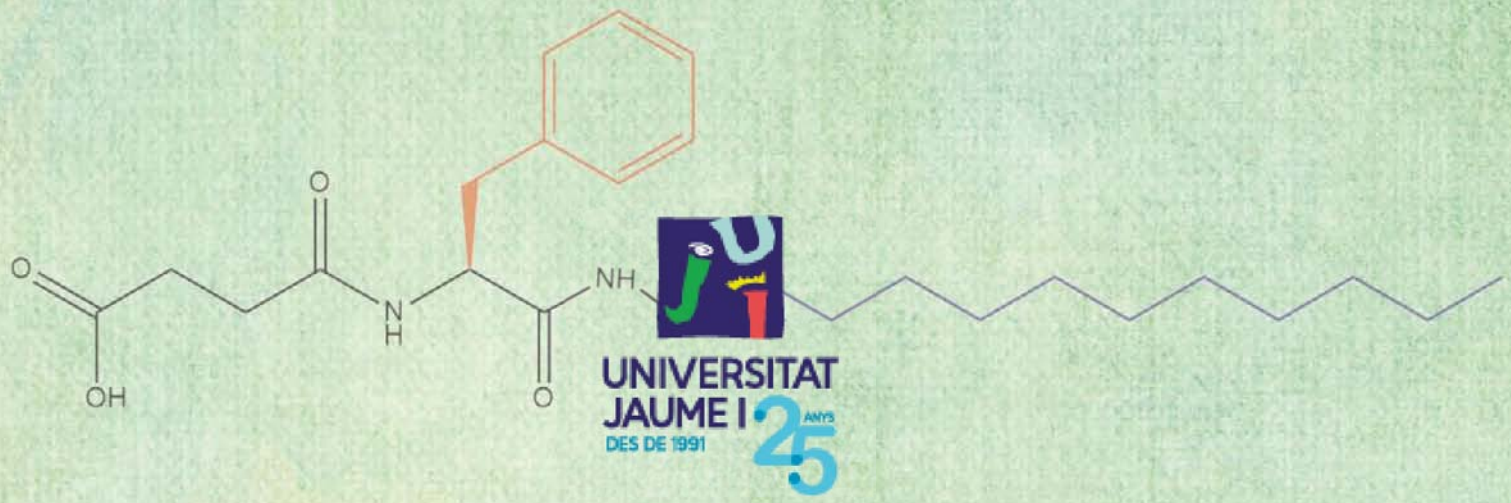

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PROTO-AGGREGATION STUDIES IN SUPRAMOLECULAR HYDROGELS DERIVED FROM SUCCINIC ACID

CARLA ARNAU DEL VALLE

CHEMISTRY DEGREE RESEARCH PROJECT
2015 – 2016

Escuela superior de Tecnología y Ciencias Experimentales
Departamento de Química Inorgánica y Orgánica
Grupo de Química Supramolecular

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Abbreviations

Cbz	Benzyloxycarbonyl
CMC	Critical micellar concentration
COSY	2D correlation spectroscopy
DCC	*N,N*-dicyclohexylcarbodiimide
DCU	*N,N'*-Dicyclohexylurea
DMSO	Dimethyl sulfoxide
HValDoc	(*S*)-2-amino-N-dodecyl-3-methylbutanamide
IR	Infrared spectroscopy
LMWG	Low molecular weight gelator
MGC	Minimum gelator concentration
NMR	Nuclear Magnetic Resonance
SucAlaDoc	(*S*)-4-((1-(dodecylamino)-1-oxopropan-2-yl)amino)-4-oxobutanoic acid
SucIleDoc	4-(((2*S*,3*R*)-1-(dodecylamino)-3-methyl-1-oxopentan-2-yl)amino)-4-oxobutanoic acid
SucPheDoc	(*S*)-4-((1-(dodecylamino)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobutanoic acid
SucValHx	(S)-4-((1-(cyclohexylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid
SucValDoc	(*S*)-4-((1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid
THF	Tetrahydrofurano
UV-Vis	Utraviolet-visible spectroscopy
ZValOH	Carbobenzyloxy-*L*-valine
ZValOSu	(*S*)-2,5-dioxopyrrolidin-1-yl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate
ZValDoc	(*S*)-benzyl (1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)carbamate

El Dr. Juan Felipe Miravet Celades, Profesor Titular del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana , y el Dr. César Augusto Angulo Pachón, Investigador de la Universitat Jaume I,

CERTIFICAN

Que el trabajo fin de grado con el título **Proto-aggregation studies in supramolecular hydrogels derivative of succinic acid** ha sido realizado por Carla Arnau del Valle bajo su dirección, en el grupo de Química Supramolecular del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana.

Lo que certificamos a los efectos oportunos en Castellón de la Plana a 15 de junio de 2016.

Fdo. Dr. Juan F. Miravet Celades

Fdo. Dr. César A. Angulo Pachón

Acknowledgements

I would like to express my deepest appreciation to all those who provided me with the possibility to complete this report. I would like to give my final year Project supervisor special gratitude, Dr. Juan F. Miravet Celades, whose contribution in stimulating suggestions and encouragement helped me to coordinate my Project. The attention and support received by him helped me to choose the best possible decisions for the project. Furthermore, I would also like to acknowledge the backing and guidance given by Dr. César Augusto Angulo Pachón. He has given me all the help and support needed to achieve success in all laboratory situations. His advice and warnings have been of vital importance in any doubt that arose, and for making important conclusions of the data obtained in the chemical process. I would like to thank the kindness, patience and familiarity received from all members of the group of Supramolecular Chemistry Department of Inorganic and Organic Chemistry.

Index

Introduction

1. Introduction

1.1. Low Molecular Weight Gelators.

In daily life most people encounter gels, often without realising it *(i.e*., shampoos, toothpastes, gelatines, contact lenses, and so forth) and they are widely applied in cosmetics, medicine, food, materials sciences, among other fields. Rigorous definitions of gel were proposed in attempts to link the microscopic and macroscopic properties of a gel. Based on these definitions a substance is a gel if it (1) has a continuous microscopic structure with macroscopic dimensions that is permanent on the time scale of analytical experiments and (2) is solid-like in its rheological behaviour despite being mostly liquid. [1]

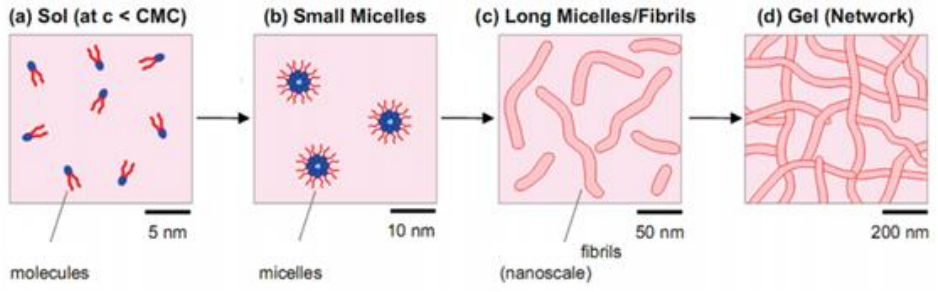
Traditionally gels were thought to be formed exclusively by polymeric species. However in the 1930s, it was found that certain low molecular weight organic compounds could turn organic solvents into a gelly-like substance. The current interest in supramolecular gels has followed a long period of silence after their original discovery. Because of this property they found widespread use as thickeners and lubricants, but at that time they did not raise much scientific interest. It took until the rise of supramolecular chemistry in the 1970s and 1980s, to realise that gel formation by these low molecular weight compounds is an example of a supramolecular system par excellence.

Supramolecular gels are thus a type of physical gels that are formed by small organic compounds as network-forming component. These small organic compounds (2000 *Da*) are called low molecular weight gelators (LMWGs). They are capable of gelling organic solvents (organogelators) or water (hydrogelators). LMWGs have in common that their self-assembly into fibrous networks is driven by noncovalent interactions, like Van der Waals interactions, π-interactions, dipolar interactions, hydrogen bonding and coulomb interactions. Also, solvophobic effects play an important role by reducing the overall solubility of the gelator in the solvent. So, molecular gel formation by LMW gelators is a complicated phenomenon, controlled by a delicate balance of intermolecular interactions both between gelator molecules and between gelator and solvent. [2]

Rational design of self-assembling systems is a very challenging task due to the large number of possibilities of intermolecular interaction that arise from the relative disposition of the molecules in the supramolecular complex and their conformational variability. A particular case of self-assembly is the formation of molecular gels. Small molecules can self-assemble into elongated, fibril-like, objects that percolate the solvent and form gels. Molecular gels have attracted wide interest due to potential applications in fields such as optoelectronics o biomedicine among others. Their intrinsic reversibility and stimuli responsiveness together with their biodegradable nature provide distinct advantages compared with many polymeric analogues.

Most gelators are amphiphilic, i.e., they have one portion that is hydrophilic or polar (capable of forming hydrogen bonds) and another portion that is considerably hydrophobic or nonpolar). But not all amphiphiles are surfactants; on the other hand, all surfactants are amphiphiles. Surfactants are molecules with a hydrophilic headgroup and one or two hydrophobic tails (surfactants with two tails are often called lipids) that have the following key property: when added to water at concentrations above their critical micelle concentration (*CMC*), surfactants will form micelles (or in the case of lipids, vesicles). So, the *CMC* is the concentration above surfactant when micelles will form spontaneously. The higher the concentration, the more micelles there are. Micelle formation also depends on the Krafft temperature. This temperature is when surfactants will form micelles. If it is below the Krafft temperature, then there is no spontaneous formation of micelles. As the temperature increases, the surfactant will turn into a soluble form and be able to form micelles from a crystalline state. Micelle formation can be summed up by thermodynamics, driven by entropy and enthalpy.

Small molecules act as gelators and gel formation is closely related to crystallization. That is, when a sol of the gelator is cooled, one set of conditions results in bulk crystals while a different set of conditions leads to a fibrillar gel.



Scheme 1-1. Schematic of gel formation in the case of surfactants.

At very low concentrations, the compound exists as individual molecules (2a). At a concentration above its *CMC*, the compound forms small reverse micelles, spherical or ellipsoidal in shape, and with a diameter < 10 nm (2b). Note that the micelle cores are formed by the compound's heads and the corona consists of the compound's tails. Then, the micelles grow to form long fibrils whose diameter is on the nanoscale and comparable to the micelle diameter (2c). Finally, these fibrils overlap or entangle to form the gel network (2d). [3]

This picture emphasizes the kinetic aspects of gelation (*i.e.*, nucleation and growth and path dependence); it suggests that the gel state may not be the thermodynamically stable one. In this perspective, surfactant-based gels fall into a different class where few of the above aspects seem to hold. Rather, the latter gels are more akin to micelles or similar assemblies, and gelation in surfactant systems appears to be a thermodynamically driven self-assembly process.

Another characteristic property of a surfactant is its micellar aggregation number. This value, giving the average number of surfactant molecules in the micelle, depends on the hydrocarbon tail length, the kind of counter-ion, and the ionic strength (as does also the *CMC*). The aggregation number will have a dependence on the number of carbon atoms in the hydrocarbon tail.

1.1.1. Properties of molecular gels: Many different techniques have been applied to establish the interactions that are involved in self - assembly leading to gelation, to elucidate the structure and morphology of the gel and gel fibres, and to determine the thermotropic and viscoelastic properties. [1]

One important characteristic of gelator is the minimum required amount of gelator to form a gel in a specific solvent, also called the minimum gelator concentration, *MGC*. It consists of two contributions: concentration of material required for the formation of a 3D network and the concentration of gelator molecules that remain in solution.

Molecular gels are most often formed by heating the solid low molecular mass gelator in an appropriate solvent to achieve complete solubilisation, and then cooling to allow gelation to occur. But molecular gel formation can be regulated by chemical or physical stimuli like changes in temperature, pH, light wavelength, oxidation state, ion bonding, or guest inclusion. These stimuli-responsive gelators have found innovative applications in a range of currently relevant areas, such as biomedicine, catalysis, nanotemplated synthesis, light harvesting, photonics, or electro-optics, by taking advantage of their switchable behaviour.

1.1.2. Parameters and techniques to gel characterization: There are techniques to assess the physical behaviour of the gels and they are particularly useful for exploring the gel - sol phase boundary, as the conversion of the material from a gel to a sol can be simply and visually assessed. There are two simple parameters that are widely used to define the macroscopic properties of molecular gels:

- Minimum gelation concentration (*MGC*). It is the minimum concentration of gelator required to form a sample-spanning, self-supporting gel at a given temperature.
- Gel-sol transition temperature (T_{gel}). It is the temperature at which the gel is converted into a sol on slow heating.

There are several ways in which these parameters can be assessed: Tube Inversion Methodology and Dropping Ball Method. In this project Tube Inversion Methodology was used. It is the simplest method of monitoring the

gel - sol transition and it involves the inversion of a vial containing the gel and the observation to see whether any flow occurs. [1]

1.1.3. Solvent effects characterization in gels: Gel-phase materials are usually constituted of 99 % of solvent, and in some cases significantly more. Because solvent is the major component of a gel, it can play a vital role in controlling the material's performance and properties. As such, the study of solvent effects on gelation is an important topic, as understanding how solvent and self-assembled fibres interface can provide significant insights into gelation.

1.1.4. Nuclear Magnetic Resonance (NMR): Over the past fifty years nuclear magnetic resonance spectroscopy, has become the preeminent technique for determining the structure of organic compounds. It is a spectroscopic method for which a complete analysis and interpretation of the entire spectrum is normally expected. Unlike in mass spectroscopy, in NMR larger amounts of sample are needed and it is non - destructive method.

NMR has been commonly used as a complementary tool for the investigation of low molecular weight gelators. In most cases attention is paid to the analysis of the variation of any chemical shift, NMR relaxation times, or intensity of the NMR signals with concentration, solvent composition, or temperature. Those data can be used to obtain useful information such as the nature of the intermolecular interactions, the critical concentration values, the change in the motion of molecules, or thermodynamic parameters associated with gel formation.

1.1.5. Fluorescence spectroscopy: Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyses fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light; typically, but not necessarily, visible light. In the special case of single molecule fluorescence spectroscopy, intensity fluctuations from the emitted light are measured from either single fluorophores, or pairs of fluorophores. Devices that measure fluorescence are called fluorometers. In a typical fluorescence (emission) measurement, the excitation wavelength is fixed and the detection wavelength varies, while in fluorescence excitation

measurement the detection wavelength is fixed and the excitation wavelength is varied across a region of interest.

The most intense and useful fluorescence is presented by compounds containing aromatic groups with $\pi \rightarrow \pi^*$ transitions low energy, since they have shorter half - life and is less likely to non-radiative deactivation processes occur. Most unsubstituted aromatic hydrocarbons fluoresce in solution and quantum efficiency generally increases with the number of rings and their degree of conjugation. Among the different phosphors used to design new chemosensory is pyrene. Its ability to carry out the phenomenon of fluorescence emission is due to its high conjugation, to be composed of four fused aromatic rings. Pyrene has interesting, such as a fluorescence emission with high quantum yield, a life time extended in the excited state and the ability to form dimers and exciplexes features. [4]

Fluorescence probe analysis is becoming an important area in biophysical studies of multi molecular aggregates such as micelles and membranes. Pyrene has several interesting photophysical properties which make it suitable for use as an effective probe, notably the long lifetime of pyrene monomers and efficient formation of excimers. There have been extensive studies on the photophysics of pyrene: its electronic spectrum and state assignments, kinetic details of excimer formation, spectral pressure effects, formation and kinetics of excited states, photoionization, delayed luminescence and quasilinear spectra.

