SYNTHESIS OF A LOW MOLECULAR WEIGHT HYDROGELATOR DERIVED FROM L-VALINE AND SUCCINIC ACID AND STUDY OF HYDROGELATION BY ITS ANION FORM

VÍCTOR POZO GAVARA

BACHELOR'S DEGREE RESEARCH PROJECT

CASTELLÓ, JULY 2020

Escuela superior de Tecnología y Ciencias Experimentales
Departamento de Química Inorgánica y Orgánica
Grupo de Nanomateriales Moleculares Orgánicos con Aplicaciones Biomédicas

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CHEMISTRY DEGREE RESEARCH PROJECT

JULY 2020

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

CERTIFICAN

Que el trabajo fin de grado con el título **SYNTHESIS OF A LOW MOLECULAR WEIGHT HYDROGELATOR DERIVED FROM L-VALINE AND SUCCINIC ACID AND STUDY OF HYDROGELATION BY ITS ANION FORM** ha sido realizado por Víctor Pozo Gavara bajo su dirección, en el grupo de Nanomateriales Moleculares Orgánicos con Aplicaciones Biomédicas 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 Castelló de la Plana a 19 de junio de 2020.

Fdo. Dr. Juan F. Miravet Celades

Fdo. Dr. César A. Angulo Pachón

Abbreviations

Suc	Succinic acid radical
Val	Valine radical
Doc	n-Dodecyl radical
CMC	Critical micellar concentration
MGC	Minimum gelation concentration
DCC	*N,N'*-Dicyclohexylcarbodiimide
DME	1,2-Dimethoxyethane
TEM	Transmission electron microscopy
DLS	Dynamic light scattering
NMR	Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
DMSO_*d6*	Dimethyl sulfoxide deuterated
T_{gel}	Transition temperature of gel to solution

Acknowledgements

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Introduction

Introduction

1.1 What are gels?

Gels are materials that we use in our daily lives, often without realizing it because we find them in a variety of forms. Some of their properties, such as flexibility, malleability, and their storage capacity make them suitable for different fields such as medicine, the cosmetics industry, materials science, pharmacology, as well as the food or electronics industry.

Gels have been studied since the 1860s, however, did not arouse a great interest until a century later. Although the study of gels began more than 150 years ago, it is still not possible today to find an adequate definition of this material that covers all its varieties.

Throughout this time, several rigorous gel definitions were proposed to link the microscopic and macroscopic properties of a gel. Dorothy Jordan Lloyd introduced in 1926 that a gel is easier to recognize than to define and made the following definition:

- Only one rule seems to hold for all gels, and that is that they must be built up from two components, one which is a liquid at the temperature under consideration and the other which, the gelling substance proper, often spoken of as the gelator, is a solid. The gel itself has the mechanical properties of a solid, i.e., it can maintain its form under the stress of its weight, and under any mechanical stress, it shows the phenomenon of strain.[1]

A few decades later, in 1974, Paul John Flory proposed a slightly more rigorous definition in which he maintained that a substance is a gel if:

- It has a continuous microscopic structure with macroscopic dimensions that is permanent on the time scale of analytical experiments.

[1] Jordan Lloyd, D. In Colloid Chemistry by Alexander, J. 1926, vol 1, 767-782, The Chemical Catalogue Co, New York.

- It is solid-like in its rheological behavior despite being mostly liquid.[2,]

Although no exact definition can be found, in general, a gel contains a 3D network formed by the gelator, which entraps the solvent. These materials are generally composed of at least 99% of solvent.

The majority of gels used daily in food or medicine are macromolecular or polymeric gels. These gels are constituted by large molecules or macromolecules. For example, alginate is an anionic polysaccharide that forms irreversible gels with diverse uses:

- Medicine: calcium alginate has healing properties in injuries. It creates a protecting layer and helps to regenerate cells.
- Cosmetics: It is useful as a thickening, gelling or stabilizing agent for the elaboration of creams, masks, or lotions.
- Alimentation: both sodium and calcium alginate are used like alimentary additives in a lot of processed meals. Moreover, sodium alginate is also used in cooking techniques such as spherification.

In this work, supramolecular gels are studied, i.e., gels formed by small molecules or low molecular weight molecules. These gels are relatively new and have been studied in detail since the end of the 20th century. [3]

1.2 Types of gels

Gels can be classified in different ways depending upon their origin, constitution, the type of cross-linking that creates their 3D network, and the medium they encompass.[4] (Figure 1.1)

If the classification is made according to the nature of the gels, they can be divided into natural gels (produced in nature) or artificial gels (produced in a laboratory).

[2] Flory, J.P. (1974). Faraday Discussions. Chemical Society, 57, 7-18.
[3] Weiss, R.G., Terech, P. (2006) Molecular gels: materials with self-assembled fibrillar networks. Springer.
[4] Sangeetha, N. M., & Maitra, U. (2005). Supramolecular gels: Functions and uses. Chemical Society Reviews, 34(10), 821–836.

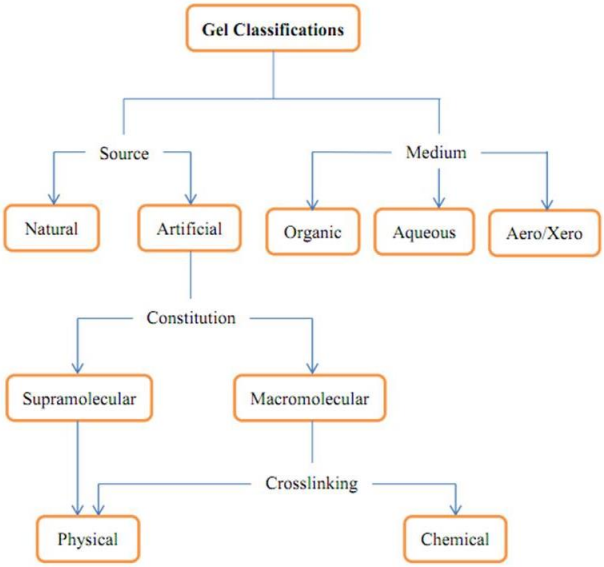


Figure 1.1 Gels classification.

Another relevant classification is according to the solvent retained inside. On the one hand, there are the organogels or organic gels and the hydrogels or aqueous gels. On the other hand, these gels can be treated by replacing or eliminating the solvent to obtain aerogels or xerogels. In the case of aerogels, the liquid phase is replaced by a gas, and the 3D structure is preserved. However, in the case of xerogels, the liquid is eliminated by evaporation, and the 3D structure commonly collapses.

Focusing on the type of structure that forms the gel, as mentioned above, they can be divided into supramolecular gels, which are made up of low molecular weight molecules (studied only in the recent decades), and macromolecular or polymeric gels which are made up of high atomic weight monomers and constitute the vast majority of gels studied and used in commercial applications.

Finally, macromolecular gels are separated into chemical and physical gels. In chemical gels, there are covalent cross-links between the polymer strands, and this results in the formation of a thermally irreversible network. On the other hand, in physical gels, the contacts in the 3D network are non-covalent, and this type of material include polymeric and supramolecular gels.

