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Formation of Multilamellar Vesicles ('Onions') in Peptide Based Surfactant

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Abstract—Concentration dependent morphological characteristics of a novel dipeptide derivative Lys-Asp-Lauryl.HBr (**1**) has been presented. Evidence for "onion" like vesicle formation at higher concentration ($>8.2\times10^{-3}$ M) of peptide (**1**) in aqueous medium was obtained from conductance and 90° light scattering measurements, and cryo-transmission electron microscopic studies.

The influence of concentration on the structure of the complex fluids (such as surfactants in solution or block copolymer melts) with lamellar arrangements has attracted immense interest in recent years. Self assembled structures that are found at higher concentration offers the opportunity to create distinct alignments of microstructures and thus lead to novel material properties.[1–15] The concentration induced alignment of the surfactant leads to the formation of multilamellar vesicles (MLVs), sometimes called 'onions' in lamellar lyotropic mesophases formed by surfactant.[7–14] The formation of such vesicles is very important from the fundamental understanding of creating discrete and higher order self-assembled structure starting from the 'closed-micelles' and also from the applied aspects of modification of the rheological properties and of encapsulation of active ingredients. As the 'onion' structures are layered, these systems provide an opportunity for the development of controlled delivery system.

Peptide derivatives containing long chain alkyl groups have proven to be a very versatile system in terms of phase behaviour and biological activity.[16,17] We have previously reported the aggregate formation characteristics of various peptide amphiphiles in aqueous and non-aqueous medium.[18]

In this communication, we present the results obtained from conductance and 90° light scattering measurements, and cryogenic transmission electron microscopy (Cryo-TEM) of a novel dodecyl amine derivative of dipeptide **1** at high concentration in aqueous medium. The 'onion' structure for peptide based system is reported for the first time.

The schematic diagram of the peptide derivative **1** is shown in Figure 1. It was prepared by the solution phase method using dicyclohexylcarbodiimide/*N*-hydroxy-succinimide strategy[19] using Boc-Lys(Boc)-OH, H-Asp (OBzl)-OH and dodecyl amine as starting materials. The deprotection of the peptide was carried out in HBr/AcOH medium. The peptide was purified and characterised using HPLC, ^{1}H and ^{13}C NMR.[20]

The specific conductance and 90° light scattering measurements at various concentration of dipeptide derivative **1** are presented in Figure 2. The conductance plot changes abruptly at 5.31×10^{-4} M indicating the primary micellisation (CMC_I) process at this concentration. The second inflection point (4.9×10^{-3} M) in the figure may

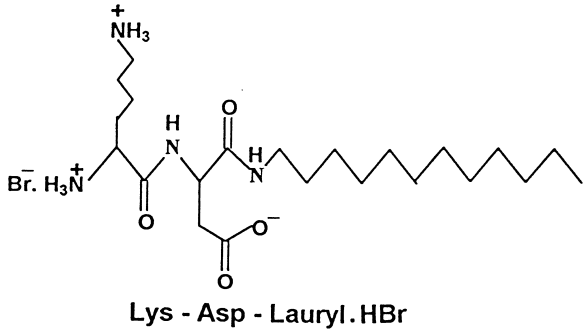

Figure 1. The structure of the dipeptide derivative (**1**).

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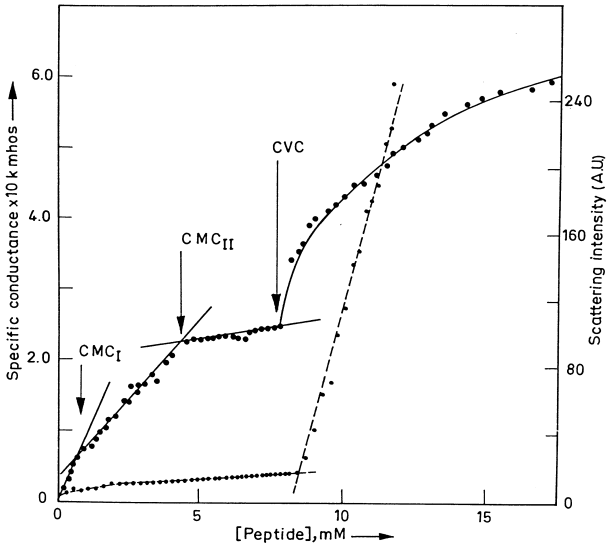