In order to explain solute - solvent interactions involved in the perturbation of the vibrionic band intensities it is necessary to examine the relative peak intensities. The intensities are normalized with reference to the 0 – 0 band (peak I). Since peak III shows maximum variations in intensity of peak III to peak I, referred to hereafter as the 1/3 ratio, will be used to discuss the environmental effects on pyrene monomer fluorescence. [5]

Fluorescence measurements allow us to determine the critical micelle concentration using the fluorescent probe pyrene. The method is based on the variation of the ratio of intensities of the emission bands 1 and 3 of pyrene, I_1/I_3 ratio, with increasing product concentration in solution as the medium becomes more hydrophobic. This ratio of the intensities reflects the polarity of the microenvironment surrounding the pyrene. So, in the presence

of micelles and other macromolecular systems, pyrene is preferentially solubilized in the interior hydrophobic regions of these aggregates.

1.1.6. Dynamic Light Scattering (DLS): [6] A novel methodology for non - destructive and real - time determination of the gelation threshold for both chemical and physical systems has been proposed. This method allows one not only to determine the gelation threshold but also to investigate critical dynamics near gelation threshold, mechanism of gelation, and architecture of gelling cluster. Dynamic light scattering (DLS) is a method to size submicron particles by measuring their thermal motion (diffusion) in suspensions and emulsions. However, the validity of the Stokes Einstein equation that relates the diffusion coefficient and the particle size is limited to spherical particles and very low concentrations.

The measurement principle is based on a time-resolved measurement of the scattered light intensity from a sample cell that contains the particle system in a solvent. Due to the erratic motion of the particles caused by non-compensated impacts of the solvent molecules (so - called Brownian motion or diffusion) the intensity oscillates round an average value. The frequency of these fluctuations contains information about the diffusion coefficient of the particles, which in turn is size-dependent. The measurement principle requires that diffusion is the only cause of motion in the sample, *i.e*. that effects of sedimentation, thermal convection and fluid flow have to be avoided. This usually limits the upper particle size that can be measured with DLS to some micrometers. If the scattered light from one particle encounters other particles before it is registered at the detector the size information of the signal is lost. The lower size limit is determined by the time resolution of the specific measurement device and lies typically in the range of a few nanometers.

DLS is routinely used to measure the diffusion of surfactant micelles. Theory and experiment suggest, however, that the microscopic concentration fluctuations monitored by DLS obey the same mutual diffusion equations that describe the decay of macroscopic concentration gradients. According to this interpretation, DLS provides mutual diffusion coefficients for the total surfactant components, including contributions from micelles, free surfactant monomers, and counterions. An attempt is made to decide the correct interpretation of DLS measurements by comparing DLS diffusion coefficients (DDLS) with mutual diffusion coefficients measured by macroscopic gradient

techniques for binary aqueous solutions of ionic and zwitterionic surfactants. Possible contributions to DDLS from free surfactant monomers are investigated by extending DLS measurements into the critical micelle (*CMC*) region where substantial portions of the surfactants diffuse as free monomers. The widely held assumption that DDLS is the micelle diffusion coefficient is tested by comparing DDLS with micelle diffusion coefficients measured unambiguously by NMR or Taylor dispersion techniques. DDLS is found to decrease sharply as the surfactant concentration is raised through the *CMC*, in agreement with the steep drop in the mutual diffusion coefficient caused by the association of free surfactant monomers. Above the *CMC*, DDLS and the micelle and mutual diffusion coefficients are nearly identical for the zwitterionic surfactants. For the ionic surfactants, DDLS and the mutual diffusion coefficients are several times larger than the micelle diffusion coefficients as a result of the diffusion of charged micelles with relatively mobile counterions to maintain electroneutrality.

Objectives

2. Objectives

Previously the research group synthetized a family of compounds derived from succinic acid with different amino acid moieties.

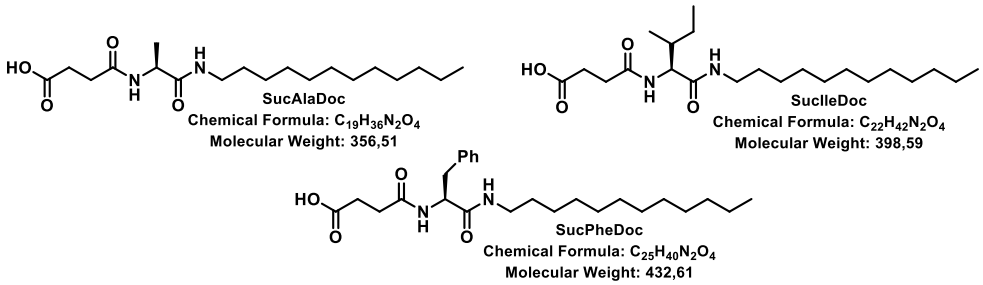


Figure 2-1. Structures of the compounds SucAlaDoc, SucIleDoc and SucPheDoc.

The minimum gelation concentrations in water of each compound were determinate by pH change and warming-cooling cycle's methodologies. For understanding those aggregation processes, we decided to determine the values of critical micellar concentration (*CMC*) and the aggregation number (N_{agg}) for these compounds.

The specific objectives are:

- Synthetize and Characterize of SucValHx and SucValDoc.

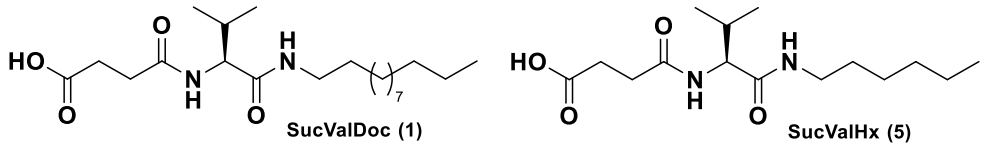
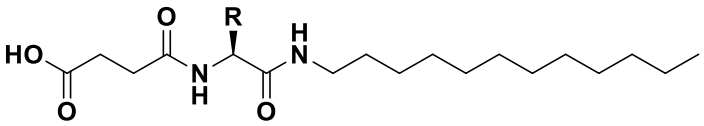


Figure 2-2. Structures of the compounds SucValDoc and SucValHx.

- Determine *CMC* of different hydrogels derived from succinic acid by fluorescence using pyrene as a probe.

- Study the effect of the amino acid (valine, isoleucine, phenylanaline or alanine) over the critical micelle concentration.



R = -CH(CH_3)$_2$ = Valine
R = -CH(CH_3)(CH_2CH_3) = Isoleucine
R = -CH_2(C_6H_6) = Phenylalanine
R = CH_3 = Alanine

- Determine N_{agg} for the SucValDoc (1) and SucValHx (5) and analyse the effect of hexyl and dodecyl residues over this.

- Determine the size of micelles form in each case by Dinamic Light Scattering (DLS).

Results and discussion

3. Results and Discussions

3.1. Synthesis of amino-acid derivatives

Previously the research group synthetized a family of compounds derived from succinic acid which presented unexpected behaviour in their gelation properties. The figure 3-1 shows the structure of these compounds.

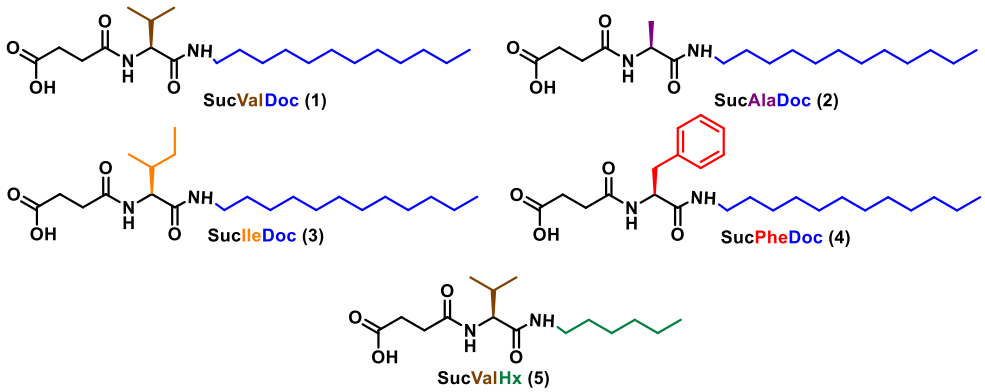
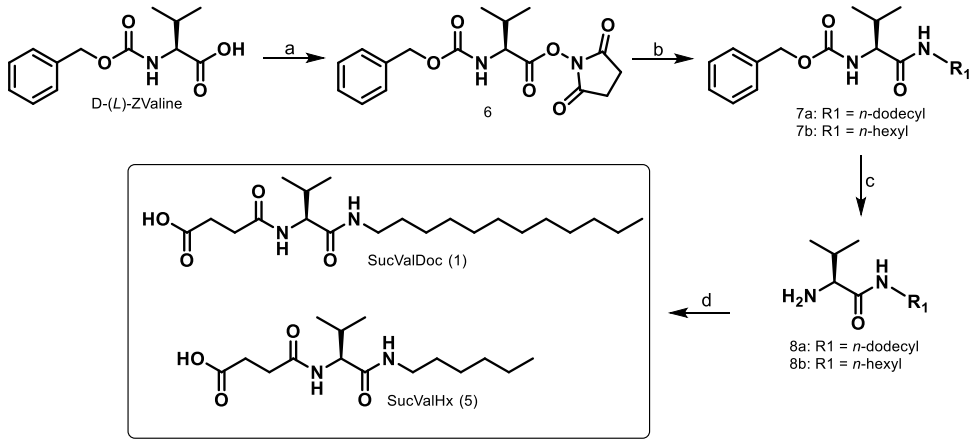


Figure 3-1. Family of compounds derived from succinic acid.

To understand this behaviour it was decided to carry out studies about the minimum concentrations of gelation and their respective minimum micellar concentrations. Therefore it was decided to synthesize two compounds with valine as central amino acid and a different amine in each case. They have been synthesized following a commonly used synthetic route by the research group. The hydrogelators were easily prepared in grams scale from amino acids by *N*-acylation with succinic anhydride and amide formation with *n*-dodecylamine or *n*-hexylamine.

To continue, the scheme 3-1 show the synthetic route to obtained the compounds **1** and **5**.



Scheme 3-1. Reagents and conditions: a) *DCC*, *N*-hydroxisuccinimide, THF, 2 h., 97 %; b) *n*-dodecylamine or *n*-hexylamine , THF, 16 h., 96 %; c) Pd / C, H_2, MeOH, 4 h., 98 %; d) Succinic anhydride, K_2CO_3, THF, 16 h., 94 – 95 %.

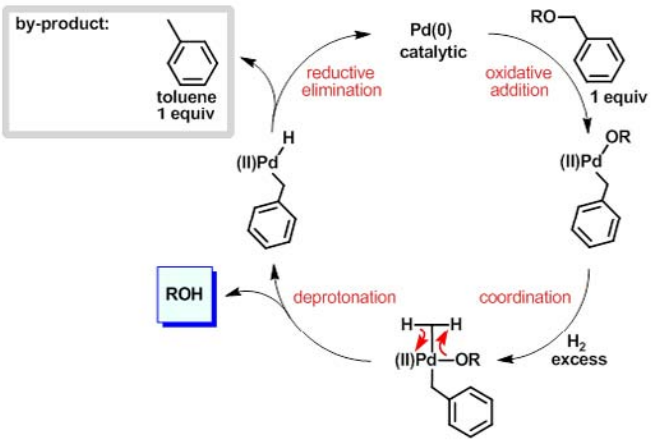
The step c is a carboxybenzyl carbamate deprotection. Benzyl alcohols and benzyl ethers can be hydrogenated catalytically, thereby breaking the bond between the benzylic carbon atom and the oxygen atom with simultaneous formation of a new bond to hydrogen. This transformation, called hydrogenolysis, is made possible through the particular reactivity of the benzyl group. Catalytic hydrogenolysis of other alcohols and ethers is not possible. Therefore, benzyl ethers are good protecting groups for alcohols, especially when the protecting group will be removed under neutral conditions. Most other alcohol protecting groups are cleaved under acidic conditions. [7]

Deprotection is normally performed as palladium-catalyzed hydrogenation, delivering the alcohol and toluene. Cleavage of benzyl ethers is also possible using strong acids, but this method is limited to acid - insensitive substrates. Alternatively, oxidation to the benzoate allows a subsequent hydrolysis under basic conditions.

Palladium on carbon (Pd/C) has been known, for more than a century, to be an effective catalyst for reductive processes and it is estimated that 75 % of industrial hydrogenation processes are performed with Pd/C catalysts. More recently, its scope has been diversified, by synthetic chemists, to include

many applications in coupling reactions for carbon – carbon and carbon – heteroatom bond formation.

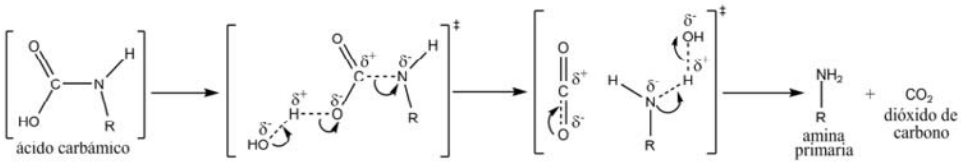
In scheme 3-2 [8] it is shown the hydrogenolysis of benzyl ether mechanism wherein the phenyl ring can be optionally substituted (*e.g.* by a methoxy or halogen) and wherein R is a residue compatible with the catalytic hydrogenation reaction under the particular conditions employed. The first step is the oxidation addition using palladium zero. Once compound has been oxidated, a coordination step takes place in which excess of hydrogen is needed. Finally, after deprotonation the alcohol is formed and by reductive elimination palladium is recuperated and 1 equivalent of toluene is formed.



Scheme 3-2. Hydrogenolysis of benzyl ether mechanism.

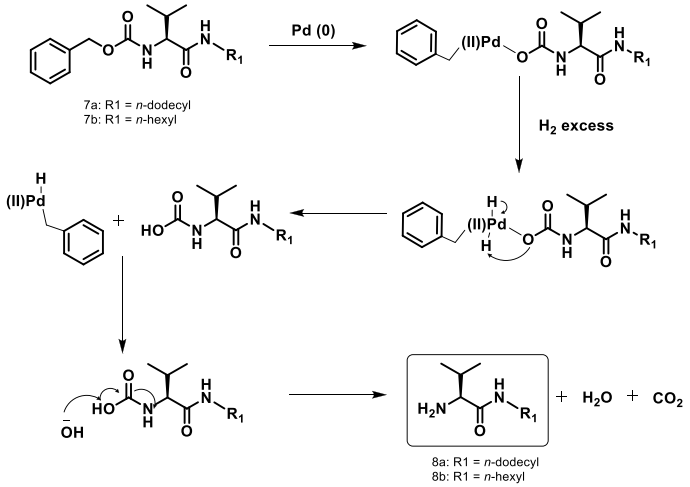
Once alcohol is formed, the carboxyl group of carbamic acid (unstable) in the alkaline solution is deprotonated (acid-base reaction) and spontaneously cleaving the molecule yielding carbon dioxide and a primary amine occurs.