1.3 Low molecular weight gelators (LMWG)

Supramolecular gels or low molecular weight gels are those formed from low molecular weight organic molecules (with a molar mass of less than 3000 Da) that self-organize into a fibrillar-shaped network (SAFINs: Self-Assembled Fibrillar Networks).[4] The characteristic feature of these gels is that the binding of their gelling molecules occurs through non-covalent interactions, such as Van der Waals interactions, π-π interactions, hydrogen bridges, Coulomb interactions, and others.

The structures generated after its organization can be broken and formed reversibly because the union between species is not covalent. Also, solvent effects play an essential role because they modulate the solubility/aggregation balance of the gelator. The formation of molecular gels from LMWGs is a complicated phenomenon, controlled by a delicate balance of intermolecular interactions between the gelator molecules and between the gelator and the solvent.

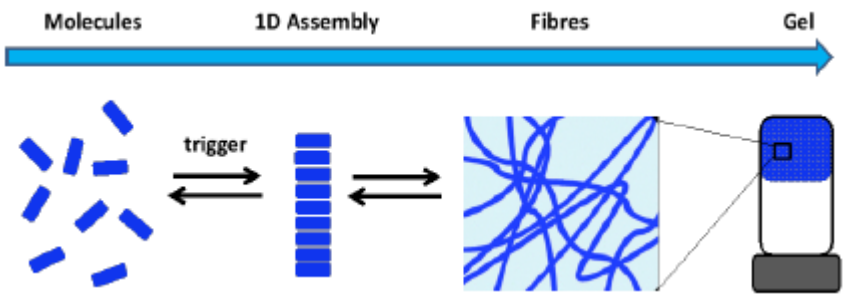


Figure 1.2 Gel formation from LMWG's self-assembly.

The most common way to form supramolecular gels is by heating the LMWG in the solvent to a solution and letting it cool down (Figure 1.2). However, the formation of molecular gels can be regulated by chemical or physical stimuli such as changes in temperature, pH, light wavelength, or oxidation state.

Supramolecular chemistry studies how more or less complex chemical species can self-assemble and self-organize thanks to the intermolecular interactions that are established between them. A prevalent and versatile type of molecule used in supramolecular chemistry is amphiphiles. They are defined as molecules with one part of their chemical structure being hydrophilic and the other hydrophobic. For

example, in the presence of water, the molecules can self-assemble because the hydrophilic component is oriented towards the water. In contrast, the hydrophobic part will try to reduce as much as possible contact with the solvent. (Figure 1.3).

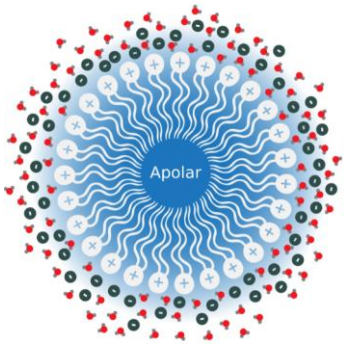


Figure 1.3 Micelle formed by amphiphilic molecules.

Surfactants are very interesting in supramolecular chemistry. They are amphiphilic molecules that reduce the surface tension that exists in a fluid. But not all amphiphilic molecules are surfactants, while all surfactants are amphiphilic. The essential property of surfactants is the critical micellar concentration (CMC), which is defined as the concentration of surfactant in a solvent above which micelles begin to form spontaneously. Above CMC, the higher the concentration, the more micelles will be created. CMC depends on the nature of the surfactant and the solvent, as well as other physical properties such as temperature.

The formation of a gel from surfactant-like molecules has been proposed to follow the mechanism outlined in Figure 1.4.[5] When the concentration of the gelator is below its CMC, the solution is full of free molecules (a). If the concentration of the gelator is increased above its CMC, intermolecular interactions provide the driving force for the molecules to self-assemble, leading to the formation of micelles (< 10 nm) (b). Over time, intermolecular interactions produce the assembly of micelles on top of each other, thus forming long fibers (c). Finally, these fibers grow and overlap or become entangled, building the three-dimensional network of the gel and trapping the solvent inside (d).

[5] Raghavan, S. R. (2009). Distinct character of surfactant gels: A smooth progression from micelles to fibrillar networks. Langmuir, 25(15), 8382–8385.

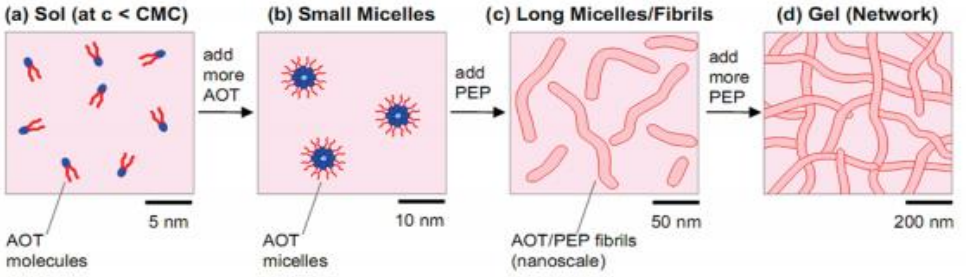


Figure 1.4 Schematic of gel formation in the case of surfactants.[5]

1.4. Parameters and characterization techniques

It is essential to define a method to differentiate when there is a gel is present. There are many ways to do this, but in this project, it is done using the Tube Inversion Methodology (Figure 1.5). It is based on turning a test tube or vial containing the sample upside down and then noting whether the sample flows under its weight.[6] Additionally, two simple parameters are widely used to define the macroscopic properties of molecular gels:

- Gel-sol transition temperature (T_{gel}). It is the temperature above which a fluid system is obtained, i.e., the self-assembled aggregates are broken. Below this temperature, gelation occurs.

- The minimum gelation concentration (MGC). It is defined as the minimum concentration of surfactant needed to form a stable gel at a given temperature.

To characterize gels, it is necessary to study their structure from the molecular scale to the macroscopic level, going through the nanometric range. A combination of different techniques such as spectroscopic, advanced microscopy, and diffraction techniques have been used to study the gels.

[6] Raghavan, S. R., & Cipriano, B. H. (2005). Molecular Gels Materials with Self-Assembled Fibrillar Network. Molecular Gels Materials with Self-Assembled Fibrillar Network, 233–244.

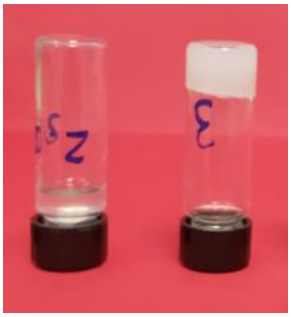


Figure 1.5 An example of Tube Inversion Methodology. There is gel in the right tube and no gel in the left tube.

1.5. Applications of supramolecular gels

Supramolecular gels have been studied and used in industry for a long time, obtaining low-cost bulk materials such as lubricants and personal products. However, it has been in the last twenty years when other types of applications have been developed. Thanks to their advantages of reversibility and biocompatibility compared to polymer gels, applications have appeared in the field of biomaterials, drug delivery, advanced materials ("smart materials") and electronic devices.

