# Chapter 2
# Low Molecular Weight Micelles

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**Abstract** Low molecular weight amphiphile micelles are formed from the self-assembly of comparatively hydrophilic amphiphiles (molecular weight <1,500 Da). These structures may be spherical or present as nanofibres in the case of peptide amphiphiles; the latter with one axis in the 5–20 nm size range. Micelles are formed from amphiphiles in aqueous media and micelle formation is driven by the need to reduce the energetically unfavourable interactions between the hydrophobic regions of the amphiphilic molecule and the bulk water molecules. Micelles are used for the delivery of hydrophobic drugs and are usually used in intravenous formulations. Hydrophobic drugs may be encapsulated within the hydrophobic micelle core, increasing the level of hydrophobic drug that may be incorporated within aqueous media by over 1,000-fold in some cases. The characterisation of micelles for pharmaceutical use involves a determination of: the critical micellar concentration (the concentration at which micellisation starts), the colloidal stability of the dispersion, micelle particle size, micelle morphology and the drug encapsulation or drug solubilisation capacity of the micellar dispersion. Micelles formed from low molecular weight amphiphiles are dynamic structures and there is continuous exchange of material between the micellar aggregate and the bulk medium; this dynamic exchange has a negative effect on the stability and biocompatibility of micellar formulations.

## 2.1 Introduction

Micelles are colloidal aggregates formed by the self-assembly of amphiphiles (Fig. 2.1) and the use of micelles in various industrial and domestic applications is not new. The solubilisation of fats for example, which essentially involves the

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I.F. Uchegbu et al. (eds.), *Fundamentals of Pharmaceutical Nanoscience*,
DOI 10.1007/978-1-4614-9164-4_2, 

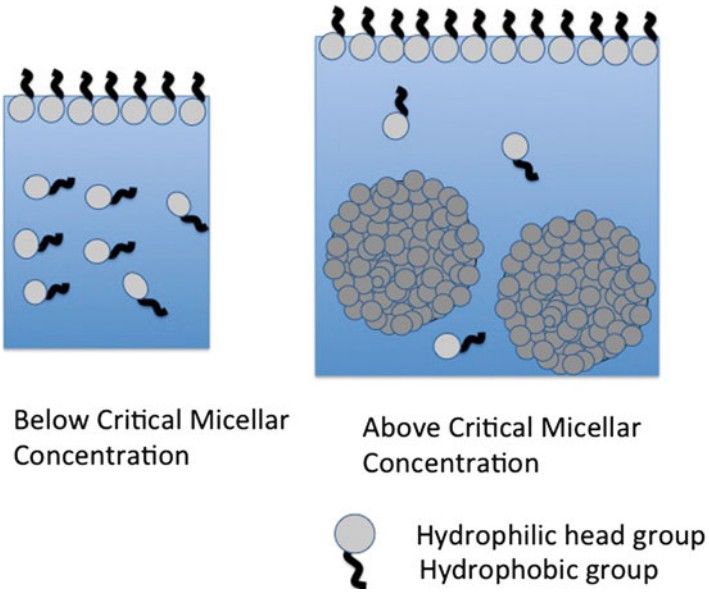


**Fig. 2.1** The self-assembly of amphiphiles into micelles. Below the critical micellar concentration, the molecules exist as monomers and above the critical micellar concentration, the monomers exist as micelles with a hydrophobic core and a hydrophilic surface. Drug solubilisation in aqueous media is achieved by the incorporation of hydrophobic drugs within the apolar micellar core

encapsulation of fats within a micellar core by amphiphiles (soaps), is many thousands of years old. However, it was at the start of the twentieth century that the first formal identification of micellar systems was made by McBain and colleagues (Laing and McBain 1920). The colloidal aggregates formed when fatty acids are added to alkalis were termed micelles and described as molecular aggregates. The micelle has since taken its place as a fundamental unit involved in the dispersion of hydrophobic drugs within aqueous media (Florence and Attwood 2006) and the dispersion of dietary fats within the small intestine (St-Pierre et al. 2001).