The global synthesis good yields (SucValDoc **1** 91 % and SucValHx **5** 90 %).



Scheme 3-3. Formation of primary amine.

Scheme 3-4 shows all the mechanism of deprotonation of carboxibenzyloxy group. $HValR_1$ is used in crude form for the next reaction.



Scheme 3-4. Deprotection of carboxibenzyl group.

3.2. Characterization of amino-acid derivatives.

3.2.1. Gelation studies: In order to know which is the capability of each compound to form a gel, one of the parameters that are necessary to study is the minor quantity required of each product to form a gel in solvent. This parameter is named minimum gelator concentration (*MGC*). It is considered that the smaller *MGC* value, the better gelator. Commonly gels are formed using a concentration of gelator around 0.1 – 1 % in weight. [9]

Different procedures were followed to form gels. One based on a heating-cooling process and other based on change of pH by γ-gluconolactone hydrolysis.

- *Heating – cooling process:*
  The procedure followed to form gels is based on a heating-cooling process. It consists on weighting an initial quantity (10 mg of compound) in a vial and adding 1 mL of solvent (water) and closing hermetically. This mixture is dissolved by heating (hot air at 240 ºC from a heat - gun) and it is allowed to stand on a cork until it reached room temperature. In the present studies a gel was formed in all cases before 24 hours of resting. A system is considered to be a gel when it passes the "inversion tube test". So, once this was cold, inversion tube test was made. The test result can be: insoluble (I), formation of aggregates (A) or gelation (G). The observations made in the laboratory for several compounds are shown in Table 3-1.

H_2O (mL)	SucAlaDoc (10.03 mg)	SucValDoc (9.97 mg)	SucPheDoc (10.05 mg)	SucIleDoc (10.05 mg)
1.0	I	I	I	I
2.0	I	A	A	A
2.5	I	A	A	A
3.0	A	A	A	A
3.5	A	A	A	A
4.0	A	A	A	A

Table 3-1. Test results of inverted tube to different amino acid derivatives.

- *pH change by γ-gluconolactone:*
  A solution 0.1 M of NH_4OH and using the different compounds, the minimum concentration of gel formation was determinate. The different solutions prepared were: SucValDoc, SucIleDoc,

SucPheDoc and SucAlaDoc 5 mg/mL. The acid used was 4 equivalents of γ-gluconolactone. It was necessary to obtain a pH around six.

To continue shows the results of *MGC* obtained for the different compounds.

SucValDoc (1)

Concentration (mg / mL)	pH	Gel/No gel	Appearance	Thixotropy	Time (min.)
5.00	6	Gel	Translucent	Yes	1
2.50	6	Gel	Translucent	Yes	1
1.25	6	Gel	Translucent	Yes	5
1.00	6	Gel	Translucent	No	-
0.75	5-6	No	-	-	-
0.50	5	No	-	-	-

Table 3-2. SucValDoc inverted tube test using γ-gluconolactone.

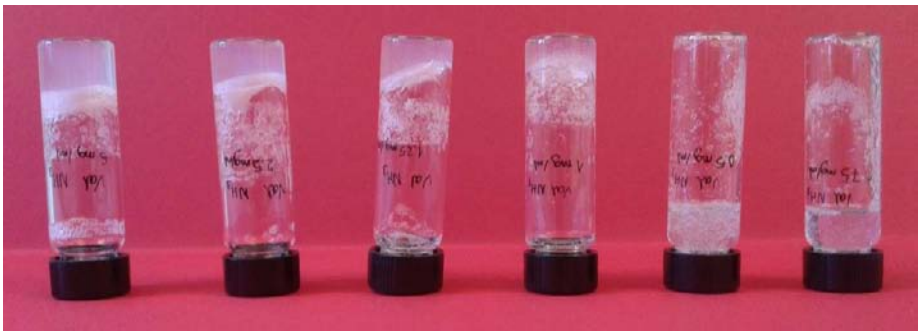


Figure 3-2. SucValDoc inversion tube test from highest concentration (left) to lowest concentration (right).

The minimum gelator concentration (*MGC*) of SucValDoc **1** was 1 mg/mL.

SucAlaDoc (2)					
Concentration (mg / mL)	**pH**	**Gel/No gel**	**Appearance**	**Thixotropy**	**Time (min.)**
5.00	5-6	Weak gel	Translucent	No	-
4.00	6	No	-	-	-
2.50	6	No	-	-	-

Table 3-3. SucAlaDoc inverted tube test using γ-gluconolactone.

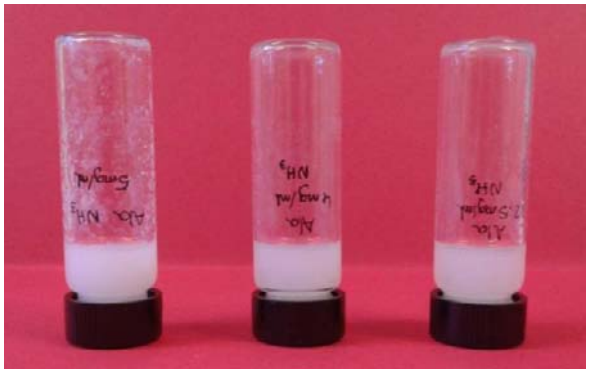


Figure 3-3. SucAlaDoc inversion tube test from highest concentration (left) to lowest concentration (right).

The minimum gelator concentration (*MGC*) of SucAlaDoc was 5 mg/mL.

SucIleDoc (3)					
Concentration (mg / mL)	**pH**	**Gel/No gel**	**Appearance**	**Thixotropy**	**Time (min.)**
5.00	5	Gel	Translucent	Yes	1
2.50	5	Gel	Translucent	Yes	1
2.20	4	Gel	Translucent	Yes	5
2.00	6	Gel	Translucent	Yes	5
1.80	6	Gel	Translucent	Yes	5
1.50	6	Weak gel	Translucent	Yes	5
1.25	5	No	-	-	-

Table 3-4. SucIleDoc inverted tube test using γ-gluconolactone.

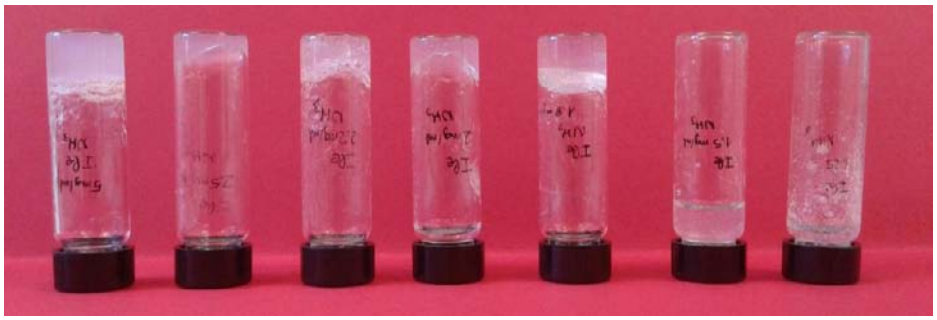


Figure 3-4. SucIleDoc inversion tube test from highest concentration (left) to lowest concentration (right).

The minimum gelator concentration (*MGC*) of SucIleDoc was 1.5 mg/mL.

SucPheDoc (4)					
Concentration (mg / mL)	**pH**	**Gel/No gel**	**Appearance**	**Thixotropy**	**Time (min.)**
5.00	5	Gel	White	No	-
4.00	6	Gel	White	No	-
3.00	6	No	-	-	-
2.50	6	No	-	-	-
1.25	6	No	-	-	-

Table 3-5. SucPheDoc inverted tube test using γ-gluconolactone.

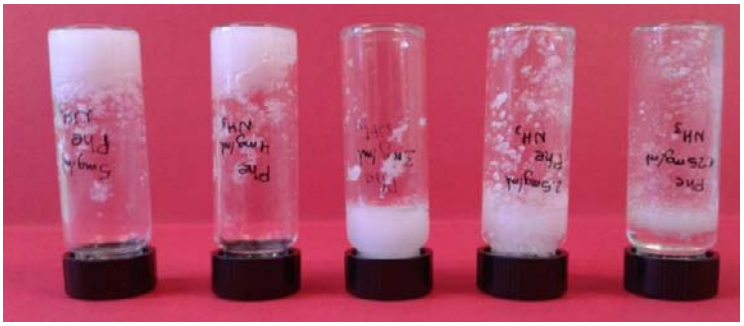


Figure 3-5. SucPheDoc inversion tube test from highest concentration (left) to lowest concentration (right).

The minimum gelator concentration (*MGC*) of SucPheDoc was 4 mg/mL.

3.2.2. Partition coefficient studies: In the physical sciences, a partition coefficient (P) is the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium. This ratio is therefore a measure of the difference in solubility of the compound in these two phases. In the chemical and pharmaceutical sciences, both phases usually are

solvents. Most commonly, one of the solvents is water while the second is hydrophobic such as 1 - octanol. Hence the partition coefficient measures how hydrophilic ("water - loving") or hydrophobic ("water - fearing") a chemical substance is. [10]

The partition coefficient is defined as a particular ratio of the concentrations of a solute between the two solvents, specifically for un - ionized solutes, and the logarithm of the ratio is thus log P. When one of the solvents is water and the other is a non - polar solvent, then the log P value is a measure of lipophilicity or hydrophobicity.

$$logP_{oct/wat} = log\left(\frac{[solute]_{octanol}^{un-ionized}}{[solute]_{water}^{un-ionized}}\right) \quad (1)$$

The partition coefficient of surfactants is theoretically calculated using the *ChemBioDraw Ultra* and the values are showed in Table 3-6.

Surfactant	ClogP in neutral pH	ClogP in basic pH
SucValDoc (1)	5.51	2.55
SucAlaDoc (2)	4.58	1.62
SucIleDoc (3)	6.04	3.08
SucPheDoc (4)	6.00	3.04
SucValHx (5)	2.33	-0.63

Table 3-6. ClogP values of surfactants in neutral and basic pH.

ClogP value greater will be the most hydrophobic compound and therefore the less water soluble as it can be deducted from the equation 1. Therefore, the compound more hydrophobic will be the first forming micelles, *i.e.*, it will form micelles at small surfactant concentration. In conclusion, the greater ClogP value the lower *CMC* value.

So according to Table 3-6 and the aforementioned deduction, all compounds will have a greater *CMC* value dissolved in basic than in neutral solution. Further, comparing the amount of carbons of the surfactants, *CMC* value for SucValHx should be higher than *CMC* value for SucValDoc. Comparing the amino acid of the surfactant, *CMC* value for SucPheDoc should be lower than *CMC* value for SucValDoc.

3.2.3. Fluorescence studies: Ionic surfactants are amphiphilic molecules with a hydrophilic charged head - group and a hydrophobic hydrocarbon tail. When dissolved in aqueous media, the salt dissociates into the bulk. If the tail is not too long, the driving force for solvation of the head - group will be strong enough to dissolve the whole molecule, even though the tail is not soluble in water. Owing to electrostatic repulsion between the head - groups, a homogeneous solution with dissolved surfactant molecules is obtained.

Increasing the surfactant concentration results in two different effects. First, the increased surfactant concentration leads to an increased ionic strength of the bulk. This in turn causes a decrease in the electrostatic repulsion between the head - groups due to screening of the charges. Second, an increase in the surfactant concentration is unfavourable for hydrophobic tails, which on their own do not dissolve in water. The latter effect works against dissolving more surfactant molecules. At this moment, two different scenarios are possible: either, if the hydrocarbon chain - length is long enough, a macroscopic phase separation will appear, or micelles will be formed. In the latter case, this special concentration is the *CMC* (critical micelle concentration). It is worthwhile to stress that micelle formation is not a macroscopic phase separation, but the formation of a thermodynamically stable, microheterogeneous supramolecular system, with surfactant molecules aggregated in micelles dissolved in the aqueous bulk.

The *CMC* is the concentration at which micelles first appear. This is actually not an exact value but a certain range of concentration, which can be relatively large if the surfactant is a mixture of significantly different chemical species each other. The *CMC* is determined as the concentration at which amphiphilic dispersions show an abrupt change in their physicochemical properties. This variation is due to differences between monomers and micelles in properties such as electrical conductivity (for surfactants ionic), surface tension, osmotic pressure, vapor pressure, freezing point, density, light scattering, spectral absorption of chromophores, speed of sound, etc.

In general, the fluorophores are considerably increased their emission by incorporating into a micelle, because it isolates them from the deactivators present in the solution. Fluorescence measurements with very apolar molecules and quencher fluorescence probes allow the determination of characteristic parameters of the micelles, as *CMC* or its aggregation number.

The photo physical behavior of pyrene in micro heterogeneous systems is complex and has been object of many detailed studies and reviews. The characteristic dependence of the fluorescence vibrational fine structure of pyrene could be used to determine the critical micelle concentrations of surfactant solutions. The pyrene 1:3 ratio method was used to determine the *CMC* and it has become one of the most popular procedures for the determination of this important parameter in micellar systems. It is well known that the ratio of the intensities of the bands of vibration pyrene 1 and 3, I_1/I_3, solubilized in a micellar solution reflects the polarity of the micro-environment surrounding the pyrene. From these spectra the intensities I_1 and I_3 were measured at the wavelengths corresponding to the first and third vibrionic band located near 373 and 384 nm, whose absolute and relative intensities, width and positions depend sensibility on the polarity of microenvironment. The ratio I_1/I_3 is the so-called pyrene 1:3 ratio index.

The plots of the pyrene 1:3 ratio as a function of the total surfactant concentration show, around the *CMC*, a typical sigmoidal decrease. Below the *CMC* the pyrene 1:3 ratio value corresponds to a polar environment; as the surfactant concentration increases the pyrene 1:3 ratio decreases rapidly, indicating that the pyrene is sensing a more hydrophobic environment. Above the *CMC*, the pyrene 1:3 ratio reaches a roughly constant value because of the incorporation of the probe into the hydrophobic region of the micelles. A problem arises from the fact that there is not an objective and unified method to obtain the *CMC* value from the plots of pyrene 1:3 ratio against surfactant concentration, and different authors seem to take different criteria to choose this point. The *CMC* values can be obtained using two approaches: (*i*) from the interception of the rapidly varying part and the nearly horizontal part at high concentration of the pyrene 1:3 ratio plots; (*ii*) from the inflection point of the pyrene 1:3 ratio plots, for surfactants with very low *CMC*s, typically bellow 1 mM. Second approach was followed in this project. [11]

The cuvettes used in the project were made of plastic. The transmission range of the spectrum is the area in which the material has a transmittance greater than 80%. The transmission range of these cuvettes is between 380 and 780 nm in the area of the visible spectrum. So, they do not interfere with

the measurement. Another advantage of using these cuvettes is that they are very cheap so it is not necessary to clean every time the same bucket. The cuvettes were used and then discarded. All fluorescence measurements were carried out at 30.0 ± 0.1 ºC.