Figure 2. The plot of specific conductance and 90° light scattering measurements at various concentration of dipeptide derivative (**1**).

be due to the appearance of a second CMC (CMC_{II}) for the peptide derivative, and this means that primary micelles of peptide can aggregate further to form secondary micelles.[21] A sharp increase in conductance beyond 8.2×10^{-3} M showed a pronounced transition indicating the presence of vesicles in the solution (Critical Vesicular Concentration, CVC). The CMC values obtained by conductivity measurements are the average of three measurements and their accuracy is ±3%.

In the conductance plot, after attaining CMC_I and CMC_{II}, the slope of the plot decreases. It may be due to the entrapment of the counter ions within the aggregate which leads to the decrease in the conductance. However after attaining the CVC, the amphiphiles reorient themselves within the aggregate in order to favour the ionic interaction (salt-bridge formation) between the monomers in the aggregates resulting in change of pK_a of the carboxylate side chain of Asp leads to the increase in the conductivity due to proton release.[18c] At higher concentrations, the salt-bridge formation between intermicellar units results in reduction of Gaussian bending modules[22] which in turn favours the bigger 'onion' like aggregates.

The 90° light scattering measurements were made by using Hitachi (model 650-40) fluorimeter by setting incident and emission wavelength as 600 nm. At this wavelength there is no absorption contribution from the peptide derivative. The principle of this experiment involves the measurement of the 90° scattered light in terms of relative intensity at various concentrations of dipeptide **1** derivative.[23] The onset of CVC is observed by sharp increase in scattering intensity at 8.4×10^{-3} M.

Since, cryogenic transmission electron microscopy (Cryo-TEM) is a method of observing macromolecules and their aggregates in their native environment, we have employed this technique to our system to study the morphological aspects. The Cryo-TEM technique has been carried out using the reported procedure.[24] The electron micrographs of peptide derivative in aqueous medium at higher concentration region (18.5×10^{-3} M) is presented in Figure 3. The spherical shape vesicles with diameter of 320±40