## 2.2 Materials Chemistry

Micelles are formed from amphiphilic compounds in which the hydrophilic and hydrophobic chemical moieties lie in geometrically distinct regions of the molecule. The low molecular weight amphiphiles, which form micelles, are relatively hydrophilic (e.g. Fig. 2.2). Israelachvili's Critical Packing Parameter (CPP) has been used to categorise the types of self-assemblies that will arise from a range of amphiphilic molecules (Israelachvili 2011). Essentially the resulting self-assembly

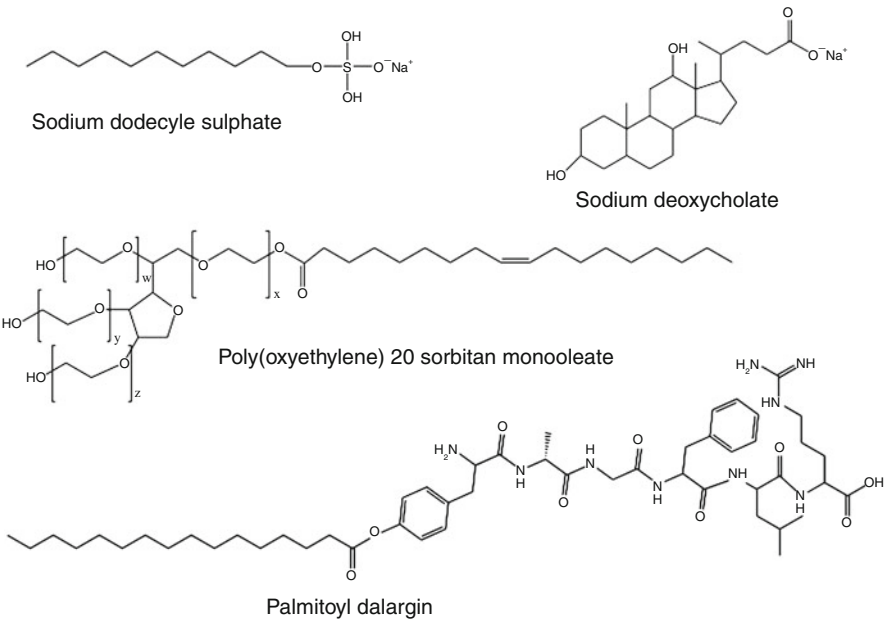


**Fig. 2.2** Micelle forming amphiphiles used in pharmaceutical formulations. Sodium dodecyl sulphate, sodium deoxycholate and poly(oxyethylene) 20 sorbitan monooleate form spherical micelles while palmitoyl dalargin forms nanofibres

is defined by the relative sizes of the hydrophobic and hydrophilic regions of the molecule (2.1).

$$CPP = \frac{v}{a_0 l_c} \tag{2.1}$$

where $v$ = the volume of the hydrocarbon, $a_0$ = the hydrophilic head group area and $l_c$ = chain length. A CPP of less than 0.33 (symptomatic of relatively hydrophilic molecules) leads to the formation of spherical micelles within aqueous media, while a CPP of between 0.5 and 1 (applicable to relatively hydrophobic molecules) leads to the formation of closed bilayers — ultimately vesicles, such as liposomes and niosomes in aqueous media. A CPP of above 1 (indicative of extremely hydrophobic molecules) results in the formation of reverse micelles in non-aqueous media. In reverse micelles, which form in non-aqueous media, the core of the micelle is hydrophilic and the surface hydrophobic and thus the hydrophilic head groups are shielded from the non-aqueous bulk phase in the self-assembly. The majority of pharmaceutical applications of micelles involve the use of spherical micelles in aqueous media. Essentially micelles are characterised by a fluid interior and form in aqueous media when the optimum hydrophilic head group area is large enough to support a high radius of curvature in the self-assembly and yet still permit the

hydrocarbon chain to be in a fluid state (Israelachvili 2011). As well as spherical micelles, amphiphilic molecules may self-assemble into fibrous (Cui et al. 2010) micellar structures.

## 2.3 Micellisation

Micellisation takes place when the mean activity of the monomer in the aggregate ($\mu_N^0$) is less than the activity of the unaggregated monomer ($\mu_1^0$) (2.2) (Israelachvili 2011).

$$\mu_N^0 < \mu_1^0 \tag{2.2}$$

Micelles may form spontaneously in aqueous media or with the input of a little energy, either by shaking or vortexing or may be formed with the input of significant energy such as that resulting from probe or bath sonication.