Figures 3-6 and 3-7 shows SucValHx spectrum when concentration is 0 mg/mL and 5 mg/mL. A change in peaks 1 (372 nm) and 3 (383 nm) of the spectra is observed. There is a decrease of the pyrene 1:3 ratio if compared below and above *CMC* value (increasing surfactant concentration).

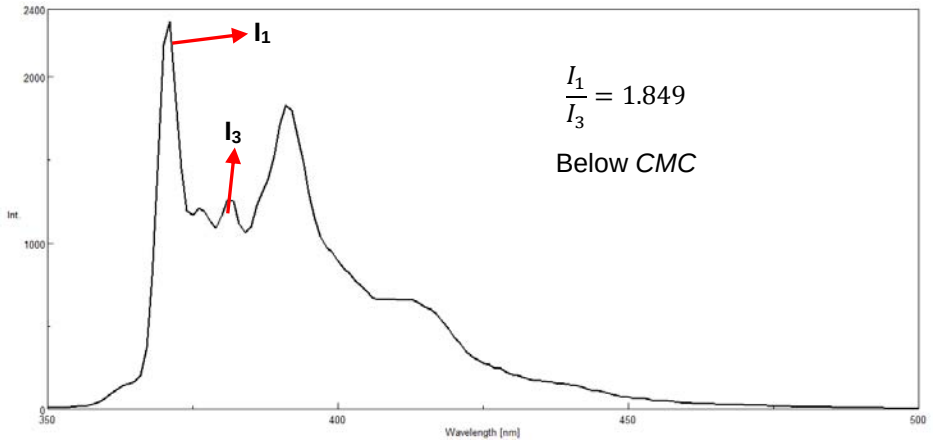


Figure 3-6. Fluorescence measurement of SucValHx at 0 mg/mL (using H_2SO_4 and stabilized during 24 h).

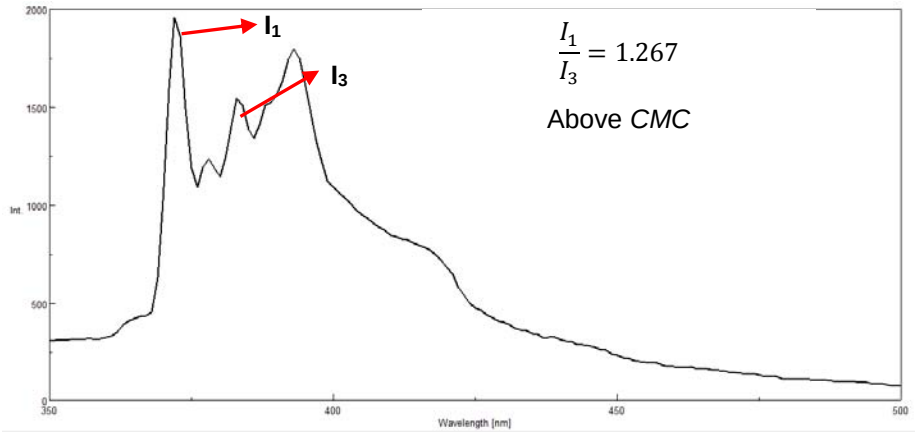


Figure 3-7. Fluorescence measurement of SucValHx at 5 mg/mL (using H_2SO_4 and stabilized during 24 h).

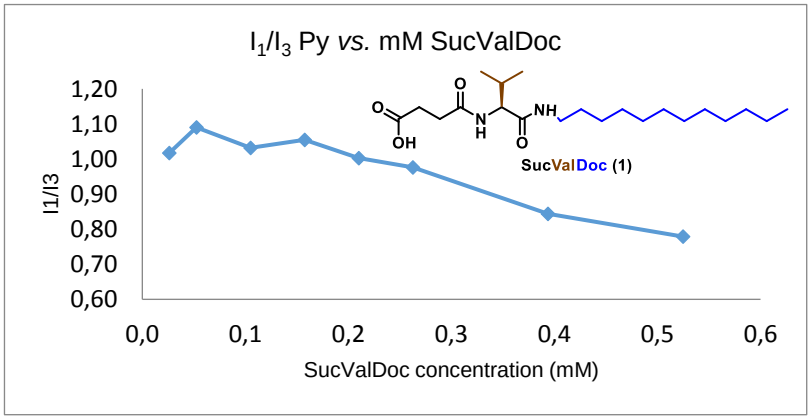
Fluorescence emission spectra of a number of different concentrations of each surfactant solutions containing pyrene were recorded using an excitation wavelength of 335 nm, and the intensities I_1 and I_3 were measured at the wavelengths corresponding to the first and the third vibrionic bands located near 373 and 384 nm. The emission spectra of these solutions were recorded and the logarithm of the ratio I_1/I_3 at the specific wavelength was plotted against the surfactant concentration. The optimum range was looked for from this screening, preparing solutions of different concentrations. At very high concentrations, aggregates were formed, thus the fluorescence measurement was not correct. So a wide range of concentrations was measured, the highest being the maximum concentration that did not form aggregates or gel. From the data obtained, and adding more measures of different concentrations, the desired range was found.

3.3. Results of the determination of CMC for different compounds.

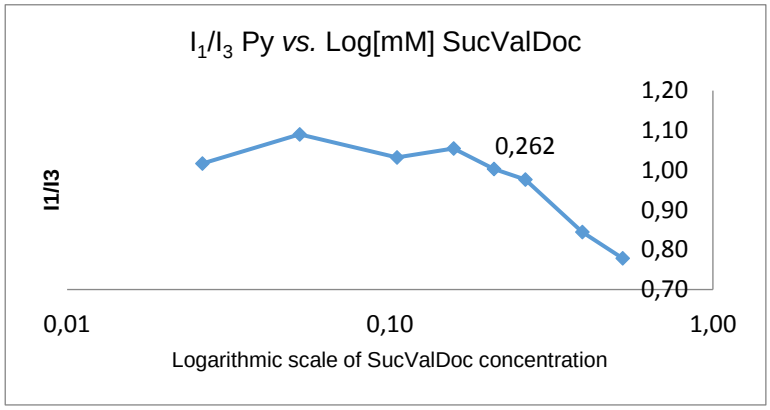
Before taking the measures the optimum range of measures for each compound was searched.

3.3.1. SucValDoc results:

Basic solution



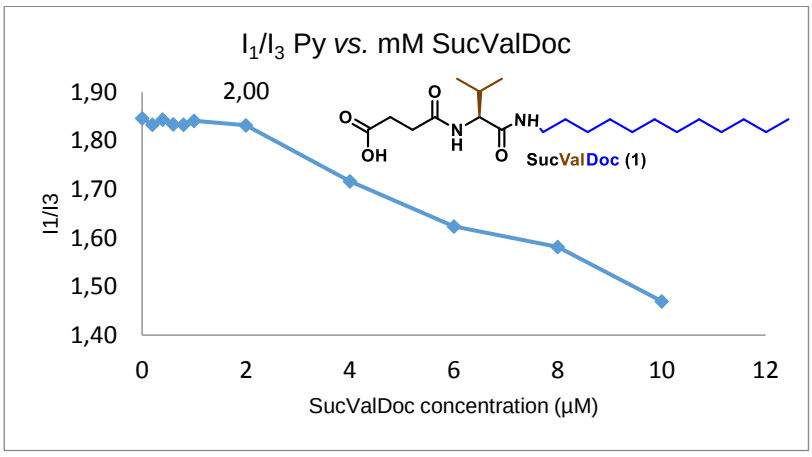
Plot 3-1. Intensity ratio (I_1/I_3) *vs* SucValDoc concentration (mM) in basic pH.



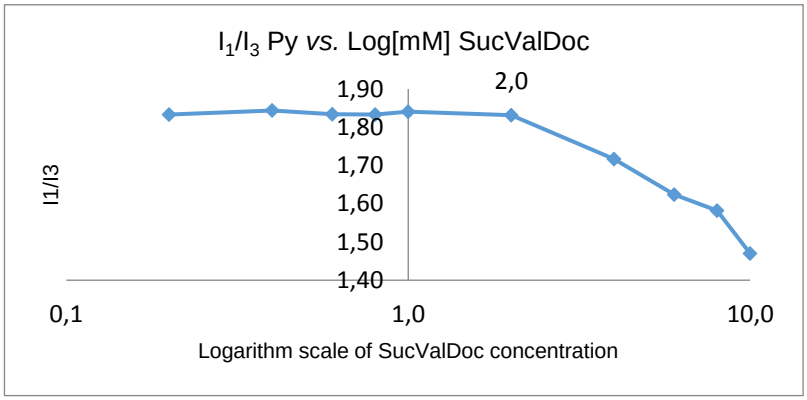
Plot 3-2. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucValDoc concentration in basic pH.

In plot 3-1, interception of the nearly horizontal part and the rapidly varying part is not seen clearly, so logarithm scale is made for concentration axis. In plot 3-2, the point where the line begins to decrease rapidly represents the starting of micellar formation. This point is the minimum micellar concentration for SucValDoc in a basic pH solution and its value is 0.26 mM.

Neutral solution



Plot 3-3. Plot of the intensity ratio (I_1/I_3) *vs* SucValDoc concentration (µM) in neutral pH solution.

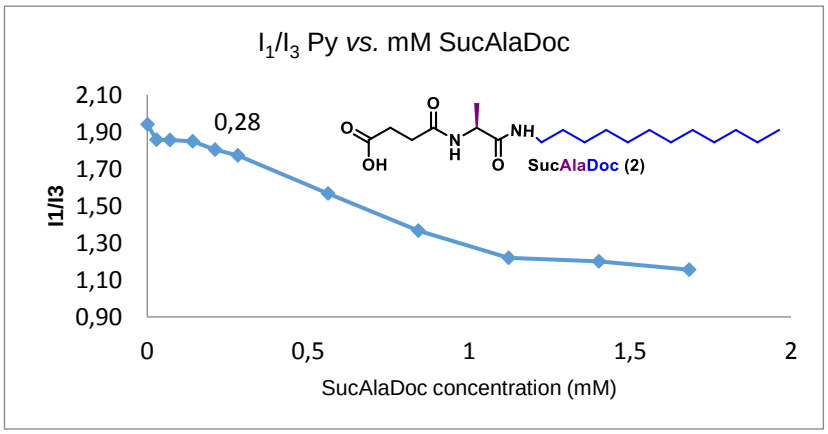


Plot 3-4. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucValDoc concentration in neutral pH solution.

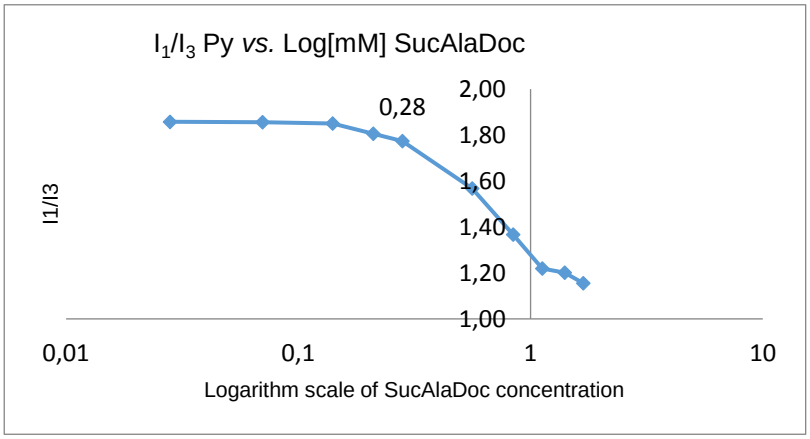
Plot 3-3, interception of the nearly horizontal part and the rapidly varying part occurs at 2 µM but logarithm scale is made for concentration axis to confirm this value. In plot 3-4, the point where the line begins to decrease rapidly represents the starting of micellar formation. This point is the minimum micellar concentration for SucValDoc in a neutral pH solution and its value is 2 µM.

3.3.2. SucAlaDoc results:

Basic solution



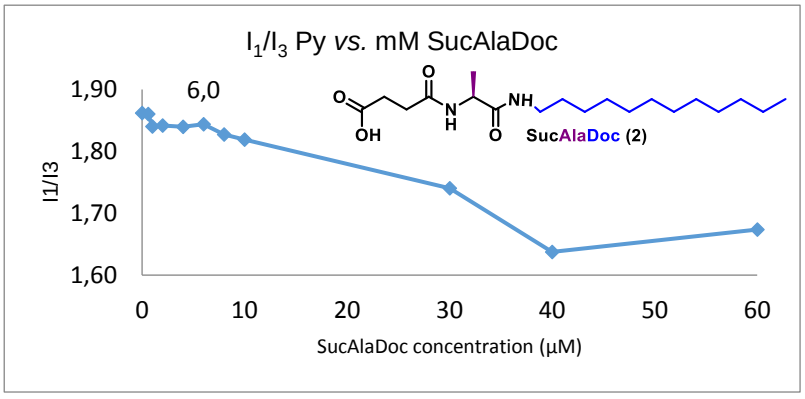
Plot 3-5. Intensity ratio (I_1/I_3) *vs* SucAlaDoc concentration (mM) in basic pH.



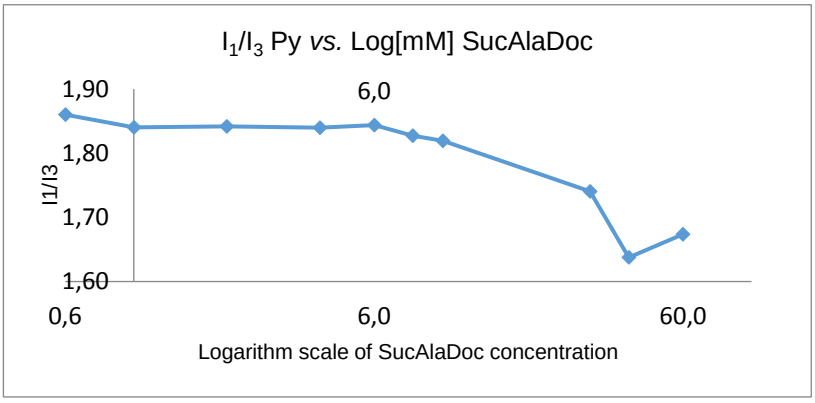
Plot 3-6. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucAlaDoc concentration in basic pH.

The interception of the nearly horizontal part and the rapidly varying part occurs at 0.281 mM but logarithm scale is made for concentration axis to confirm this value. Plot 3-6, the point where the line begins to decrease rapidly represents the starting of micellar formation. This point is the minimum micellar concentration for SucAlaDoc in a basic pH solution and its value is 0.281 mM.

Neutral solution



Plot 3-7. Intensity ratio (I_1/I_3) *vs* SucAlaDoc concentration (mM) in neutral pH.