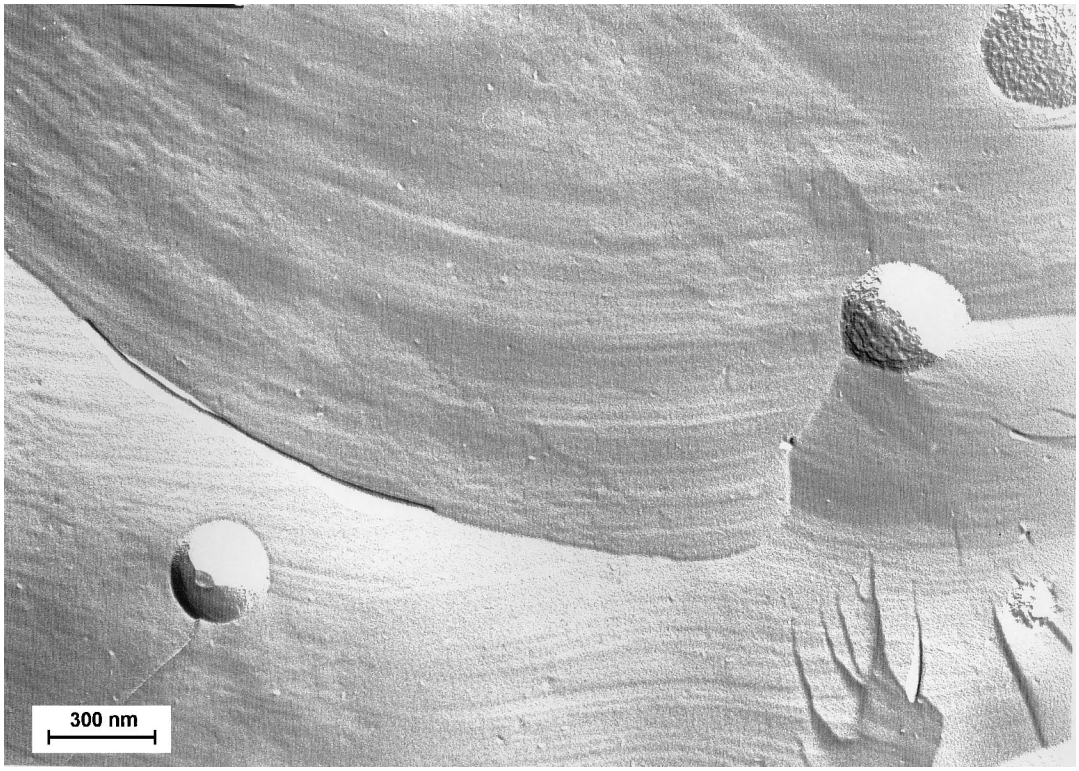

Figure 3. Cryo-TEM of dipeptide derivative (**1**) in aqueous solution at higher concentration (18.5×10^{-3} M).

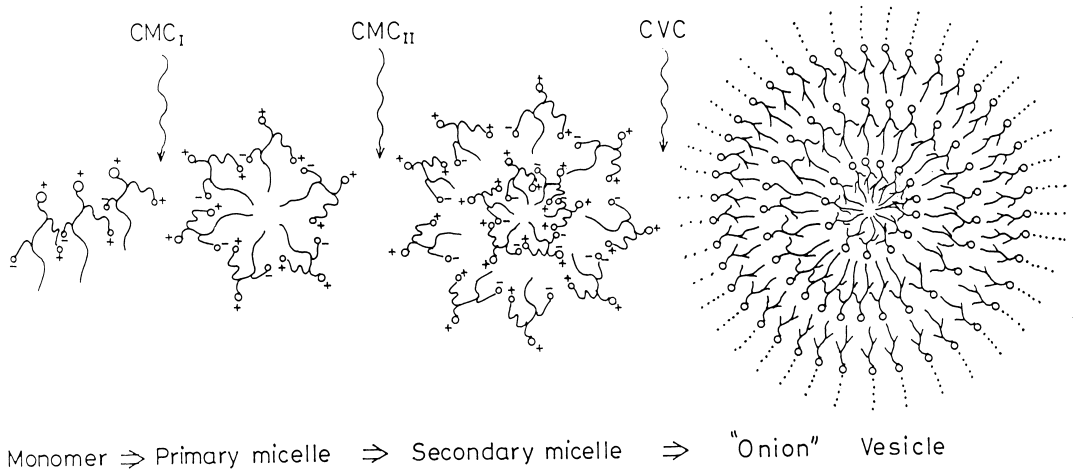

Figure 4. Representative diagram of structural transition of (**1**) during its concentration variation in aqueous medium.

nm are observed, and the smaller spherical structure of ~20 nm may be the secondary micelles. The structure of the aggregate depend upon the packing parameter[25,26] P which is represented by the following equation.

$$P = v/la_o$$

where v represents the volume of the hydrophobic portion, l is the length of the hydrophobic chain and a_o is the effective area per head group. The P values for spherical micelles, cylindrical and multilamellar vesicles are 0.33, 0.5 and 1, respectively. Increasing the concentration of the surfactant decreases the a_o value in which some cases the P take smooth transition from 0.33 to 0.5 (spherical to discoid and then rods). However in the present case at lower concentration, the peptide aggregates take micellar structure indicating the packing parameter attains the value 0.33. Increasing the concentration (above CVC) leads to salt-bridge formation, which is expected to drastically reduce a_o and favours the formation onion like vesicle. This type of micellar-lamellar behaviour is also observed in the case of cetyltrimethylammonium-3-hydroxy-2-naphthalenecarboxylate.[26]

Figure 4 is the representative diagram for various morphological transitions of dipeptide derivative **1** during the increase in its concentration. It is shown that the dipeptide derivative **1** exists as monomer in the premicellar region and a further increase in concentration of peptide **1** leads to the formation of peptide micelles. The primary micelles are assembled further into secondary micelles and at higher concentration they transform into a giant 'onion' like vesicle. These corresponding transition concentrations are designated as primary micellar concentration (CMC_I), secondary micellar concentration (CMC_{II}) and critical vesicular concentration (CVC) respectively.

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