At low concentrations in water (below the critical micellar concentration — CMC) amphiphilic molecules, with a hydrophobic moiety and a hydrophilic moiety, orient themselves at the air–water interface and in the bulk disperse phase they exist as monomers (Fig. 2.1). As the concentration of the amphiphiles increases, the amphiphilic molecules aggregate, such that the hydrophobic moiety is shielded from the aqueous medium. The driving force for the micellisation at room temperature is usually the release of water molecules adjacent to the hydrophobic moiety, which are now free to hydrogen bond with one another and thus experience an entropy gain (Tanford 1980). Micellisation occurs at a critical concentration, the CMC and a critical temperature, the critical micellar temperature, and is the point at which the amphiphile is no longer soluble in the aqueous medium as a monomer. Once the monomer's intrinsic solubility is exceeded, micelle forming molecules then proceed to aggregate and shield the hydrophobic moieties in the molecule from the aqueous disperse phase. The micelles thus consist of a hydrophobic core and a hydrophilic surface and the hydrophobic core may be used to encapsulate drug molecules. Spherical micelles are typically 5–20 nm in diameter and so are at the smaller end of the nanoparticle spectrum (Florence and Attwood 2006). The final size of the micelle is ultimately controlled by a desire to minimise the entropy deficit associated with the formation of a large-sized structure (Israelachvili 2011). Micelles may either be produced in aqueous media or in non-aqueous media and in the latter case, they are then known as reverse or inverted micelles. Although the CPP appears to define the molecules which form micelles within a relatively narrow range (2.1), micelles may be formed from a number of chemical entities, e.g. molecules with the same hydrophilic head group but with different hydrophobic groups. Micelles formed by a homologous series of alkyl substituents sharing a common hydrophilic head group exhibit CMCs which vary linearly with the number of carbon atoms ($m$) in the alkyl or acyl unit (2.3) (Florence and Attwood 2006).

$$\log[CMC] = A - Bm \tag{2.3}$$

Where *A* and *B* are constants specific for the particular homologous series. As becomes immediately apparent, the more hydrophobic an amphiphile the lower, of course, is its CMC. The formation of micelles from amphiphiles is not limited to low molecular weight compounds and indeed polymers also form micelles (Wang et al. 2004; Siew et al. 2012); however, the current chapter is limited to a discussion of micelles formed from low molecular weight amphiphiles (Molecular weight <1,500 Da). One notable thing about low molecular weight micelles is that since they are formed from relatively hydrophilic compounds (Fig. 2.2), the structures are relatively dynamic. The aggregate residence time of the monomers ($t_R$) is comparatively low ($t_R = 10^{-4}$ s) when compared to the residence time of monomers within a bilayer ($t_R = 10^4$ s) (Israelachvili 2011). This dynamic nature has a negative impact on micelle stability and also contributes to the toxic effects encountered with micellar formulations. The dynamic nature of the aggregate and relatively high monomer aqueous solubility means that micelle monomers will be present in relative abundance in the aqueous bulk and thus may easily integrate within membranes and cause haemolysis for example; integrating within membranes by simple diffusion in response to a concentration gradient. A study of poly(oxyethylene) 20 sorbitan monooleate micelles compared to polymeric micelles formed from *N*,*N*,*N*-trimethyl, *N*,*N*-dimethyl, *N*-monomethyl, *N*-palmitoyl, 6-*O*-glycol chitosan (quaternary ammonium palmitoyl glycol chitosan—GCPQ) revealed that while poly(oxyethylene) 20 sorbitan monooleate micelles caused 80 % haemolysis at a concentration of 1 mg $mL^{-1}$, GCPQ micelles caused no haemolysis at a concentration of 1 mg $mL^{-1}$ and less than 20 % haemolysis at a concentration of 10 mg $mL^{-1}$ (Siew et al. 2012). Both poly(oxyethylene) 20 sorbitan monooleate and GCPQ have CMCs in the micromolar range (Patist et al. 2000; Siew et al. 2012), indicating that the toxicity observed with poly(oxyethylene) 20 sorbitan monooleate may be attributed to the dynamic nature of the low molecular weight amphiphile micelles. Additionally when the micelle forming amphiphile Solulan C24 is contained within a non-ionic surfactant bilayer, it is less toxic to Caco-2 cell monolayers when compared to Solulan C24 micelles or Solulan monomers (Dimitrijevic et al. 1997).

## 2.4 Preparation of Micellar Formulations

At the laboratory scale, micellar formulations are prepared by dissolving the amphiphile and drug in organic solvents, evaporating the organic solvent to form a film and then hydrating the film with agitation (Brajtburg et al. 1994a, b; Javali et al. 2012). In a minor variation of this more commonly used technique, the drug may be added to the preformed micelles (formed by hydrating an amphiphile film subsequent to evaporation of an organic solution of the amphiphile), followed by filtration to remove unentrapped drug (Li et al. 2011). To avoid the use of organic solvents, however, micelle forming drugs may simply be added to aqueous media and the mixture probe sonicated and filtered to yield the micellar formulation (Cheng et al. 1999). Likewise peptide nanofibres may be produced by probe sonication of the

peptide amphiphile in aqueous media (Mazza et al. 2013). Additionally at the industrial scale, the methods used to make liposomes (Gregoriadis 2006) may also be employed for the preparation of micelles; namely the size reduction of a coarse dispersion resulting from the hydration of a thin film using high pressure homogenisation, microfluidisation and high pressure extrusion.