Focusing on drug delivery, this term refers to the technology used to present the drug to the desired body site for drug release and absorption, or the subsequent transport of the active ingredients across the biological membranes. The main problem of modern pharmacology is the low selectivity of drugs. Typically, only 1% of the administered dose enters the target cell. The rest of the drug is distributed throughout the body, causing side effects. The encapsulation of a medicinal substance into nanoparticles can considerably help in solving the problem of selectivity. Many strategies have been investigated and used to overcome these problems, including the formulation of drugs with delivery systems such as liposomes, micelles, emulsions, and polymers. In recent years, LMWGs have emerged as an alternative to previously reported polymeric gels. Currently there are three strategies for drug delivery using LMWGs (Figure 1.6)[7]:

- The compound can be physically entrapped within an inert gelator matrix (defined as a "scaffold") and then released from the gel via diffusion or during gel degradation (A).

- The therapeutic agent can be covalently conjugated to a functional group, creating a prodrug with amphiphilic characteristics; this will self-assemble and slowly degrade upon enzymatic cleavage (B).

- A functionalized linker can be covalently conjugated with a therapeutic to form a prodrug. The functionalized part of this structure is removed by an enzymatic cleavage to obtain an amphiphilic prodrug. Finally, these amphiphilic structures can self-assemble (C).[7]

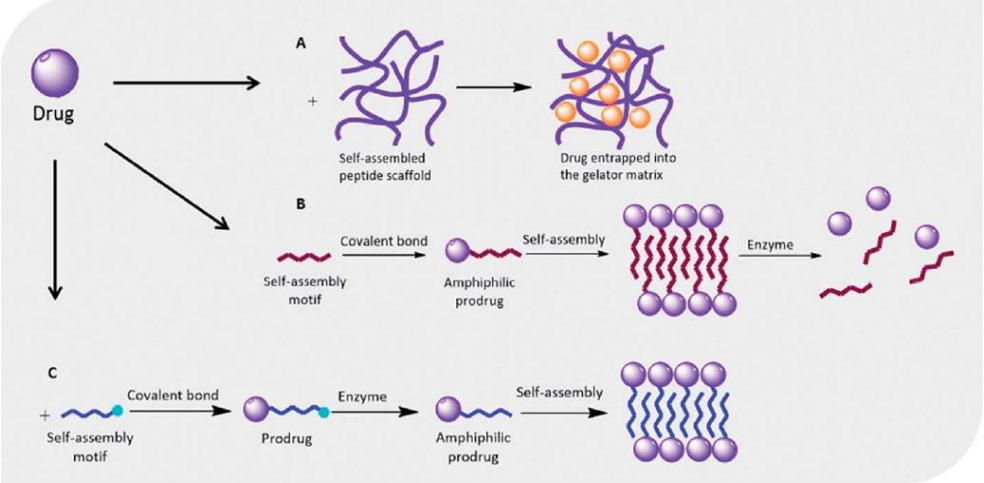


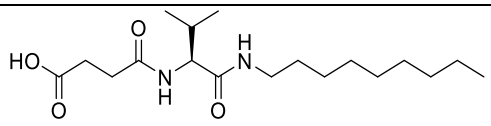
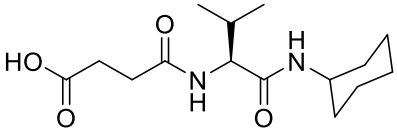
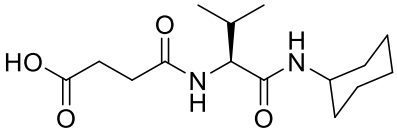
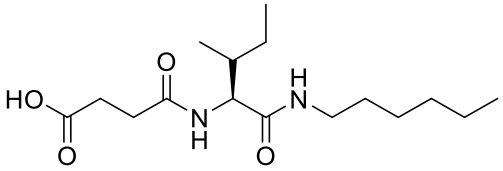
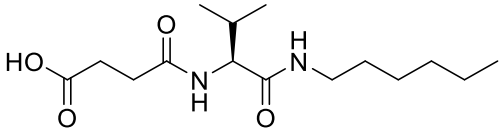
Figure 1.6 Methods of physical gelation of low molecular weight gelators.[7]

1.6. Supramolecular gels in this laboratory.

In recent years, this research group has studied a type of gelator derived from amino acids that form gels in different conditions and media. The formation of gels was investigated for the neutral, non-ionized form of gelators that contain carboxylic acid moieties. Some examples are shown in Table 1.1.

[7] Skilling, K. J., Citossi, F., Bradshaw, T. D., Ashford, M., Kellam, B., & Marlow, M. (2014). Insights into low molecular mass organic gelators: A focus on drug delivery and tissue engineering applications. Soft Matter, 10(2), 237–256.

Table 1.1 Examples of gelators and MGC of compounds required for gelation in different solvents.

Ref.	Compound	Solvent	MGC
8		water	5 mg ml^{-1}
9		water	5 mg ml^{-1}
9		CH_2Cl_2	2 mg ml^{-1}
9		water	5 mg ml^{-1}
9		$CHCl_3$	8 mg ml^{-1}

[8] Torres-Martínez, A., Angulo-Pachón, C. A., Galindo, F., Miravet, J. F. (2019). In between molecules and self-assembled fibrillar networks: Highly stable nanogel particles from a low molecular weight hydrogelator. Soft Matter. 15(17):3565-3572.

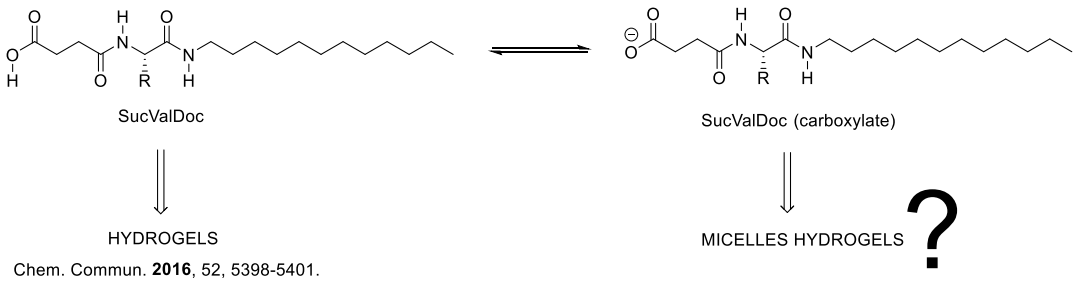
[9] Angulo-Pachón, C. A., Gascó-Catalán, C., Ojeda-Flores, J. J., & Miravet, J. F. (2016). Improved Efficiency of Molecular-Gel Formation by Adjusting Preorganization of Amino-Acid-Derived Flexible Molecules: A NMR and Thermodynamic study. ChemPhysChem, 2008–2012.

Objectives

Objectives

Molecule SucValDoc (Scheme 2.1) had been studied previously as a low molecular weight hydrogelator in the research group where this work has been carried out. SucValDoc, and related molecules, form gels in water in its neutral form.[10] However, accidentally it was found that its ionic form, carboxylate, present at basic pH values, tended to form also hydrogels. This fact is, a priori, unexpected due to the improved solubility in water of the ionic form compared to the neutral one. With this consideration mind, the following objectives were established.