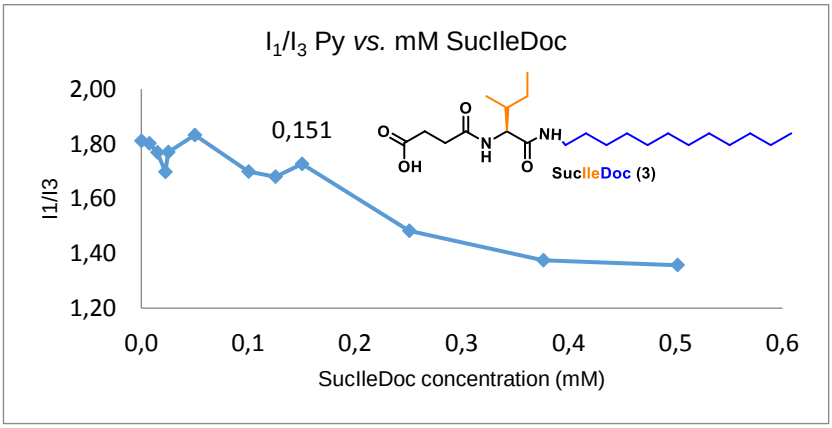


Plot 3-8. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucAlaDoc concentration in neutral pH.

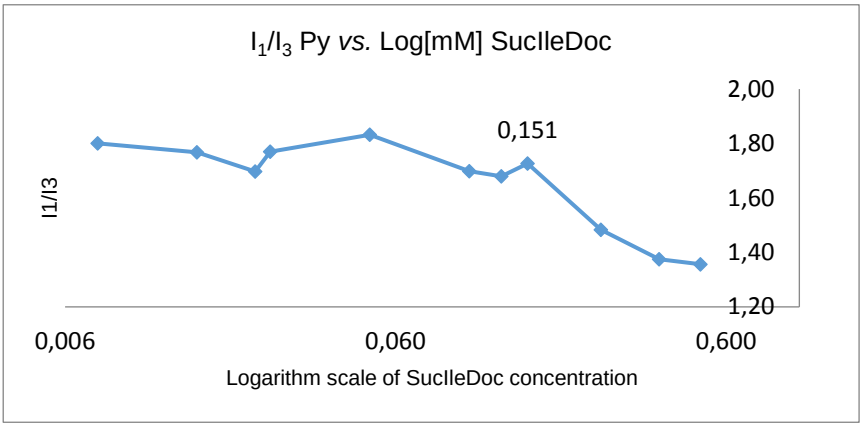
Plot 3-7 and 3-8, shows to the point where the line begins to decrease rapidly represents the starting of micellar formation. This point is the minimum micellar concentration for SucAlaDoc in a neutral pH solution and its value is 6 µM.

3.3.3. SucIleDoc results:

Basic solution



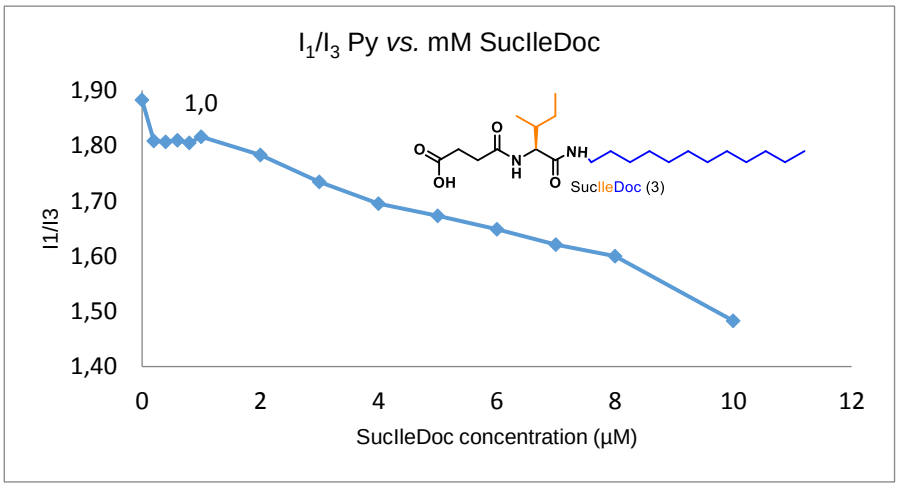
Plot 3-9. Intensity ratio (I_1/I_3) *vs* SucIleDoc concentration (mM) in basic pH.



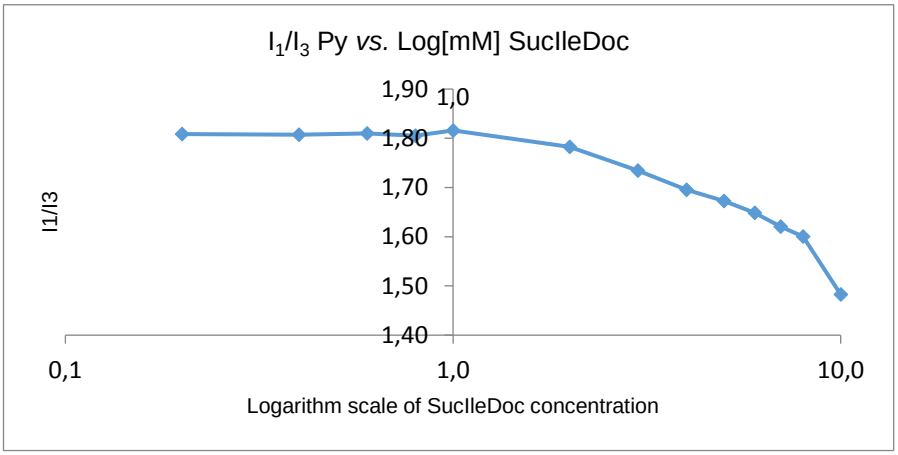
Plot 3-10. Plot of the intensity ratio (I_1/I_3) *vs* logarithm scale of SucIleDoc concentration in basic pH.

In plot 3-10 and 3-9, the point where the line begins to decrease rapidly represents the starting of micellar formation. This point is the minimum micellar concentration for SucIleDoc in a basic pH solution and its value is 0.152 mM.

Neutral solution of SucIleDoc



Plot 3-11. Intensity ratio (I_1/I_3) *vs* SucIleDoc concentration (mM) in neutral pH.

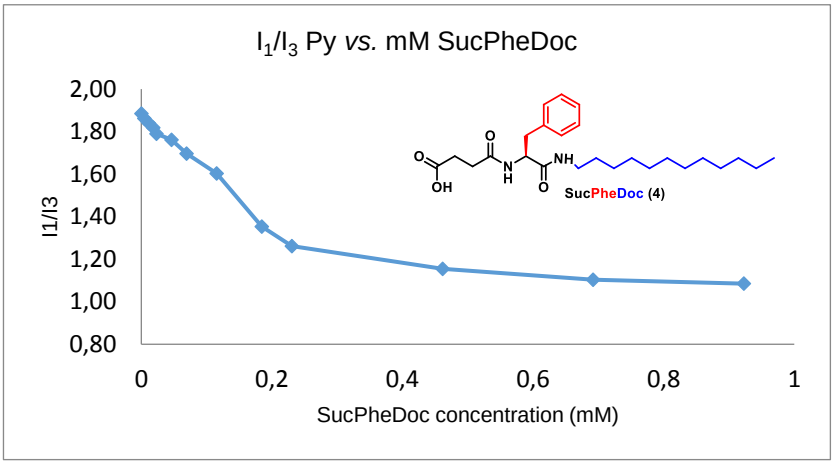


Plot 3-12. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucIleDoc concentration in neutral pH.

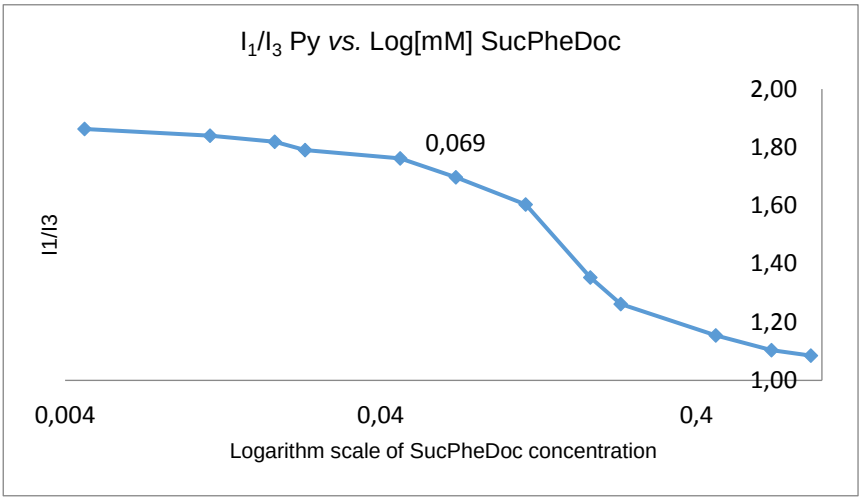
The interception of the nearly horizontal part and the rapidly varying part occurs at 1 µM for SucIleDoc in plot 3-11 as plot 3-12.

3.3.4. SucPheDoc results:

Basic solution



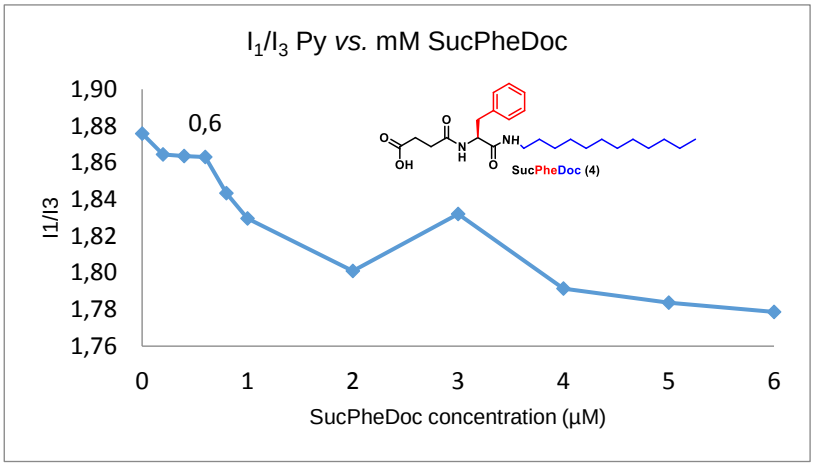
Plot 3-13. Intensity ratio (I_1/I_3) vs SucPheDoc concentration (mM) in basic pH.



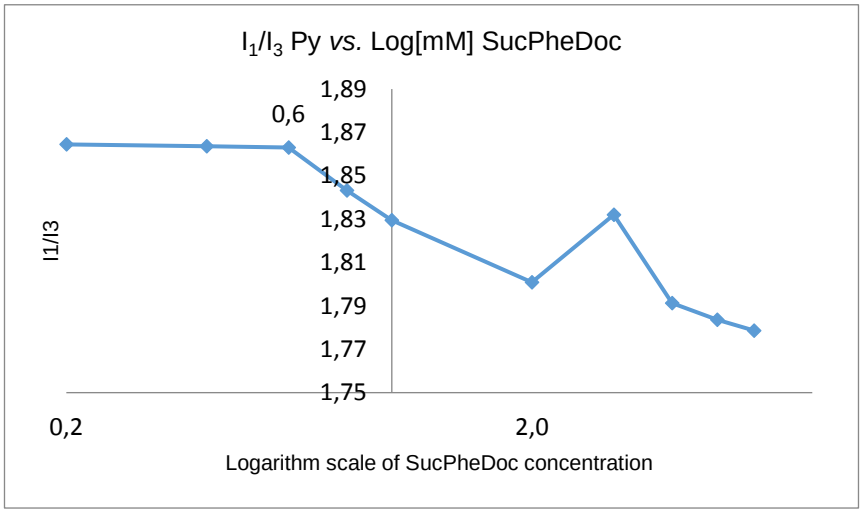
Plot 3-14. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucPheDoc concentration in basic pH.

Plots 3-13 and 3-14 that the minimum micellar concentration for SucPheDoc in a basic pH solution and its value is 0.069 mM.

Neutral solution



Plot 3-15. Intensity ratio (I_1/I_3) *vs* SucPheDoc concentration (mM) in neutral pH.

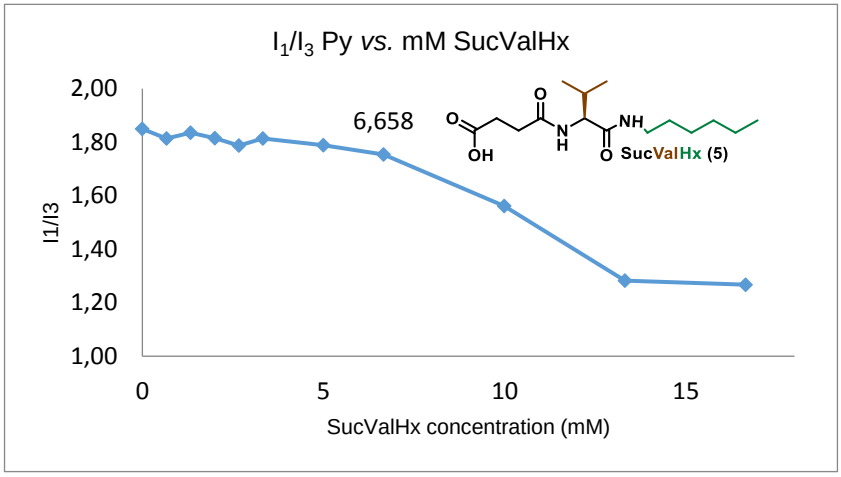


Plot 3-16. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucPheDoc concentration in neutral pH.

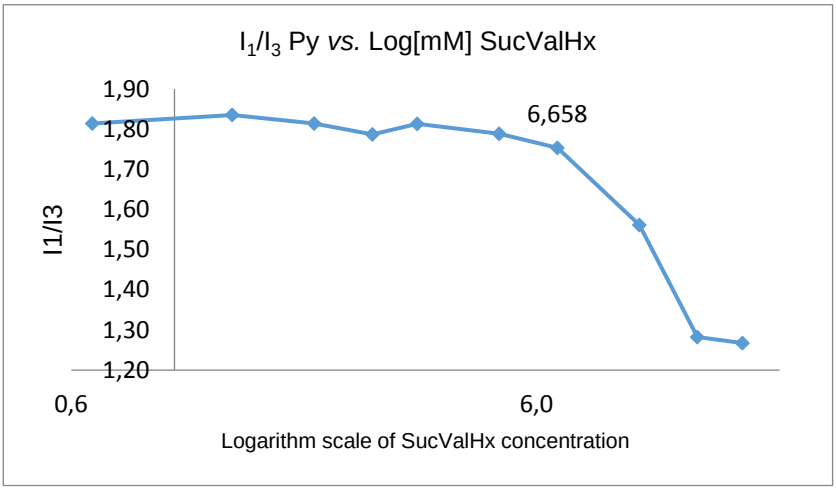
Analysing the plots 3-15 and 3-16 the minimum micellar concentration for SucPheDoc in a neutral pH solution and its value is 0.6 µM.