## 2.5 Characterisation of Micellar Formulations

To characterise micellar formulations, it is necessary to first determine the CMC of the amphiphilic molecules. This provides a contextual basis for most, if not all of the relevant properties. Generally a lower CMC is preferable as this will ensure that the micelles are not destabilised on dilution. There are various methods that are used to measure the CMC; these methods exploit the fact that the macroscopic properties of an aqueous dispersion of micellar aggregates changes profoundly at the CMC. For example the surface tension progressively decreases as molecules associate at the air aqueous interface (Fig. 2.1); this continues until the surface tension reaches a limiting value and a point at which the addition of further molecules to the aqueous dispersion does not result in the molecules associating at the surface but results in the molecules aggregating into micelles (Fig. 2.1). Surface tension measurements as a function of concentration thus provide a means of determining the CMC (Nagadome et al. 1992). Additionally fluorescent and colorimetric probes may be used to determine the CMC. These probes alter their spectroscopic characteristics on being solubilised within the micelle. For example the hypsochromic shift experienced by methyl orange, as it is solubilised within the micellar apolar core, may be monitored (Karukstis et al. 1998) as the concentration of the amphiphile increases (Fig. 2.3) (Wang et al. 2004). Furthermore isothermal calorimetry may be used to determine the CMC by preparing demicellisation enthalpograms. With this method, a dispersion of the micelles is progressively added to the calorimetry cell and the enthalpy change of micellisation monitored; once the CMC is reached inside the calorimetry cell, demicellisation is halted and micelles begin to form, causing a break point in the enthalpy change with concentration data. Table 2.1 details the various methods which may be used to determine the CMC of a pharmaceutical amphiphile.

The CMCs of the most commonly used pharmaceutical low molecular weight amphiphiles are shown in Table 2.2.

Following determination of the CMC, methods such as light scattering to measure molecular weight may be used to determine the aggregation number of micelles (Okano et al. 2000; Wang et al. 2004). Micelle size may be determined by photon correlation spectroscopy (Dal Bo et al. 2012) or electron microscopy (Mclean et al. 1989). The zeta potential of a micellar dispersion may also be determined by measuring the electrophoretic mobility of the micelles in the dispersion. The solubilisation capacity of the micelles must also be determined, i.e. number of moles of drug solubilised per mole of micelle forming amphiphile. The micellar solubilisation capacity depends on the nature of the drug, nature of the micelle forming surfactant and other environmental factors such as temperature, and pH

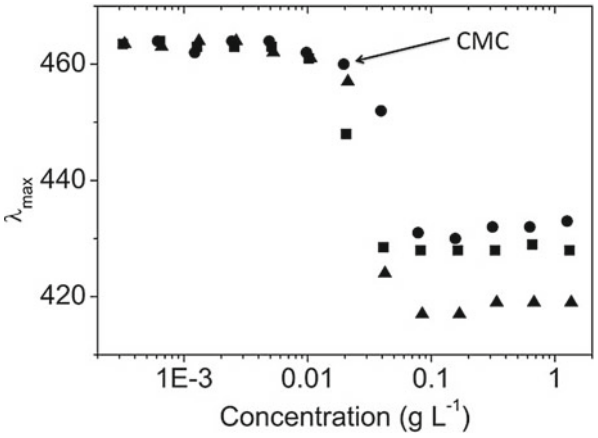


**Fig. 2.3** Determination of the critical micellar concentration of low molecular weight cetyl poly(ethylenimine) by measuring the hypsochromic shift in methyl orange dispersions: *filled circle* = LCPEI14 [linear *N*-cetyl poly(ethylenimine), 14 mol% cetylation, molecular weight = 740 Da], *filled square* = LCPEI23 [linear *N*-cetyl poly(ethylenimine), 23 mol% cetylation, molecular weight = 870 Da], *filled triangle* = LCPEI42 [linear *N*-cetyl poly(ethylenimine), 42 mol% cetylation, molecular weight = 1,320 Da]

(with ionic amphiphiles or ionic drugs) (Florence and Attwood 2006). In essence the more hydrophobic the amphiphile, and hence the lower the CMC, the more drug that is encapsulated within the micelle.

As micellar dispersions are metastable structures, data on the colloidal stability of the micellar drug formulation must also be collected. This may be achieved by monitoring the micelle particle size, drug encapsulation and micellar morphology over time.

The European Medicines Agency (EMA) recently outlined the relevant factors to consider when developing a micelle formulation for intravenous administration (European Medicines Agency 2012). As well as requiring applicants for a product licence to provide information on the CMC, solubilising capacity and stability of the micelles/ micelle formulations; the EMA also requires applicants to establish the ratio of free and encapsulated drug present in the formulation and the fate of encapsulated drug and micelles in vivo, with a requirement to quantify encapsulated vs. non-encapsulated drug in vivo or at least model the trajectories of these formulation components.