1) Reproduce the synthesis and characterization of SucValDoc, using conventional organic synthesis procedures.
2) Evaluate gel formation of the carboxylate form of SucValDoc. In this study, the variables assayed will be the nature of alkaline cations present in the medium (Li^+ Na^+ or K^+) and their concentration. Also, the thermal stability of the gels will be tested.
3) Study of the crystallinity of the gels by powder X-ray diffraction.
4) Characterization of the aggregates, presumably micelles, which are precursors of the gels, by electron microscopy and dynamic light scattering.



Scheme 2.1 Pictorial representation of the main objective of the project

[10] Angulo-Pachón, C. A., Miravet, J. F. (2016). Sucrose-fueled, energy dissipative, transient formation of molecular hydrogels mediated by yeast activity. Chemical Communications, 52(31), 5398–5401.

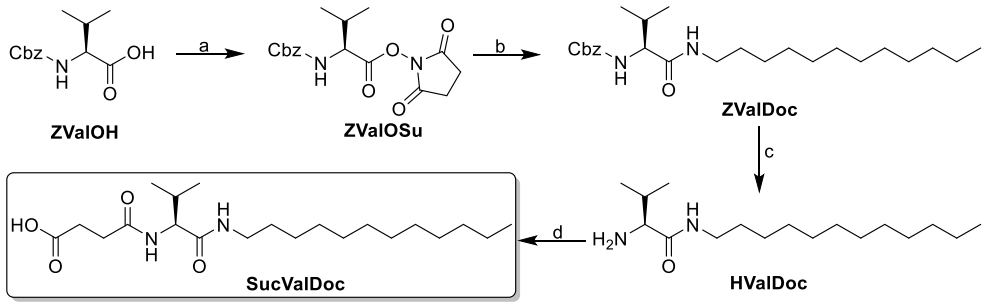
Results and Discussion

Results and Discussion

3.1 Synthesis of SucValDoc

SucValDoc was obtained by reaction of L-valine with n-dodecylamine and succinic anhydride following the procedure previously published shown in Scheme 3.1.[10] The process is divided into four steps:

- Step *a* corresponds to the activation of carboxybenzyl-L-valine with DCC and N-hydroxysuccinimide to produce the activated ester, ZValOSu.
- Step *b* involves the nucleophilic attack of n-dodecylamine to ZValOSu to form a peptide bond. In this reaction, N-hydroxysuccinimide was the leaving group.
- Step *c* consists of the carboxybenzyl group removal through hydrogenolysis, using Pd/C as a catalyst.
- Step *d* affords the final product and consists of the nucleophilic attack of HValDoc to succinic anhydride to obtain SucValDoc. The overall yield of the synthesis was 62%.



*Scheme 3.1 Reagents and conditions in the synthesis of the SucValDoc: a) DCC, N-hydroxysuccinimide, DME, 1 h., 80%; b) n-*dodecylamine*, DME, 16h., 90%; c) Pd/C, H_2, MeOH, 4h., 100%; d) Succinic anhydride, K_2CO_3, THF, 16 h., 86%.*

3.2. Evaluation of gel formation by the ionic form of SucValDoc

3.2.1. Influence alkaline cation on MGC, and T_{gel}.

The minimum concentration required to form a gel, MGC, gives a measure of how effective a gelator is. It is considered that the smaller the MGC value, the better the gelator. Usually, commercial gels are built using a concentration of gelator around 0.1 – 1% wt. The compounds that form gels at a concentration below 0.1% wt. are known as supergelators.

The gel formation studies were carried out by dissolving SucValDoc in basic aqueous medium using alkaline (Li, Na, or K) hydroxides with a final pH of ca. 12, which assures the formation of the anionic carboxylate species. The amount of alkaline cation was regulated by the addition of the corresponding chlorides.

The influence of the alkaline cations studied in MGC values and the gel melting temperature, T_{gel}, are collected in Tables 3.1, 3.2, and 3.3, as well as in Figure 3.1. As can be seen, there are significant differences in MGC and T_{gel}, depending on the cation used and its concentration. The results obtained demonstrate that when the concentration of cation is increased, MGC of gelator decreases. For example, in the case of gels formed in the presence of Li^+, Table 3.1, MGC decreases from 7.6 mM to 2.3 mM upon increasing the concentration of Li^+ from 0.2 to 1 M. The same tendency is observed for gels formed in the presence of Na^+ or K^+ (Tables 3.2 and 3.3). As for thermal stability, namely, T_{gel} values, sodium and potassium gels start disassembling at similar temperatures, ca. 30-40 ºC. The gels formed in the presence of Li^+ show a substantial increase in thermal stability, presenting a melting onset of ca. 70 ºC.

Figure 3.1 permits to visualize the data regarding MGC values graphically. It is noticeable that the MGC shows a critical dependence on the nature of the cation. Especially evident is the case of systems with a 0.4 M concentration of cation. The MGC values for systems with K^+, Na^+, and Li^+ are respectively 6, 10, and 49 mM.

Table 3.1 MGC and T_{gel} for SucValDoc gels at different concentrations of Li^+

[Li^+] (M)	MGC (mg ml^{-1})	MGC (mM)	T_{gel} (ºC)
0.2	2.91	7.6	68
0.4	2.30	6.0	68-70
0.6	1.68	4.4	70
0.8	1.49	3.9	70
1.0	0.88	2.3	70-72

Table 3.2 MGC and T_{gel} for SucValDoc gels at different concentrations of Na^+

[Na^+] (M)	MGC (mg ml^{-1})	MGC (mM)	T_{gel} (ºC)
0.2	11.2	29	35
0.4	3.85	10	35
0.6	1.50	3.9	37
0.8	1.50	3.9	37
1.0	1.50	3.9	37

Table 3.3 MGC and T_{gel} for SucValDoc gels at different concentrations of K^+

[K^+] (M)	MGC (mg ml^{-1})	MGC (mM)	T_{gel} (ºC)
0.2	No gel	No gel	No gel
0.4	19	49	33-35
0.6	14	36	37
0.8	8	21	37-39
1.0	6	16	37-39

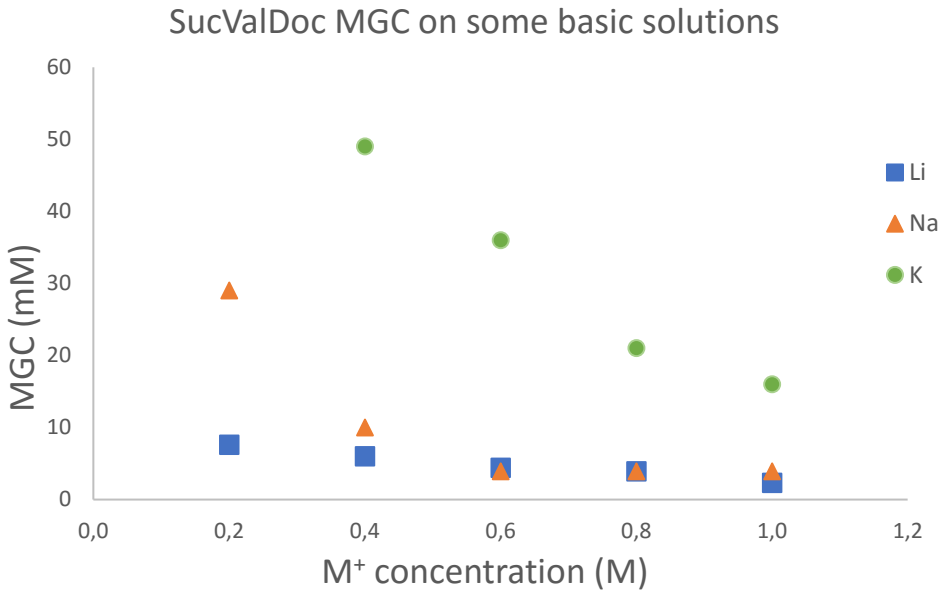


Figure 3.1 Graphical representation of MGC in front of cation concentration, pH >12