3.3.5. SucValHx results:

Basic solution

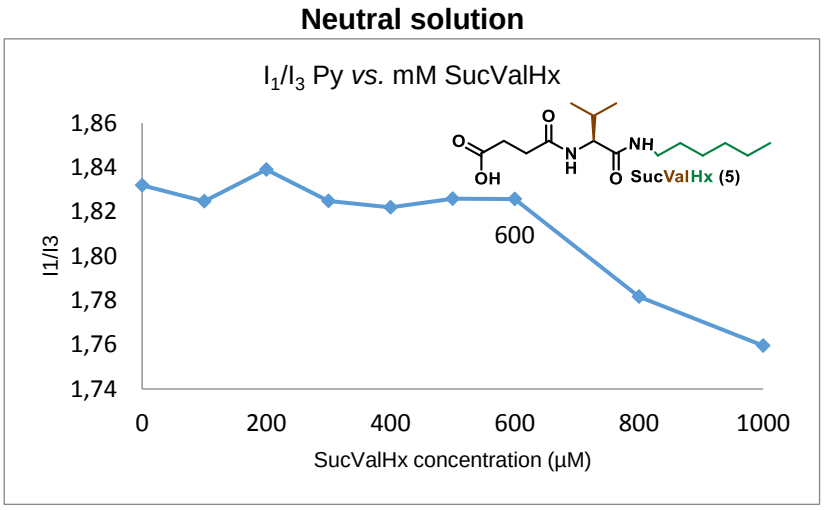


Plot 3-17. Intensity ratio (I_1/I_3) *vs* SucValHx concentration (mM) in basic pH.

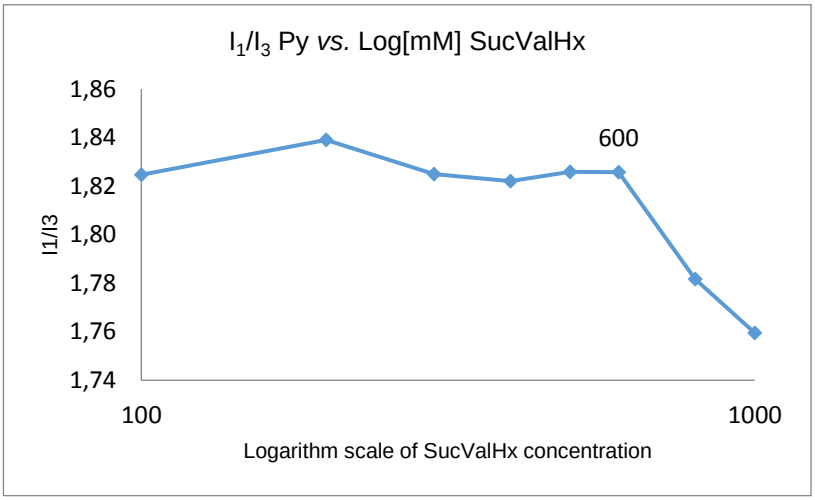


Plot 3-18. Intensity ratio (I_1/I_3) *vs* logarithm scale of SucValHx concentration in basic pH.

The plots showed that minimum micellar concentration for SucValHx in a basic pH solution and its value is 6.658 mM.



Plot 3-19. Intensity ratio (I_1/I_3) *vs* SucValHx concentration (µM) in neutral pH.



Plot 3-20. Ratio (I_1/I_3) *vs* logarithm scale of SucValHx concentration in neutral pH.

The minimum micellar concentration for SucValHx in a neutral pH solution and its value is 600 µM.

From the above data it can be summarized that in all cases whereas the micellar formation is accompanied by an abrupt decrease in the pyrene 1:3 ratio values. This behaviour can be explained by two ways: (*i*) pyrene is associated with some kind of pre-micellar aggregates, which at higher surfactant concentrations are converted into micelles; and (*ii*) pyrene and surfactant may self - associate into small aggregates that do not exist in the absence of pyrene, pyrene being responsible for their formation.

3.4. Comparative between Valine derivatives.

The micelles formed by surfactants with a tail of moderate length (approximately C10 – C16) are thought to be spherical or nearly spherical – at least close to the *CMC:* [12]

- Surfactants with longer tails will have a lower *CMC* and a larger aggregation number than analogues with shorter tails.

- Adding salt to an ionic micellar solution will decrease the *CMC* and increase the aggregation number owing to the screened electrostatic repulsion.

- Counterions that are more strongly bound to the surfactant will induce a lower *CMC* and a higher aggregation number.

- Owing to the amphiphilic character of the micellar surface, it can interact with both hydrophilic and hydrophobic species dissolved in the aqueous bulk.

3.5. Micelle aggregation number (N_{agg}) determination.

The process used to determine the micelle aggregation number was the one appears in the literatura. [13] A 1 x 10^{-3} mol/dm^3 solution of pyrene in ethanol was prepared. A small aliquot was transferred with a pipet to a flask, and the solvent was allowed to evaporate. Water and surfactant were then added to this flask and stirred to dissolve the pyrene. The final concentration of surfactant was different in each case: $[S]_{SucValDoc}$ = 1 mM and $[S]_{SucValHx}$ = 20.8 mM. The concentration of pyrene in both cases was [P] = 0.05 µM. Working mixtures for fluorescence measurements were made up by mixing 1 mL samples of the surfactant/pyrene solution with appropriate volumes of an 10 µM aqueous solution of the quencher for SucValDoc and an 0.1 mM aqueous solution of the quencher for SucValHx, and making up to 5 mL with water to give the final quencher concentrations. Optimum range of quencher concentration was found in each case. An excitation wavelength of 337 nm and emission wavelength of 394 nm were used to obtain the results.

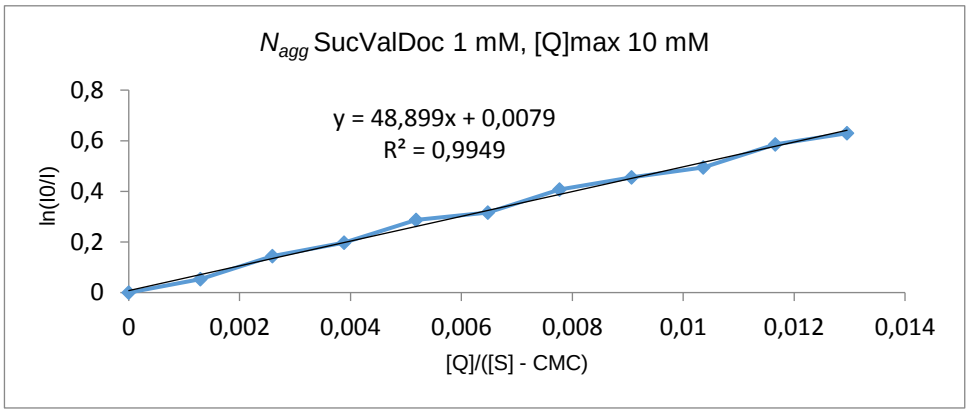
If fluorescence intensity measured at various quencher concentrations with fixed surfactant concentration, the aggregation number can be calculated from the slope of the straight line obtained by ploting ln (I_0/I) against [Q]/([S]-[*CMC]*), provided that the *CMC* is known.

$$ln\left(\frac{I_0}{I}\right) = \frac{[Q]\cdot N_{agg}}{[S]-[CMC]} \qquad (2)\ [13]$$

I is the intensity of fluorescence in the presence of quencher, I_0 is the intensity in the absence of quencher, [Q] is the bulk concentration of quencher, [S] is

the bulk concentration of surfactant, [*CMC*] is the critical micelle concentration and N_{agg} is the micelle aggregation number.

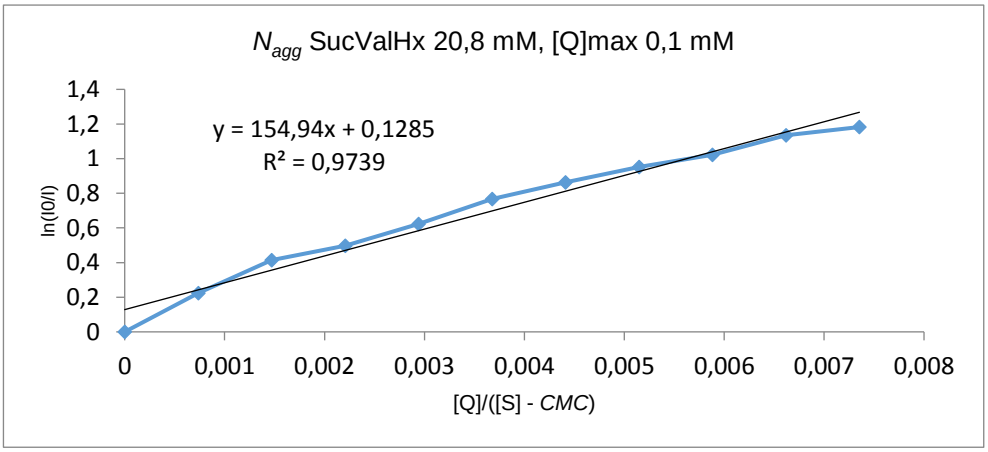
3.5.1. SucValDoc micelle aggregation number:



Plot 3-21. ln (I_0/I) *vs* [Q]/([S]-*CMC*) for SucValDoc.

It can easily be deduced from the plot and the equation (2) that the slope of the straight line will be the aggregation number of SucValDoc. So, the aggregation number for SucValDoc is 48.9.

3.5.2. SucValHx micelle aggregation number:



Plot 3-22. ln (I_0/I) *versus* [Q]/([S]-*CMC*) for SucValHx.

It can easily be deduced from the plot and the equation (2) that the slope of the straight line will be the aggregation number of SucValHx. So, the aggregation number for SucValHx is 154.94.

3.6. DLS studies. [6]

Dynamic light scattering is a technique well suited for the determination of the *CMC*. In DLS one measures the time dependence of the light scattered from a very small region of solution, over a time range from tenths of a microsecond to milliseconds. These fluctuations in the intensity of the scattered light are related to the rate of diffusion of molecules in and out of the region being studied (Brownian motion), and the data can be analyzed to directly give the diffusion coefficients of the particles doing the scattering. When multiple species are present, a distribution of diffusion coefficients is seen. Traditionally, rather than presenting the data in terms of diffusion coefficients, the data are processed to give the size of the particles (radius or diameter). The relation between diffusion and particle size is based on theoretical relationships for the Brownian motion of spherical particles. The hydrodynamic diameter or Stokes radius, *Rh*, derived from this method is the size of a spherical particle, and the data is commonly presented as the fraction of particles as a function of their diameter.

Next figures show an example of data analysis using DLS with good results. It represents the results of the analysis of a phospholipids' type. The Figure 3-8 shows the raw correlation data. The Figure 3-9 shows the relation between size distributions by number and Figure 3-10 the relation between size distributions by intensity.

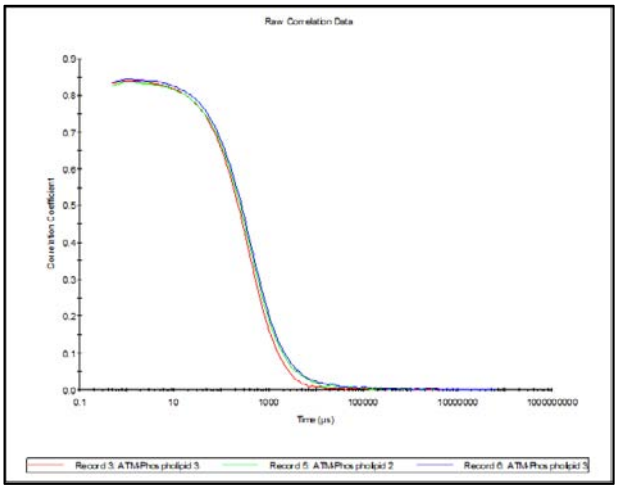


Figure 3-8. Concentration coefficient *vs* time (µs) of an example of good result quality.

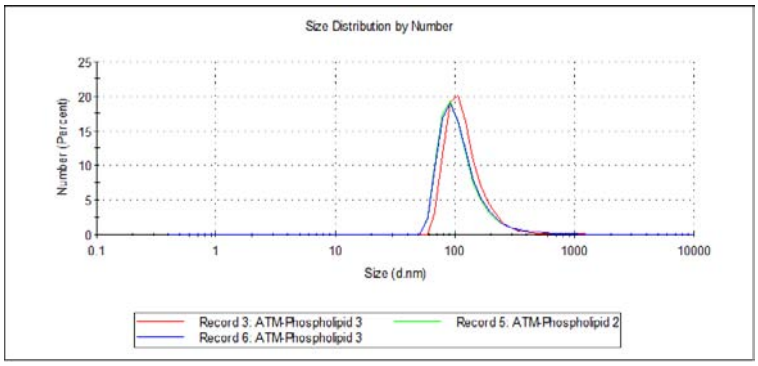


Figure 3-9. Number (%) *vs* size (diameter in nm) of good result quality example.

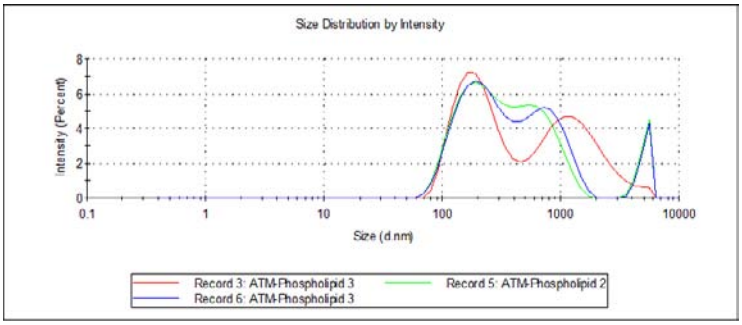


Figure 3-10. Intensity (%) *vs* size (diameter in nm) of good result quality example.

In biological research, it is recommended to measure the *CMC* in the real conditions, including buffer, temperature, and salt etc. Any changes in the volume of the scatterer dramatically increase the intensity of light scattered. In this project, it was proposed to obtain the *CMC* using DLS analysis and compare it with the results obtained by fluorescence. DLS experiments provide simultaneously a rapid evaluation of the total scattering intensity and the hydrodynamic radii spectrum with concentration in a non-invasive fashion. [6]

Figure 3-11. DLS instrument (Malvern Zetasizer Nano ZS).

Figures 3-12 and 3–13 show raw correlation data obtained from the results of SucValDoc. So, if the example figure and results obtained in the laboratory are compared, it can be said that the results are not quite good. Z–average in all cases were too low and therefore difficult to interpret. So data obtained was too poor for distribution analysis. Therefore, conditions to measure the *CMC* of amino acid derivatives must be optimized. It was the objective once the results were observed but the method was not optimized because lack of time. One possible cause of obtaining these data could be that filtration cause elimination of micelles. Samples could contain large particles, aggregates or dust. So, the idea will be make the measurements again without performing filtration.

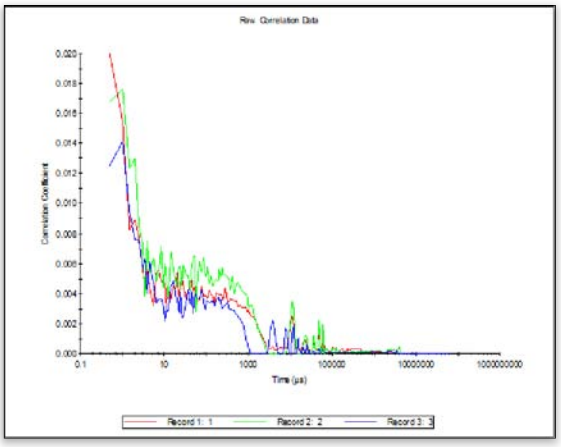


Figure 3-12. Concentration coefficient *vs* time (µs) of results obtained for 0.1 mg/mL SucValDoc with pyrene.