## 2.6 The Application of Micellar Formulations to Pharmacy

### *2.6.1 Established Formulations*

Micelle forming low molecular weight amphiphiles such as the poly(oxyethylene) sorbitan esters, bile salts and sodium dodecyl sulphate (Fig. 2.2) are used in pharmaceutical formulations as tablet lubricants, in topical formulations as emulsifiers

**Table 2.1** Methods for the determination of the critical micelle concentration

| Method | Principle | Reference | Advantages | Comments |
|---|---|---|---|---|
| Surface tension | Measures the change in surface tension caused by progressively more molecules associating at the air—aqueous interface; a limiting value signals the onset of micellisation | Nagadome et al. (1992) | Simple methodology | Large quantities of sample required (>100 mg) |
| Conductivity | Measures the change in conductivity as ionic surfactants aggregate into micelles | Mehrotra and Jain (1992); Okano et al. (2000) | Simple methodology | Only suitable for ionic amphiphiles |
| Capillary electrophoresis | Measures the conductivity change when ionic amphiphiles aggregate into micelles | Cifuentes et al. (1997) | Low sample requirements <10 mg | Only suitable for ionic amphiphiles |
| Cyclic voltammetry | Measures the change in current associated with an electrochemical probe, at varying potential and in the presence of a micelle forming amphiphile | Mandal et al. (1988) | Relatively simple experimentation | Only suitable for ionic amphiphiles |
| Isothermal calorimetry | Measures the enthalpy of demicellisation as the micelles are diluted | Hildebrand et al. (2004) | Accurate method provides additional information on other thermodynamic parameters (e.g. the entropy and free energy change of micellisation) | Expensive instrumentation |
| Nuclear magnetic resonance spectroscopy | Measures the chemical shift changes of a relevant proton as the amphiphile self assembles into micelles | Zhao and Fung (1993) | Small sample requirements — mg | Expensive instrumentation |
| Colorimetry—methyl orange | Measures the change in the absorption spectrum, the hypsochromic shift, experienced by methyl orange as it associates with the hydrophobic micelle core | Karukstis et al. (1998); Buwalda and Engberts (2001); Wang et al. (2004) | Rapid analysis | Tends to overestimate the CMC value (Siew et al. 2012) |
| Flourescence spectroscopy—pyrene | Measures the change in the emission spectra of pyrene as it associates with the hydrophobic core of the micelle | Kalyanasundaram and Thomas (1977); Chooi et al. (2010) | Rapid analysis | Errors may arise if pyrene associates with the monomers in solution, leading to an underestimation of the CMC (Chooi et al. 2010) |

**Table 2.2** The critical micellar concentration of pharmaceutical low molecular weight amphiphiles

| Compound | CMC (mM) | Temperature of determination (°C) | CMC methodology | Reference |
|---|---|---|---|---|
| Sodium dodecyl sulphate | 8.2 | 20 | 1. Conductivity<br>2. Dye solubilisation | Williams et al. (1955) |
| Poly(oxyethylene) 20 sorbitan monooleate | 0.018 | 22 | Surface tension | Patist et al. (2000) |
| Sodium deoxycholate | 6.5[a] | 25 | Pyrene fluorescence | Matsuoka and Moroi (2002) |

[a]This is the second CMC when stable micelles begin to form. Sub-micellar aggregation is seen at 2.5 mM (Matsuoka and Moroi 2002)

(sodium dodecyl sulphate) or as emulsifiers, solubilisers and suspending agents in topical, oral or parenteral formulations [poly(oxyethylene) sorbitan esters and sodium deoxycholate] (Rowe et al. 2009). Parenteral formulations provide the best examples of the use of low molecular weight amphiphiles as micellar dispersions (Strickley 2004). Poly(oxyethylene) 20 sorbitan monooleate (Molecular Weight = 1,310 Da) (Rowe et al. 2009) is the most widely used micelle forming low molecular weight amphiphile in pharmaceutical formulations (Nema et al. 1997; Powell et al. 1998). An example of a micellar dispersion comprising poly(oxyethylene) 20 sorbitan monooleate as the micelle forming amphiphile is Hectorol, a doxercalciferol intravenous formulation (Table 2.3). Poly(oxyethylene) 20 sorbitan monooleate has a comparatively low CMC of 18 μM (Table 2.2), making it an ideal agent for parenteral formulations in particular, where stability to dilution in the plasma is critical. Only aqueous formulations may be administered intravenously and poly(oxyethylene) 20 sorbitan monooleate is the excipient of choice for formulation of drugs with poor aqueous solubility for intravenous use. Although there are numerous experimental studies outlining the use of low molecular weight amphiphiles to solubilise hydrophobic drugs (Lasic 1992; Alvarez-Nunez and Yalkowsky 2000; Jain et al. 2010), the actual commercial exploitation of micellar systems is not particularly widespread, despite the fact that most new chemical entities originating from drug discovery programmes are hydrophobic molecules (Kirkpatrick 2003). Examples of some commercial micelle formulations are given in Table 2.3.