These results can be rationalized considering the tentative model proposed in Figure 3.2, which is based on the existing literature about the aggregation of surfactant type molecules.[11] In basic medium, SucValDoc behaves like a surfactant with one ionic end, the carboxylate, and a hydrophobic tail, the dodecyl group. Micelles would be formed that present a fluid like nature. The presence of alkaline cations in a sufficient concentration would provoke the formation of disk-like crystalline species that would evolve into crystalline micellar rods, namely, cylindrical micelles, that would be further transformed into an entangled network, responsible for gelation.

[11] Fuhrhop, J. H., & Helfrich, W. (1993). Fluid and Solid Fibers Made of Lipid Molecular Bilayers. Chemical Reviews, 93(4), 1565–1582.

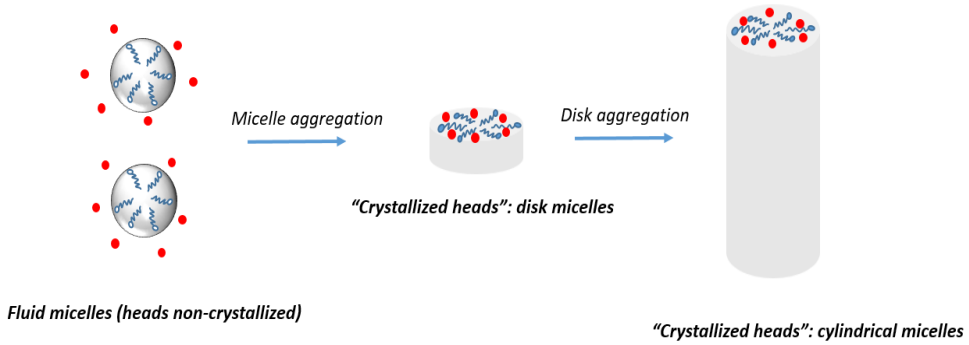


Figure 3.2 Micelles self-assembly to form disk-like crystalline species.

3.2.2. Transmission electron microscopy study of the aggregates

Transmission electron microscopy (TEM) is a powerful and unique technique for structural characterization.[12] Transmission electron microscopy is based on the emission of an electron beam onto a sample that reflects or absorbs these electrons. The image is obtained from the particles that cross the sample, with a resolution in the nanometre scale.

Images were obtained for systems containing SucValDoc at pH>12 in the presence of lithium cation (Figure 3.3 and 3.4). Noteworthy, the pictures permitted to confirm the presence of micelles in these systems, with a diameter in the range of 3-6 nm.

[12] Wang, Z. L. (2000). Transmission electron microscopy of shape-controlled nanocrystals and their assemblies. Journal of Physical Chemistry B, 104(6), 1153–1175.

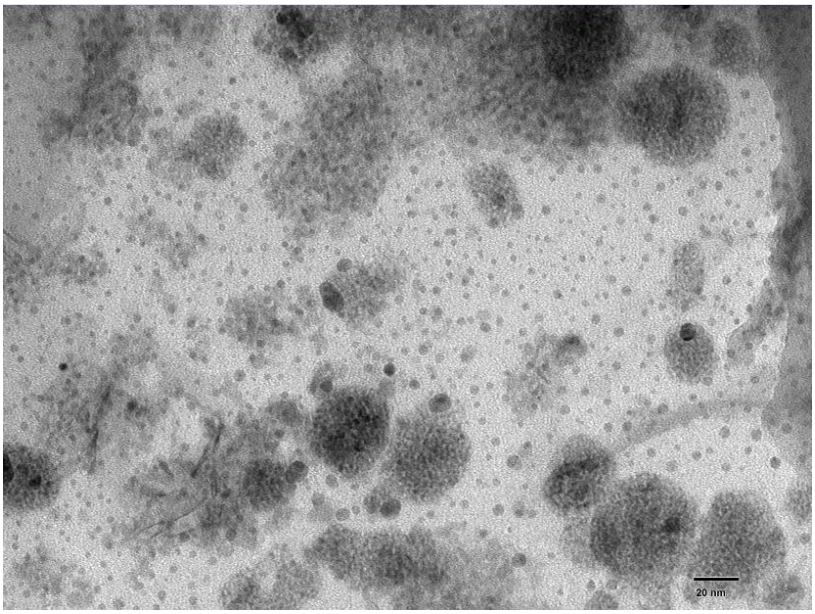


Figure 3.3 TEM image from a dried solution of SucValDoc 10 mM. Concentration of lithium 0.2M. Bar scale is 20 nm long.

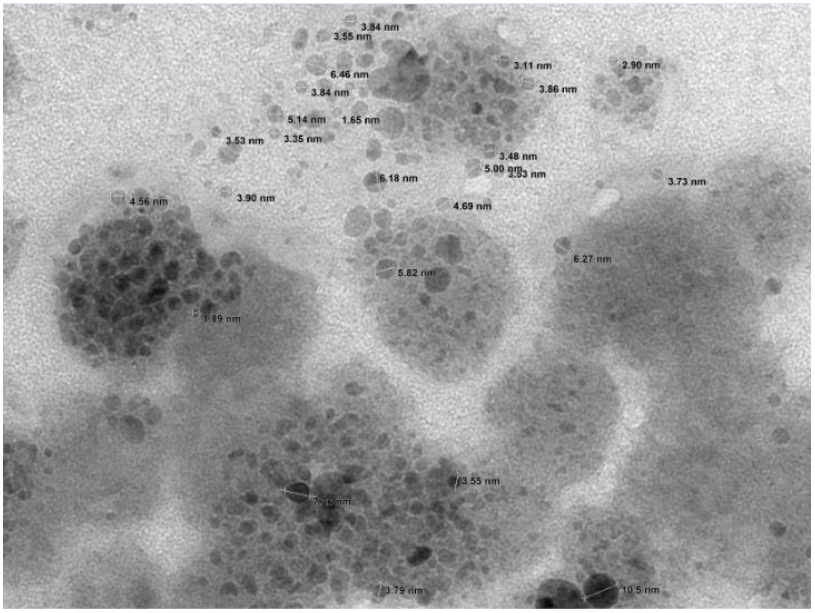


Figure 3.4 TEM image from a dried solution of SucValDoc 7.8 mM. Concentration of lithium 0.6M

Figures 3.5 and 3.6 show the fibrillar networks formed in gels that contain respectively Na^+ and K^+. They reveal the standard entangled fibrillary network, usually found in supramolecular gels. The fibers are not uniform in diameter and have values ranging from 90 to 150 nm.

