA residues were coupled to the dendrimer by ester linkages so that ALA could be released within the cells for subsequent metabolism to protoporphyrin IX. Efficient porphyrin sensitization and cell death following light exposure were demonstrated [72]. The efficacy of this dendrimer for inducing protoporphyrin IX synthesis was also studied. The dendrimer was more efficient *in vitro* than ALA for porphyrin synthesis at low concentrations in good correlation with higher cel-

lular ALA dendrimer accumulation. However, *in vivo*, the porphyrin kinetics from ALA exhibited an early peak between 3 and 4 hours in most tissues, whereas the dendrimer induced sustained porphyrin production for over 24 hours [73].

8. BORON NEUTRON CAPTURE THERAPY

Boron neutron capture therapy (BNCT), an experimental approach to treat brain tumors, uses a two-step process. First, a patient is injected with a non-radioactive pharmaceutical which selectively migrates to cancer cells. This component contains a stable isotope of boron (^{10}B). Next, the patient is irradiated by a beam of low-energy or thermal neutrons. The neutrons react with the boron in the tumor to generate alpha particles, which destroy the tumor leaving normal cells unaffected [74]. In order to sustain a lethal reaction, a large number of ^{10}B atoms must be delivered to each cancer cell. The selective delivery is achieved by using boronated antibodies directed to tumor related antigens. First tests on these compounds gave positive results [75–77]. The level of antibodies was enough to sustain a lethal reaction against tumor cells. Barth *et al*. (2002) were the first to show *in vivo* efficacy of BNCT using boronated PAMAM G4 dendrimers. Conjugating the system with folic acid residues made the delivery of ^{10}B more selective [78]. Studies using folate receptor cells *in vitro* demonstrated receptor-dependent uptake of the conjugate. Biodistribution studies revealed that boronated PAMAM G5 dendrimers conjugated with monoclonal antibody accumulated very selectively in the tumor [37].

9. RADIOTHERAPY WITH DENDRIMERS

Recently, poly(^{198}Au)-dendrimer composites of distinct sizes (diameters between 10 and 29 nm) have been reported for a cancer therapy in a melanoma mouse model. A single-intratumoral injection of the poly(^{198}Au)-dendrimer composite in phosphate-buffered saline, delivering a dose of 74 μCi, resulted in a 45% reduction in tumor volume after 8 days. The difference was statistically significant when compared with either the untreated mice or those injected with the "cold" composite. No clinical toxicity was observed during the experiments. This study provides the first proof that radioactive dendrimers can deliver therapeutic doses to tumors [79].

CONCLUSIONS

In recent years the pace of development of new anti-cancer therapies based on dendrimers has seen sharp growth. When these approaches are optimized, dendrimers may offer hope for the improved treatment options for cancer that are so urgently sought. The search will focus on new strategies for targeting to improve delivery at the tumor level while decreasing toxicity to normal tissues.

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