### *2.6.2 Experimental Formulations*

Micelles are dynamic structures in which there is a fairly vigorous movement of the amphiphilic molecules from the micelle to the bulk liquid as outlined above; this dynamic nature is often associated with an inherent instability. In an effort to produce more stable delivery systems, a number of researchers have prepared mixed

**Table 2.3** Commercial micellar formulations

| Drug | Trade name | Indication(s) | Manufacturer | Formulation and administration | Reference |
|---|---|---|---|---|---|
| Calcitriol | Calcijex | Hypocalcemia associated with chronic renal dialysis | Abbott | A micellar dispersion containing: calcitriol (1 μg $mL^{-1}$), poly(oxyethylene) 20 sorbitan monolaurate (4 mg $mL^{-1}$), sodium ascorbate (10 mg $mL^{-1}$), sodium chloride (1 mg $mL^{-1}$), ethylene diamine tetraacetic acid (1.1 mg $mL^{-1}$), sodium phosphate (9.2 mg $mL^{-1}$), pH = 6.5–8.0<br>Administered as an intravenous bolus | Strickley (2004) |
| Doxercalciferol | Hectorol | Secondary hyperparathyroidism associated with chronic renal dialysis | Bone care | A micellar dispersion containing: doxercalciferol (2 μg $mL^{-1}$), poly(oxyethylene) 20 sorbitan monooleate (4 mg $mL^{-1}$), sodium ascorbate (10 mg $mL^{-1}$), sodium chloride (1.5 mg $mL^{-1}$), disodium ethylene diamine tetraacetic acid (1.1 mg $mL^{-1}$), sodium phosphates (9.2 mg $mL^{-1}$)<br>Administered as an intravenous bolus | Strickley (2004) |
| Amphotericin B | Fungizone | Life-threatening fungal infections, mucocutaneous leishmaniasis | Bristol Myers Squibb | A lyophilised solid containing: amphotericin B (50 mg), sodium desoxycholate (41 mg), sodium phosphate (20.2 mg)<br>Reconstituted in 10 mL water for injection and further diluted to an amphotericin B concentration of 0.1 mg $mL^{-1}$ in dextrose (5 % w/v) injection. Administered as a slow infusion | Dailymed (2012) |

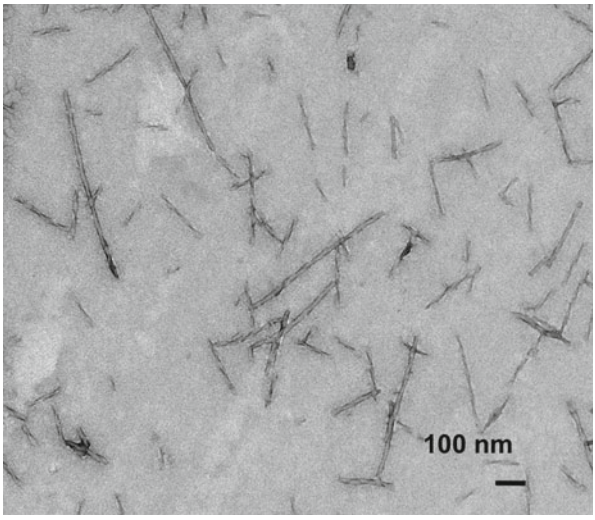


**Fig. 2.4** Palmitoyl dalargin peptide nanofibres [reproduced with permission from (Lalatsa et al. 2012)]

micelles in which the micelle is composed of hydrophilic micelle forming amphiphiles and the less hydrophilic bilayer forming phospholipids.

Mixed micellar formulations composed of sodium cholate, an undisclosed phospholipid and the poorly soluble compound silybin (solubility in water < 50 μg $mL^{-1}$), produced a 150 % increase in the oral bioavailability of the drug in dogs, when compared to silybin *N*-methyl glucamide (Yu et al. 2010). Silybin is an extract of milk thistle (*Silybum marianum*), with antifibrotic and anti-inflammatory properties (Loguercio and Festi 2011). A further example of the application of mixed micelles is in the transdermal delivery of diclofenac in sodium deoxycholate — lecithin mixed micelles (Hendradi et al. 2003). This micellar formulation was an effective anti-inflammatory agent in preclinical studies and showed a reduction in swelling when applied to the skin of a carrageenan-induced hind paw oedema rat model. The reduction in oedema was increased by the incorporation of cyclic terpenes such as D-limonene and L-menthol. The positive effects on the inflammation model were attributed to the solubilisation of diclofenac within the mixed micelles and the greater flux of diclofenac through the skin (Hendradi et al. 2003).