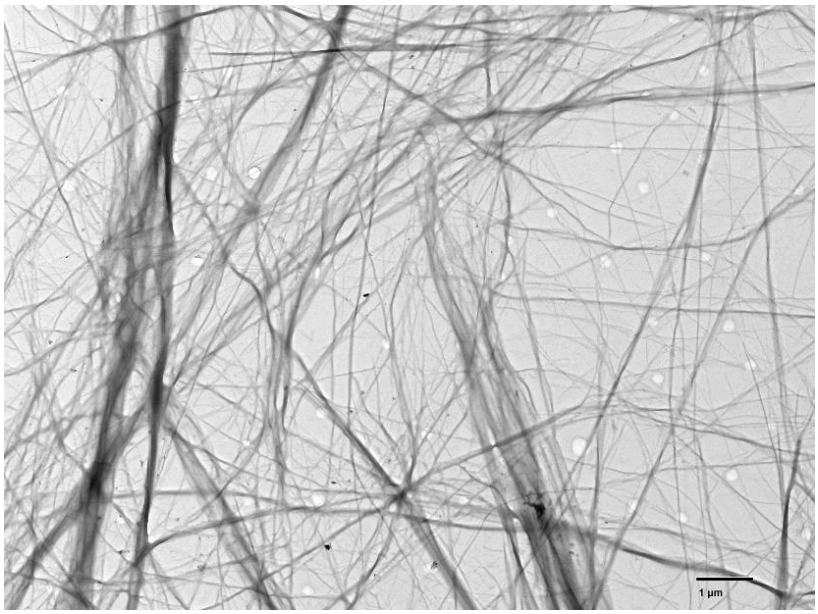


Figure 3.5 TEM image of a dried sample of SucValDoc 5.2 mM. The concentration of sodium is 0.6M. Scale bar is 1 µm long.

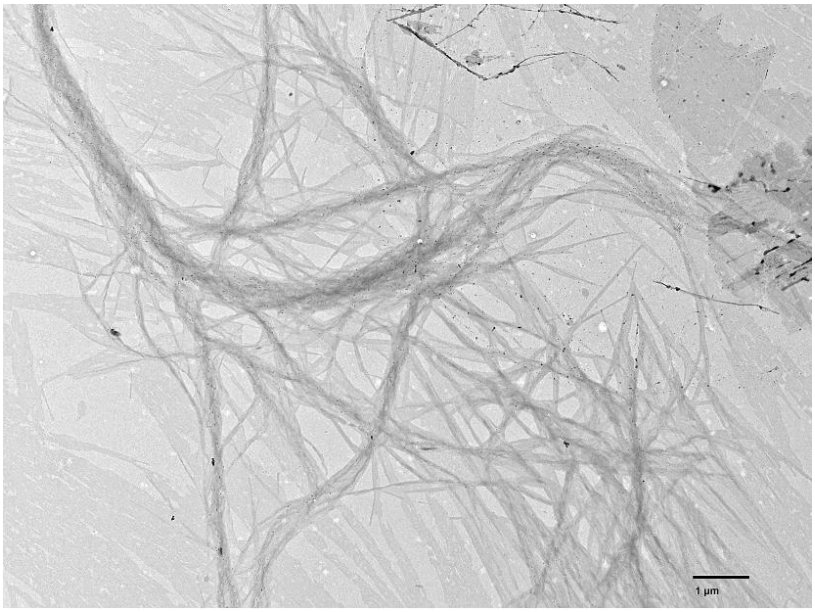


Figure 3.6 Xerogel of dissolution of SucValDoc 20.8 mM.
The concentration of potassium is 1.0M. Scale bar is 1 µm long

3.2.3 Assessment of the crystallinity of the gel fibers by powder X-ray diffraction

Another technique for analyzing the fibrillar networks of gels is powder X-ray diffraction. This method is based on Bragg's law, and it is useful for the determination of the crystalline structure of the sample. When the X-rays strike an atom, they interact with its electronic cloud, and this incident radiation is redirected in different directions with small frequency changes. This process is known as Rayleigh scattering. The radiation scattered by each atom interferes with that from adjacent atoms, causing diffraction patterns.

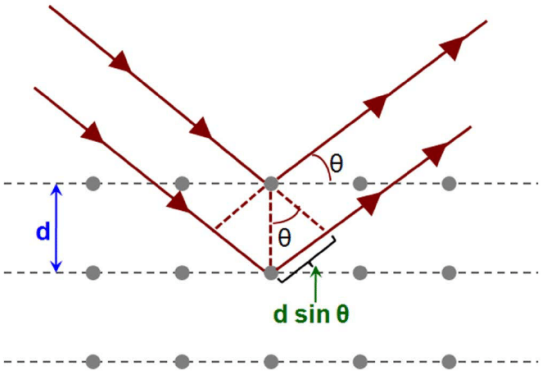


Figure 3.7 Basic scheme of diffraction.

Bragg's law is a mathematical equation described in 1913 that relates the wavelength (λ) of X-rays to the distance of the planes from the crystal lattice (d) and the angle of diffraction (θ) (Equation 3.1). Knowing the wavelength of the rays and the diffraction angles, the distance between planes can be calculated using Bragg's law.

$$n\lambda = 2\mathrm{d}\,\mathrm{sen}\,\theta$$

Equation 3.1 Bragg's law.

Figures 3.8 to 3.10 are X-ray diffraction patterns obtained from lyophilized hydrogels, respectively, formed in the presence of Li^+, Na^+, and K^+. All the samples show crystallinity being the longest interplanar distances observed, respectively, 38 Å, 36 Å, and 37 Å. In a first estimation, this distance could be ascribed to the diameter of the presumably formed cylindrical micelles. The gel formed in the presence of sodium shows three interplanar distances, related between by ½, namely, 36 Å, 18Å and 9 Å. This pattern would agree with orthogonal phases (orthorhombic, tetragonal, or cubic), presenting three 90º angles in the cell unit. Alternatively, the pattern could come from a hexagonal phase with three 120º angles in the cell unit. More diffraction peaks would be required to ascertain the crystalline system.