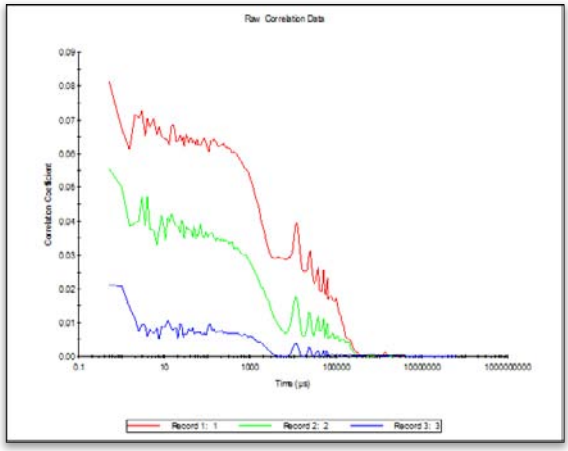


Figure 3-13. Concentration coefficient *vs* time (µs) of results obtained for 0.1 mg/mL SucValDoc without pyrene.

Conclusions

4. Conclusions

- SucValDoc (1) and SulValHx (5) were successfully synthesized and characterized.

- The values of *CMC* and N_{agg} obtained in this project agree well with theoretical assumptions mentioned.

- The table below shows the data of the partition coefficients theoretically calculated and the values of critical micelle concentrations obtained in the laboratory of each compound in both, basic and neutral pH.

- The surfactant tail has a controlling role in the formation of micelles. Tails directly influence the packing parameters and therefore the aggregation number.

Summarizing, the compounds that showed the highest ClogP were those with lower *CMC* (SucIleDoc and SucPheDoc). Curiously, the ClogP of SucValDoc is slightly lower than the SucIleDoc. This could be explained because in the aggregation process influencing factors such as solvent polarity, packing of the molecule, hydrogen bonding, Van der Waals bonds, chirality, solubility, etc. The SucPheDoc compound possesses a benzyl group which makes it more polar when providing values for determining the ClogP. It also has better packing due to π-stacking; [4] apparently, it has a greater influence on the value of *CMC* than on the value of ClogP.

Surfactant	*CMC* (μM) basic pH	*CMC* (μM) neutral pH	ClogP basic pH	ClogP neutral pH
SucValDoc (1)	260	2	2.55	5.51
SucAlaDoc (2)	280	6	1.62	4.58
SucIleDoc (3)	150	1	3.08	6.04
SucPheDoc (4)	69	0.6	3.04	6.00
SucValHx (5)	6658	600	- 0.63	2.33

Table 4-1. *CMC* and ClogP of different surfactants.

Experimental Section

5. Experimental Section

5.1. General Methods.

$^{1}H/^{13}C$ NMR spectra were recorded at 500/125 MHz or 300/75 MHz in the indicated solvent at 30 ºC. Signals of the deuterated solvent (DMSO-*d6* in all case, unless otherwise indicated) were taken as the reference in DMSO-*d6*, the singlet at δ 2.50 and the quadruplet centred at 39.52 ppm for ^{1}H and ^{13}C NMR, respectively. ^{1}H and ^{13}C signals were assigned with the aid of 2D methods (COSY, HSQC and HMBC). Reactions which required an inert atmosphere were carried out under N_2. Commercially available reagents were used as received. In the characterization of the spectra the abbreviations s, d, t, q, p, m, br and dd mean singlet, doublet, triplet, quadruplet, quintet, multiplet, broad and doublet of doublets. Unless detailed otherwise, “work-up” means filter to vacuum the reaction crude, followed by washing with of residue; if the reaction medium was acidic, an additional washing with NaOH 0.1 M was performed. If the reaction medium was basic, an additional washing with HCl 0.1 M was performed. New washing with water, finally the residue was drying over-night to vacuum at 60 ºC.

Where solutions were filtered through a Celite® pad, the pad was additionally washed with the same solvent used, and the washings incorporated to the main organic layer.

Mass spectra were run by the electro- spray mode (ESMS). Masses spectra were recorded at Mass Spectrometry triple Quadrupole Q-TOF Premier (Waters) with simultaneous Electrospray and APCI Probe.

The compounds SucPheDoc, SucAlaDoc, SucIleDoc were previously prepared with a good yields and purity.

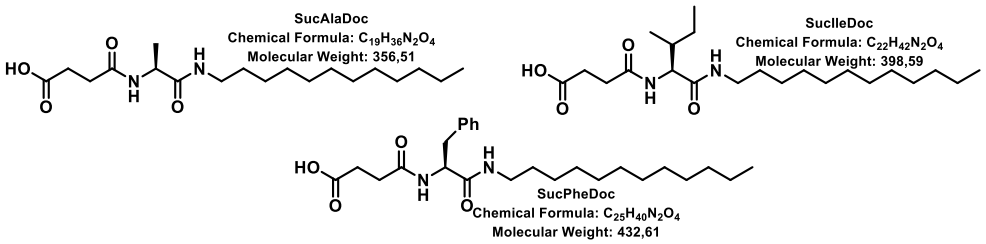


Figure 5-1. Structures of the compounds SucAlaDoc, SucIleDoc and SucPheDoc.

5.2. Fluorescence Measurements.

Excitation was performed at 334 nm and the emission intensity at 350 − 500 nm monitored in a *Jasco* FP-8300 spectrofluorimeter at 30 ºC.

5.2.1. Determination of Critical Micellar Concentration (*CMC*) by Fluorescence:

A stock solution of 0.1 mM pyrene in absolute ethanol, a known volume was pipetted into a volumetric flask. The ethanol was evaporated and distilled water added, and the solution was sonicated two hours. The final pyrene concentration was 1–2 μM. From the aqueous pyrene solution, was prepared a solution of NaOH 0.1 M and from this solution the surfactant stock solutions were prepared with surfactant concentrations wlth the respective cmc. For the calculation of *CMC,* we used the procedure showed by M. Flor Rodríguez Prieto and *et. al*. in *J. Chem. Edu.* **1995**, *77*, 662.

For determinate *CMC* in neutral conditions, the NaOH 0.1 M was neutralized with equal volume of solution of sulfuric acid 0.1 M.

Surfactant	*CMC* (µM) basic pH	*CMC* (µM) neutral pH
SucValDoc (1)	260	2
SucAlaDoc (2)	280	6
SucIleDoc (3)	150	1
SucPheDoc (4)	69	0.6
SucValHx (5)	6658	600

Table 5-1. Summary of *CMC* values.

5.2.2. Determination of Aggregation Number (N_{agg}) by Fluorescence: In the laboratory stock solution of 0.1 mM pyrene was prepared as well above. For determinate N_{agg} we used the procedure showed by Jan van Stam and *et. al*. in *J. Chem. Edu.* **1998**, *75*, 93.

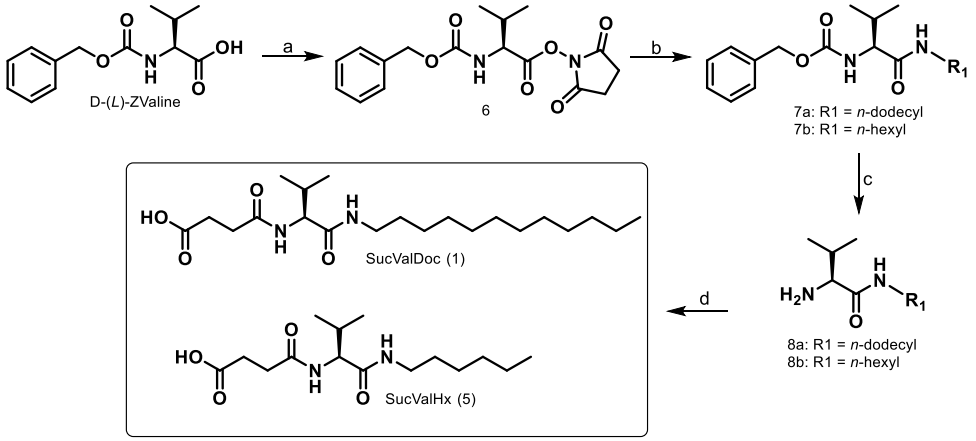
Surfactant	N_{agg}
SucValDoc (1)	48.9
SucValHx (5)	154.9

Table 5-2. Summary of N_{agg} values.

5.3. Dynamic Light Scattering (DLS).

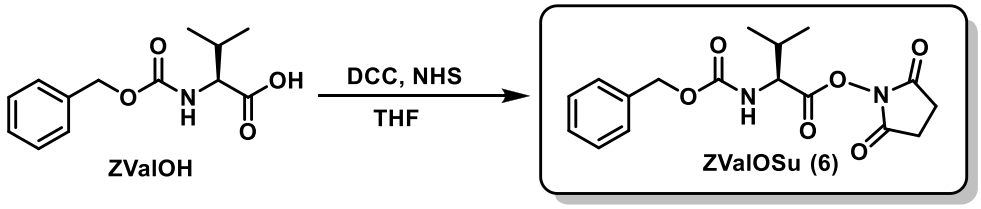
Measurements were made of different concentrations of SucValDoc. Two methods were followed, using pyrene solution with NH_4OH and without pyrene. In both cases 80 µL of H_2SO_4 (2 M) were added in each sample. Before making the measurements, all samples were filtered. It was done to remove unwanted coarse particles that may originate from drying agglomerates, dispersion tool abrasion or dust contamination, and thus to measure only the micelles present in each sample. All measurements were made at room temperature. The instrument used was Malvern Zetasizer Nano ZS (Figure 3-11). For a typical DLS experiment, 200 µL of a sample solution was slowly pipetted into a clean cuvette (tetragonal prism, 1 cm width and deep, PMMA cuvettes). This instrument is ideal, in fact, for the characterization of phase transitions and structures in micellar polymeric systems.

5.4. Experimental Procedures.



Scheme 5-1. Reagents and conditions: a) *DCC*, *N*-Hydroxisuccinimide, THF, 2 h., 97 %; b) *n*-dodecylamine or *n*-hexylamine , THF, 16 h., 96 %; c) Pd / C, H_2, MeOH, 4 h., 98 %; d) Succinic anhydride, K_2CO_3, THF, 16 h., 94 – 95 %.

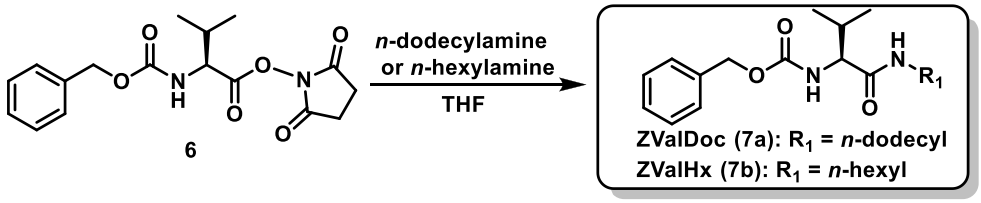
5.4.1. Synthesis of (S)-2, 5-dioxopyrrolidin-1-yl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate *(ZValOSu – 6):*



A solution of commercial available carbobenzyloxy-*L*-amino acid (**ZValOH**) (40 mmol) and *N*-hydroxysuccinimide (40 mmol, 1.0 eq.) in dry THF (150 mL) was added dropwise under N_2 at 0 ºC with a dropping funnel to a solution of *N,N'*-dicyclohexylcarbodiimide (10.9 mmol, 1.01 eq.) in dry THF (75 mL). The mixture was further stirred for 1 h at 0 ºC. The solution was then allowed to stand into refrigerator for 2 h, which caused precipitation of *N,N'*-dicyclohexylurea. After this time, the mixture was filtered under vacuum, and the filtrate was removed under reduced pressure and the crude residue was purified by crystallization in isopropanol to yield the respective activated ester.

*2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-valinate (**6**)*: A white solid was obtained (yield 97%); the NMR spectra were consistent with those described in the literature. [1]

5.4.2. Synthesis of (S)-benzyl (1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)carbamate (ZValDoc – 7a) and *(S)-benzyl (1-(hexylamino)-3-methyl-1-oxobutan-2-yl)carbamate (ZValHx – 7b) :*



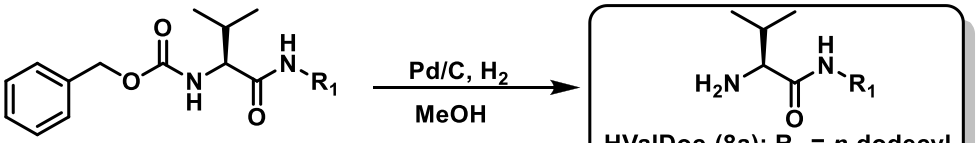
[1] J., Becerril; M., Bolte; M., I., Burguete; F., Galindo; E., García-España; S., V., Luis; J., F., Miravet. *J. Am.Chem. Soc*. **2003**, *125*, 6677 – 6686.

General procedure for coupling between activated ester and amine: A solution of carbobenzyloxy-*L*-amino ester activated **6** (38.8 mmol) in THF (200 mL) was added dropwise under N_2 at room temperature with a dropping funnel to a solution of commercial available *n*-dodecylamine or *n*-hexylamine (40.5 mmol, 1.1 eq.) in THF (100 mL). The mixture was further stirred for 5 h at 55 ºC. After this time, the mix was cooled to room temperature and solvent was removed under reduced pressure and the residue was poured into dissolution aq. HCl 0.1, then the mix was sonicated during 5 minutes. It was filtered under vacuum, and the residue was washed with water until pH = 7. The residue was dried under reduced pressure at 50ºC overnight.

*(S)-benzyl (1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)carbamate (**7a**):* A white solid was obtained (yield 96%); the NMR spectra were consistent with those described in the literature.[2]

*(S)-benzyl (1-(hexylamino)-3-methyl-1-oxobutan-2-yl)carbamate (**7b**):* Compound **7b**, was obtained following the same procedure as above except that *n*-hexylamine was used. A white solid was obtained (yield 96%). The NMR spectra were consistent with those described in the literature.[3]

5.4.3. Synthesis of (S)-2-amino-N-dodecyl-3-methylbutanamide (HValDoc – 8a) and (S)-2-amino-N-hexyl-3-methylbutanamide (HValHx – 8b):



ZValDoc (7a): R_1 = *n*-dodecyl
ZValHx (7b): R_1 = *n*-hexyl

HValDoc (8a): R_1 = *n*-dodecyl
HValHx (8b): R_1 = *n*-hexyl

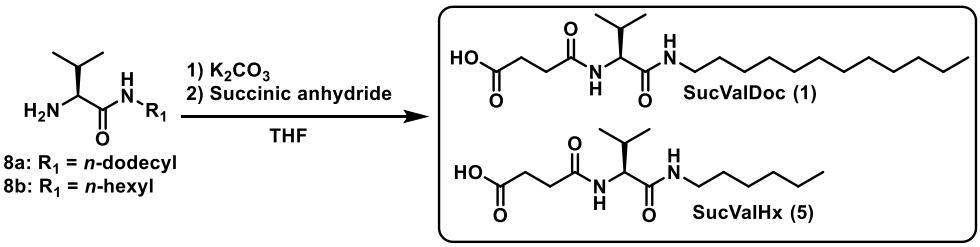
General procedure for deprotection of carbobenzyloxy group: Palladium catalyst (10% *w/w*) was suspended in MeOH (250 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of carbobenzyloxy amino compound in MeOH (150 mL) was added via syringe, followed by stirring under H_2 at room temperature for 4 h. The reaction

[2] C. A., Angulo-Pachón; J. F., Miravet. *Chem. Comm*. **2016**, *52*, 5398 – 5401.
[3] C. A., Angulo-Pachón; C., Gascó-Catalán; J. J., Ojeda-Flores; J. F., Miravet. *Chem. Phys. Chem*. **2016**, *17*, 1 – 17.

mixture was then filtered through Celite®, and the solvent was removed under reduced pressure to yield respective amine.