As well as spherical micelles, there have been recent reports of drug delivery using nanofibres—essentially micelles with a long axial ratio. Amphiphilic poly(amino acids) form a wide range of structures on self-assembly in aqueous media: micelles, bilayer vesicles and nanoparticles; the specific structure formed is driven by the amphiphile's chemistry (Lalatsa et al. 2012). Amphiphilic peptides, comprising a peptide attached to a fatty acid moiety at one end (e.g. palmitoyl dalargin—Fig. 2.1), form peptide nanofibres (Cui et al. 2010; Mazza et al. 2013) of ~20 nm in diameter and up to 1 μm in length (Fig. 2.4). The nanofibre morphology consists of a hydrophobic shaft with the peptide beta sheet wrapped tightly around

this central shaft (Mazza et al. 2013). The lipidised peptides adopt a cylindrical morphology as opposed to a spherical morphology, as the hydrophobic association of the lipidic moieties on the molecules is balanced, not by electrostatic or steric repulsion by the molecules' hydrophilic head groups as would be seen with a spherical morphology, but by the formation of a peptide beta sheet; the beta sheet prevents the formation of a spherical morphology as the hydrated amphiphiles are not cone-shaped (as is seen with spherical micelle forming molecules), but are cylindrical in shape and unable to support the radius of curvature required for a spherical morphology. The arrangement of molecules at the tip of the nanofibre is not entirely clear but it is possible that the molecules are more loosely associated at the end of the fibres. It can be envisaged that the molecules at the end of the fibres could adopt a different intermolecular arrangement in an effort to escape the entropy penalty associated with uncontrolled fibre elongation.

Recently we reported that peptide nanofibres, such as palmitoyl dalargin, enable the delivery of peptides to the brain (Mazza et al. 2013). When the analgesic peptide dalargin was intravenously administered to mice, the peptide was not detected in the brain and there was no pharmacological activity observed as a result. On the contrary, when palmitoyl dalargin peptide nanofibres (20 nm in diameter and 500 nm in length) were administered intravenously, the peptide was delivered to the brain and was pharmacologically active (Mazza et al. 2013). The plain peptide, dalargin, was rapidly degraded in the plasma, whereas the peptide nanofibres, by virtue of the fact that the beta sheet is wrapped tightly around the nanofibre core, were not degraded rapidly in the plasma and were able to cross the blood brain barrier, leading to activity from the peptide (dalargin) once it was cleaved from its peptide prodrug (palmitoyl dalargin).

Peptide nanofibres are also useful for the delivery of hydrophobic drugs. Peptide nanofibres prepared from the amphiphilic peptide: palmitoyl-$A_4G_3E_3$ (12 nm in diameter and several microns in length) increased the level of camptothecin present in aqueous media by 50-fold by encapsulating the hydrophobic drug within the hydrophobic peptide nanofibre core (Soukasene et al. 2011). The peptide nanofibre camptothecin formulation also inhibited tumour growth in a mouse orthotopic breast cancer model (Soukasene et al. 2011). This peptide nanofibre camptothecin formulation was just as efficacious as a solution of camptothecin in PEG 400, propylene glycol and polysorbate 80.

Peptide nanofibres are just emerging as biomaterials (Cui et al. 2010) and no doubt additional applications will be forthcoming in the very near future.

### *2.6.3 Toxicology*

The short aggregate residence times ($t_R$) enjoyed by micelle forming molecules (Israelachvili 2011) and the presence of a relatively high level of solubilised monomers in the bulk medium enable micelle monomers to interact deleteriously with

cell membranes. The short micelle $t_R$ also contributes to the instability of micellar formulations. The haemolysis caused by micellar amphiphilic molecules such as poly(oxyethylene) 20 sorbitan monooleate (Siew et al. 2012) is one of the reasons why the levels of micelle forming amphiphiles injected intravenously are controlled; acceptable levels are typically less than 10 % w/v (Rowe et al. 2009). As would be expected from the foregoing, haemolytic potential varies inversely with the CMC and hence haemolysis follows this trend with the commonly used amphiphiles of decreasing CMC: sodium dodecyl sulphate > sodium deoxycholate (Ross et al. 2004). In essence the more hydrophilic micelle forming amphiphiles have a higher CMC and so there is a relatively high concentration of monomers external to the micelle in equilibrium with the micelle; such monomers are able to incorporate within and lyse membranes