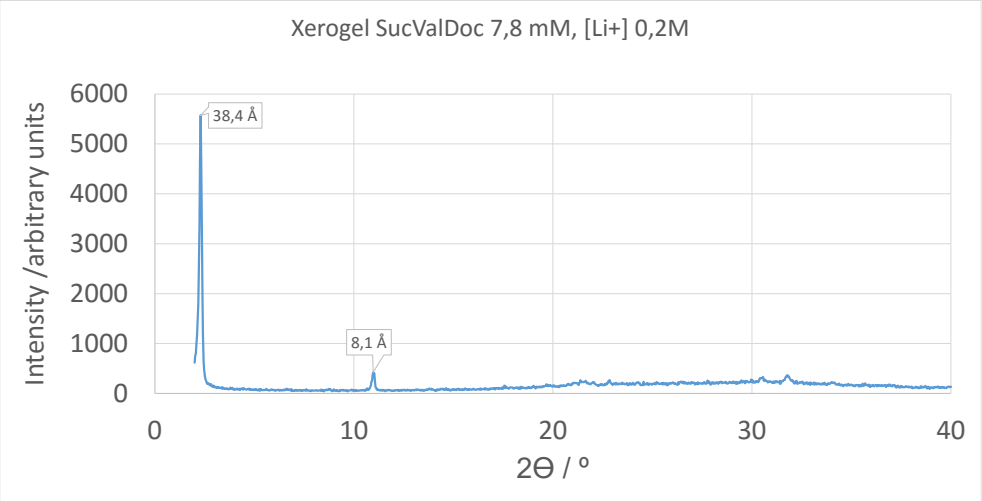


Figure 3.8 X-ray diffraction pattern obtained for xerogel of SucValDoc 7.8mM in Li^+ 0.2M.

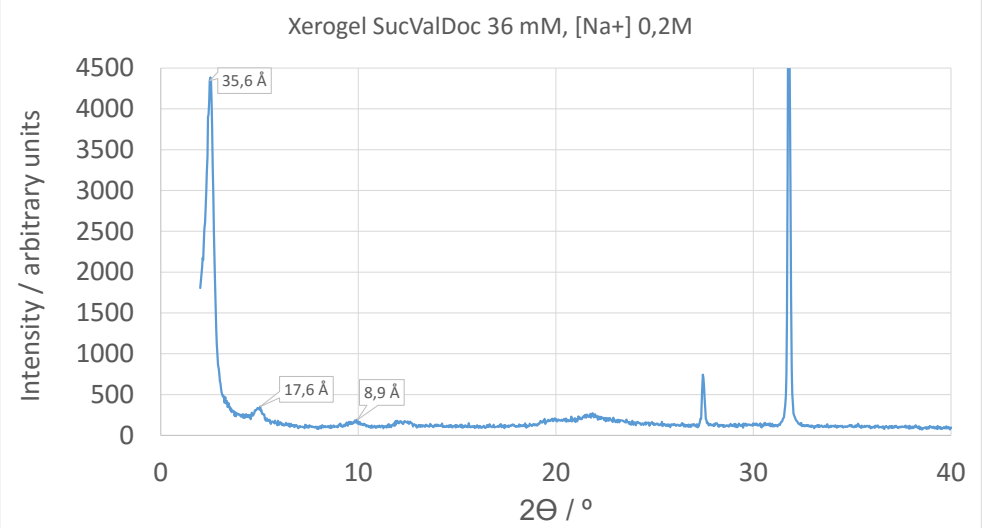


Figure 3.9 X-ray diffraction pattern obtained for xerogel of SucValDoc 36mM in Na^+ 0.2M.

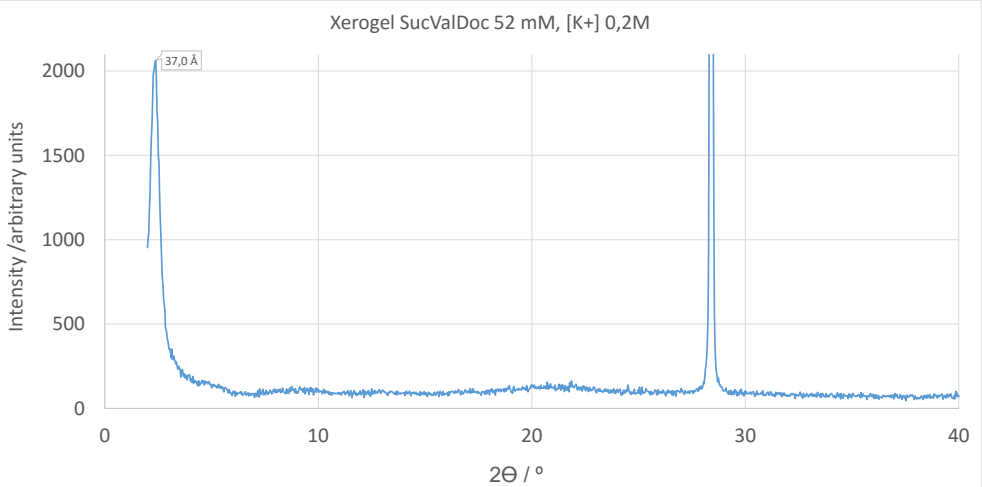


Figure 3.8 X-ray diffraction pattern obtained for xerogel of SucValDoc 52mM in K^+ 0.2M

3.2.4 Analysis by DLS of gel formation

Dynamic light scattering (DLS) is based on the Brownian motion of dispersed particles. The principle of Brownian motion is that particles are continually colliding with solvent molecules. These collisions cause a certain amount of energy to be transferred, which induces particle movement. In DLS, one measures the time dependence of the light scattered from a tiny region of the solution. A fundamental requirement is that the evolution of the particles needs to be solely based on Brownian motion.

The 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. The data can be analyzed to directly give the diffusion coefficients of the particles responsible for the scattering. The relation between the speed of the particles and the particle size is provided by the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta R_H}$$

D Translational diffusion coefficient [m²/s]
k_B Boltzmann constant [m²kg/Ks²]
T Temperature [K]
η Viscosity [Pa.s]
R_H Hydrodynamic radius [m]

Equation 3.2 Stokes-Einstein equation

Note that the diameter that is measured in DLS is the so-called hydrodynamic diameter, namely, the diameter of a sphere that has the same translational diffusion coefficient as the particle. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and is therefore known as an intensity size distribution.[13]

As the results of DLS measurements are intensity-based (the intensity fluctuations over time are detected), this is the primary weighting model displayed in a DLS software. The intensity-based distribution can be re-calculated to a number-based distribution. However, the results obtained are not the same. Intensity-based techniques show an emphasis on larger particles (they scatter more light than smaller particles). Number-based distributions will show a tendency to smaller particle fractions. It is important to note that both size distributions are just different representations of the same physical reality of distribution of differently sized particles. Consequently, the averaged diameter by intensity, Di, is bigger than the averaged diameter by number, Dn.

Four different DLS experiments were performed with SucValDoc samples in 0.05M, 0.2M, 0.5M, and 1.0M of Na^+. The count rate can be defined as the number of photons dispersed by the sample in the scattering. As can be seen in Table 3.4 and Figure 3.11, upon increasing the concentration of Na^+ larger objects are formed because the sample disperses more photons. In the range of 0.05M to 0.5M, the count rate for different temperatures is similar, but, at 1.0 M, the count rate is three times bigger than before.

Table 3.4 Count rate for different sodium concentration values (kilo counts per second). [SucValDoc] = 5.2 mM.

Na concentration (M)	30ºC	40ºC	60ºC	70ºC
0.05	487	662	523	469
0.2	776	753	590	539
0.5	427	607	742	788
1.0	3470	3068	3256	2469

[13] Malvern Panalytical Ltd. (2018). Dynamic light scattering: An introduction in 30 minutes. Tecnical note (MRK656-01). 1–8.