*(S)-2-amino-N-dodecyl-3-methylbutanamide (**8a**):* White solid was obtained (yield 98%). The compound was used in crude form for the next reaction. The NMR spectra were consistent with those described in the literature.2
*(S)-2-amino-N-hexyl-3-methylbutanamide (**8b**):* Colorless oil was obtained (yield 98%). The compound was used in crude form for the next reaction. The NMR spectra were consistent with those described in the literature.3

5.4.4. Siynthesis of *(S)-4-((1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (SucValDoc - 1) and (S)-4-((1-(cyclohexylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (SucValHx - 5):*



General procedure for preparing the final compounds: A solution of respective amine (16.3 mmol) in THF (150 mL) was treated at 0 ºC under N_2 with solid K_2CO_3 (61.9 mmol, 3.8 eq.). The mixture was stirred for 15 minutes at 0 ºC, after with a dropping funnel to a solution of commercial available succinic anhydride (32.6 mmol, 2.0 eq.) in THF (50 mL). The mixture was further stirred vigorously for 16 h at room temperature. After this time, the solution was concentrated under reduced pressure and the crude residue was dissolved in water (100 mL); then hydrochloric acid concentrate was added dropwise at 0 ºC until observe the formation of a white precipitate to pH = 4. The white solid obtained was filtered under vacuum, and the residue was washed with water (300 mL). The compound was dried under reduced pressure at 50 ºC overnight.

*(S)-4-((1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**1**):* A white solid was obtained (yield 95%).6 mmol, 95%) as a white solid.

^{1}H NMR (500 MHz, DMSO-*d6*): δ 12.0 (br s, 1H), 7.90 – 7.82 (m, 2H), 4.07 (t, *J* = 16 Hz, 1H), 3.12 – 2.92 (br m, 2H), 2.44 – 2.36 (m, 4H), 1.93 (sext, *J* = 10, 16 Hz, 1H), 1.40 – 1.31 (m, 2H), 1.30 – 1.18 (m, 18H), 0.85 (t, *J* = 5 Hz, 3H), 0.82 (d, J = 5 Hz, 6H).

^{13}C NMR (125 MHz, DMSO-*d6*): δ 173.8, 171.0, 170.7 (C=O), 57.9 (CH), 38.3, 31.3 (CH_2), 30.4 (CH), 29.9, 29.3, 29.0 (x3), 29.0 (x2), 28.7, 26.3, 22.0 (CH_2), 19.15, 18.10, 13.89 (CH_3).

HR ESMS: *m/z*: calcd for $C_{21}H_{40}N_2O_4$: 383.2914; found: 383.2910 [$M - H^+$].

*(S)-4-((1-(cyclohexylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**5**):* A white solid was obtained (yield 94%).

^{1}H NMR (300 MHz, DMSO-*d6*): δ 12.00 (br s, 1H), 7.83 (d overlapped, 2H), 4.17 – 3.97 (dd, *J* = 6.9, 9.5 Hz, 1H), 3.16 – 2.90 (m, 2H), 2.41 (s, 4H), 2.02 – 1.82 (m, 1H), 1.53 – 1.11 (m, 8H), 0.98 – 0.71 (t app, 9 H).

^{13}C NMR (75 MHz, DMSO-*d6*): δ 173.4, 171.5, 171.2 (C=O), 58.3 (CH), 38.8 (CH_2), 31.4 (CH), 30.8, 30.4, 29.8, 29.4, 26.5, 22.5 (CH_2), 19.6, 18.6, 14.3 (CH_3).

HR ESMS: *m/z*: calcd for $C_{15}H_{28}N_2O_4$: 323.1947; found: 323.1950 [$M + Na^+$].

NMR Spectra

6. NMR Spectra

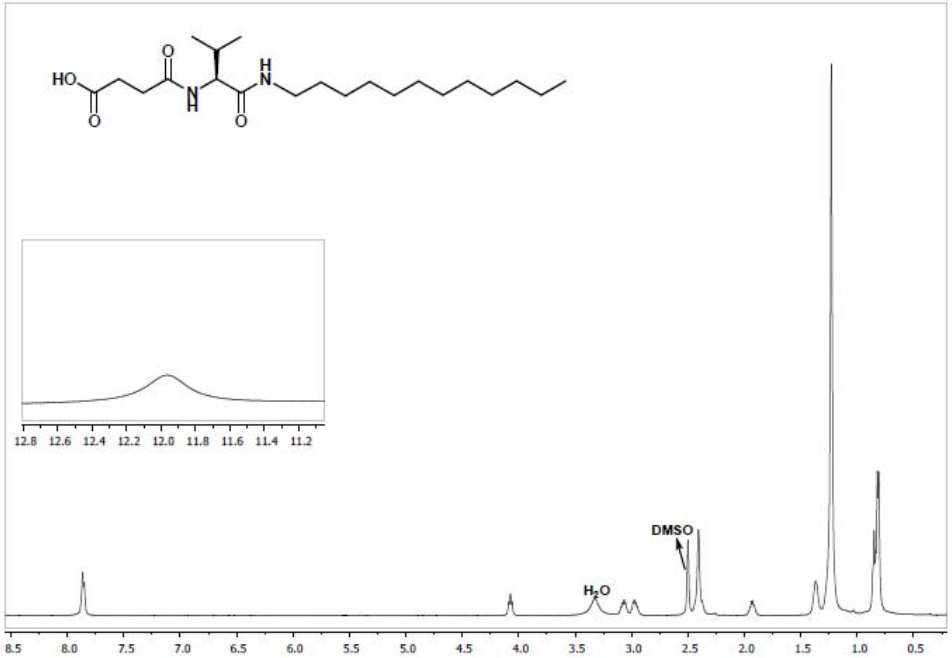


Figure 6–1. ^{1}H NMR spectrum of compound **1**.

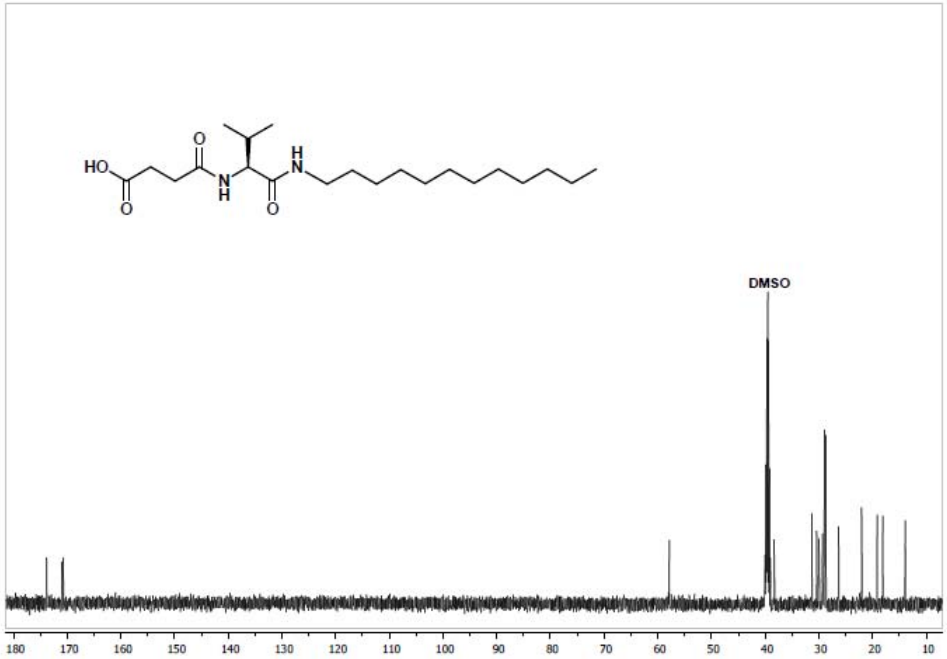


Figure 6–2. ^{13}C NMR spectrum of compound **1**.

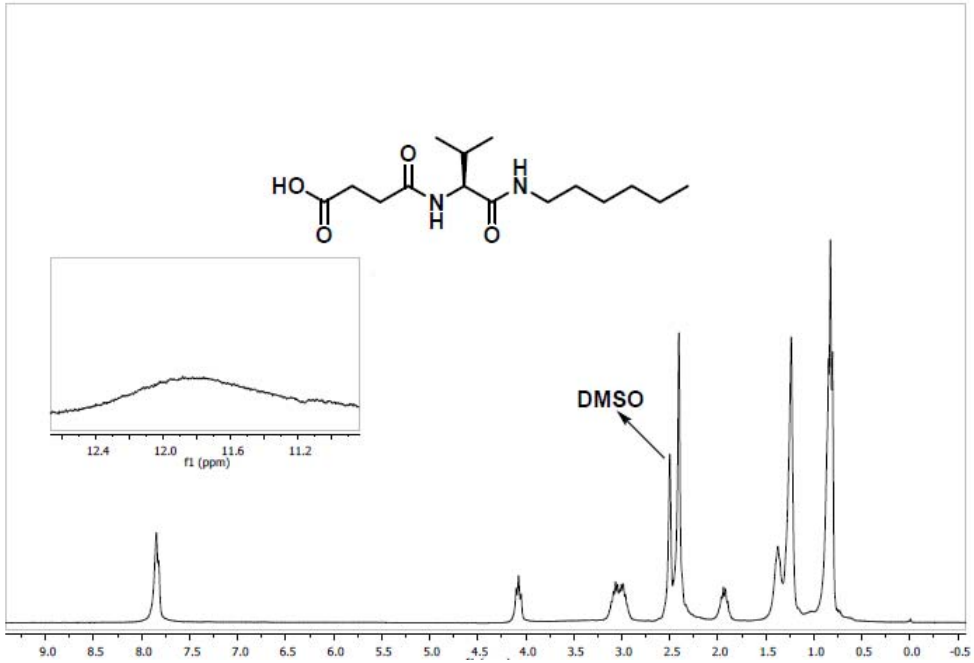


Figure 6–3. ^{1}H NMR spectrum of compound **5**.

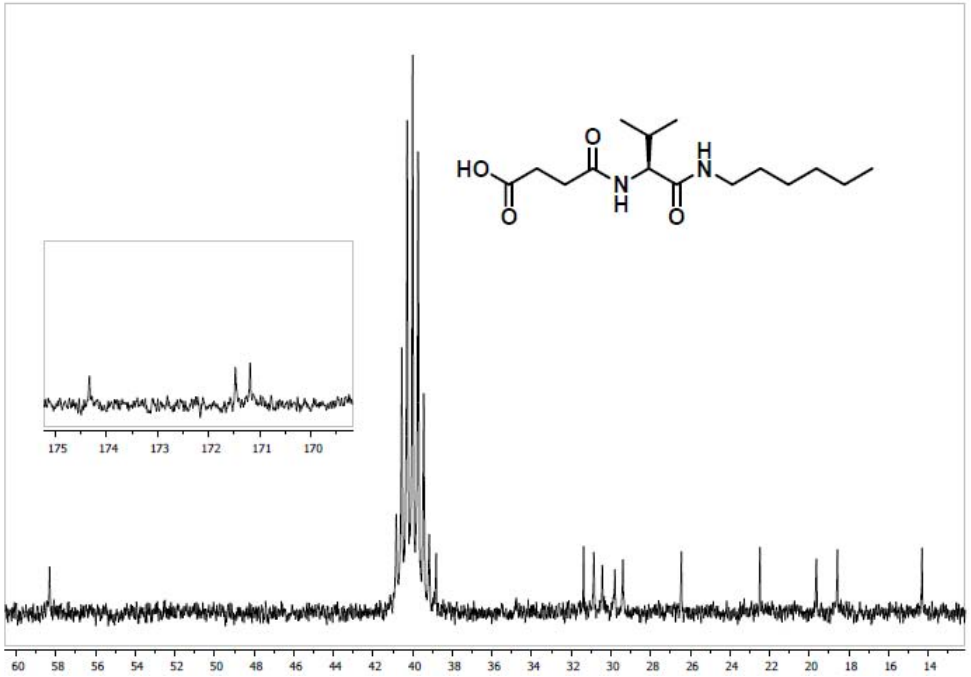


Figure 6–4. ^{13}C NMR spectrum of compound **5**.

References

7. References

[1]
Escuder, B.; Miravet J. F. *Functional Molecular Gels.* RSC Soft Matter Series. **2014**, Cambridge. UK.

[2]
Weiss, R. G.; Terech, P. MOLECULAR GELS Materials with Self-Assembled Fibrilar Networks **2006**, *Springer ed. Netherlands*.

[3]
Raghavan, S. R. *Langmuir* **2009**, *25, 8*382.

[4]
Kang, M.; Zhang, P.; Cui, H.; Loverde, S. *Macromolecules* **2016**, *49*, 994.

[5]
Kalyanasundaram, K, Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

[6]
Tatkiewicz W, Elizondo E, Moreno E, Díez-Gil C, Ventosa N, Veciana J, Ratera I. Methods for characterization of protein aggregates. Methods Mol Biol. 2015; 1258: *387*–401. doi: 10.1007/978-1-4939-2205-5_22 [PubMed].

[7]
www.chemgapedia.de/vsengine/vlu/vsc/en/ch/2/vlu/oxidation_reduktion/red_spal_benyzl.vlu.html

[8]
http://www.commonorganicchemistry.com/Rxn_Pages/Benzyl_Protection/Benzyl_Protection_H2_PdC_Mech.htm

[9]
Fontanillo, M.; Angulo-Pachón, C. A. *J. Colloid Interv. Scl.* **2013**, *412*, 65.

[10]
https://en.wikipedia.org/wiki/Partition_coefficient

[11]
Aguiar, J.; Carpena, P.; Molina-Bolívar, J. A.; Carnero-Ruiz, C. *J. Colloid Inter. Sci*. **2002**, *258*, 116.

[12]
Van Stam, J.; Depaemelaere, S.; De Schryver, F. C. *J. Chem. Ed.,* **1998**, *75*, 93.

[13]
Rodríguez, M. F.; Ríos, M- C.; Mosquera, M.; Ríos, A. M.; Mejuto, J. *C. J. Chem. Ed*. **1995**, *7*, 72.