The instability of micellar formulations, as a result of the high $t_R$, also contributes to their toxicological profile. For example the intravitreal injection of 1-*O*-octadecyl-sn-glycerol-3-phosphonoformate, indicated for the treatment of cytomegalovirus infections, as a self-assembled micelle results in retinal damage in rabbits with the drug micelles (Cheng et al. 1999). No retinal damage was observed when 1-*O*-octadecyl-sn-glycerol-3-phosphonoformate was formulated as a dioleyl phosphatidyl choline and cholesterol liposome. In the case of the micelles however, 1-*O*-octadecyl-sn-glycerol-3-phosphonoformate precipitated from the micellar formulation resulting in local opacities. This opacity lasted for up to 8 weeks with higher drug doses (0.1 mL of an 885 mM formulation) and resulted in optic nerve oedema in the high-dose animals. One case of retinal detachment was recorded out of four animals.

Additionally micellar Fungizone formulations of amphotericin B (Table 2.3) are more toxic to mammalian cells than liposomal formulations of amphotericin B as there is more self-associated amphotericin B unbound to the micelles in the micellar formulations and this self-associated and non-micellar amphotericin B is toxic to mammalian cells (Brajtburg et al. 1994a, b). The toxic effects of amphotericin B are caused by the extraction of membrane cholesterol and subsequent $K^+$ leakage. These micellar toxicities were not limited to in vitro situations as the intravenous injection of Fungizone caused more kidney toxicity (increase in plasma creatinine levels) when compared to the intravenous injection of liposomal and polycarprolactone/polaxamine 188 particulate amphotericin B formulations (Echevarria et al. 2000) even though there was less exposure to total amphotericin B with the Fungizone formulation. Interestingly, heating amphotericin B — deoxycholate micelles (4 nm in diameter and tubules) at 70 °C for 20 min reduced its toxicity by about tenfold (van Etten et al. 2000). Mice died when given an intravenous dose of 0.8 mg/kg of the standard formulation, but only died when given a 7 mg/kg intravenous dose of the heated formulation. Ultimately this reduced toxicity leads to the ability to administer higher doses and ultimately improves the efficacy of the formulation. The heating altered the micelles and produced larger 300 nm aggregates, which presumably were an entirely different arrangement of the molecules.

## 2.7 Conclusions

Low molecular weight amphiphile spherical micelles are 5–20 nm in diameter and formed from relatively hydrophilic amphiphiles. Low molecular weight amphiphile micelles are generally used in pharmacy for the delivery of hydrophobic drugs within an aqueous disperse phase, with the drug residing within the hydrophobic core of the micelle. The vast majority of micellar formulations on the market are the result of a need to administer a hydrophobic drug into the aqueous blood compartment. The level of micelle forming amphiphile must be controlled to limit the toxicity that arises as a result of the dynamic nature of the micellar self-assembly. A new type of pharmaceutical micelle has emerged recently, i.e. the peptide nanofibre. Peptide nanofibres are cylindrical micelles of 10–20 nm in diameter and 500 nm to a few microns in length. They are formed from short chain amphiphilic peptides in which the peptide chain is attached to a lipidic group. Peptide nanofibres, prepared from a lipidic prodrug of the peptide, enable the delivery of peptides to the brain and peptide nanofibres may also be used for the intravenous administration of hydrophobic drugs encapsulated within the peptide nanofibre core.

*Problem Box*

Q1: What are the main molecular attributes of a micelle forming low molecular weight amphiphile?

Answer

Low molecular weight micelle forming amphiphiles have a molecular weight of less than approximately 1,500 Da and contain geometrically distinct hydrophobic and hydrophilic regions. Generally molecules that form micelles in aqueous media are hydrophilic and are usually miscible with water while molecules that form reverse micelles in non-aqueous media are usually relatively hydrophobic.

Q2: How many low molecular weight amphiphile micelles be used in pharmacy?

Answer

Low molecular weight amphiphile micelles may be used for the incorporation of hydrophobic drug compounds in aqueous media. An example of a pharmaceutical product relying on micellar drug delivery is Hectoral, a doxercalciferol intravenous injection formulated with poly(oxyethylene) 20 sorbitan monooleate micelles.

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http://www.springer.com/978-1-4614-9163-7

Fundamentals of Pharmaceutical Nanoscience

Uchegbu, I.F.; Schätzlein, A.G.; Cheng, W.P.; Lalatsa, A. (Eds.)

2013, XIV, 598 p. 155 illus., 99 illus. in color., Hardcover
ISBN: 978-1-4614-9163-7