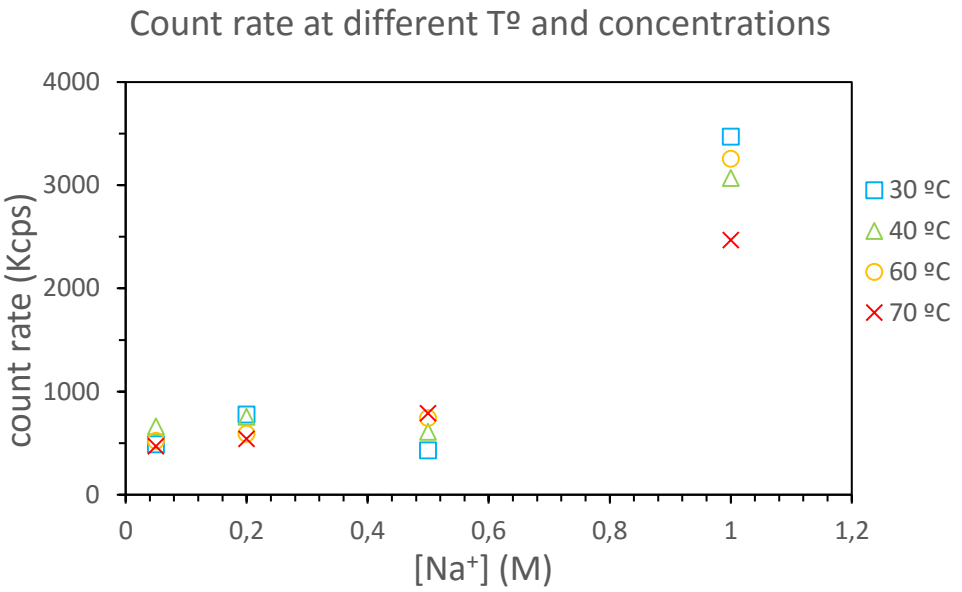


Figure 3.11 Count rate at different temperatures and concentrations of Na^+. [SucValDoc] = 5.2mM

Figure 3.12 shows an example of size distribution (Di) variation with temperature. Upon increasing the temperature, the fibrillary network of the gel breaks. Consequently, at 30 ºC, a peak of about 10 µm is observed, which disappears as the temperature increases. Finally, above 60 ºC the fibrillary network breaks down entirely, and there two peaks at 100 and 10 nm corresponding to aggregates, presumably micelles and cylindrical micelles, are observed.

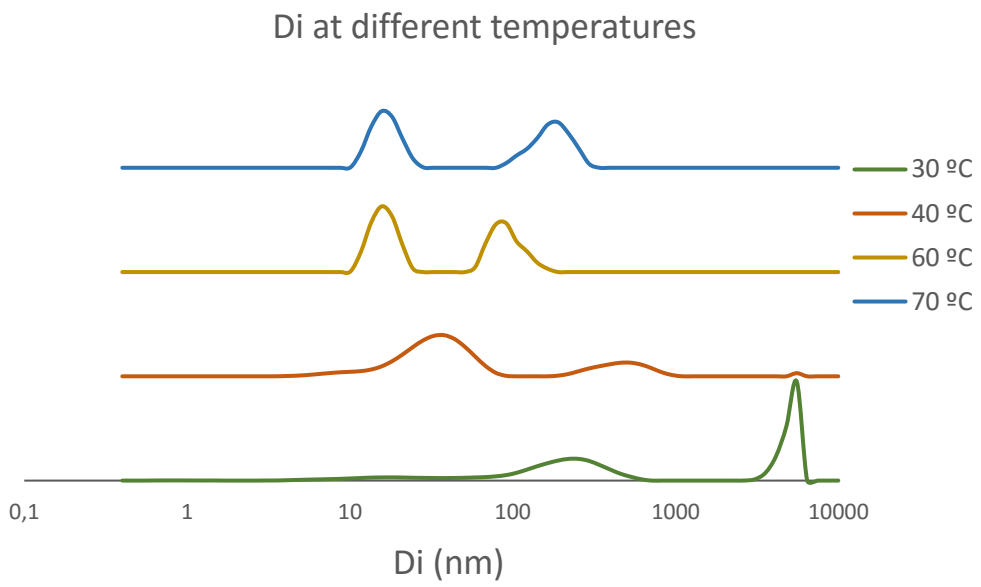


Figure 3.12 Averaged diameter by the intensity at different temperatures in 1,0M Na.). [SucValDoc] = 5.2 mM.

As can be seen in Figure 3.13, at 70 ºC, the fibrillar network is not formed (no peaks in the micrometer region), and there are only small particles of 100 nm and less than 10 nm. It seems that at these temperatures, there are micelles, whose diameter is around 5 nm, and nanotubes formed by merging of micelles with a diameter of ca. 100 nm.

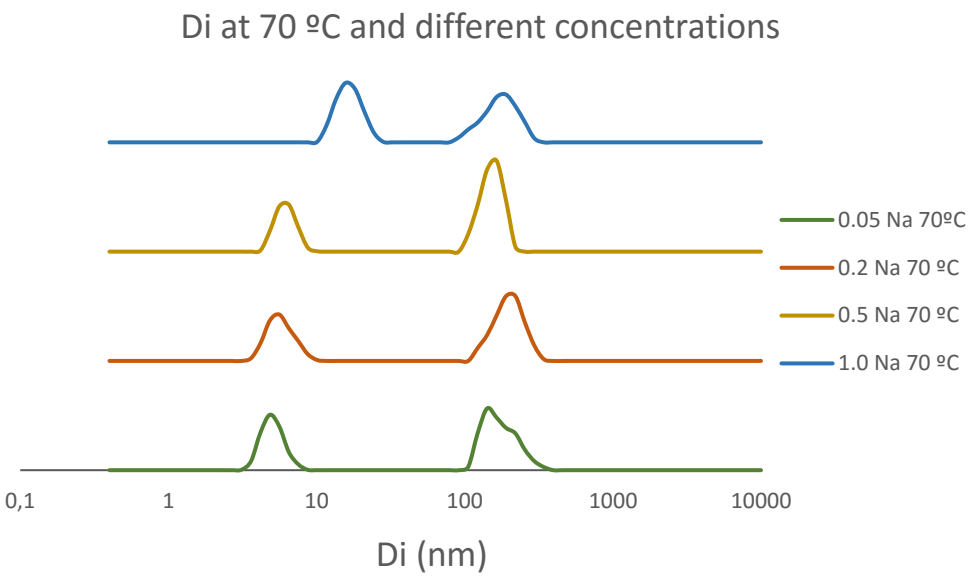


Figure 3.13 Averaged diameter by intensity at 70 ºC and different concentrations Na^+. [SucValDoc] = 5.2mM

In Figure 3.14, it is possible to see the self-assembly of gelator micelles. At low concentrations of sodium cation, the SucValDoc molecules are forming independent micelles and nanotubes with average diameters of ca. 5 nm and 100 nm, respectively. Upon increasing the concentration of Na^+, the free gelator molecules can form more micelles, and the intensity of the peak at ca. 5 nm becomes higher. Finally, at high concentrations of the cation, a gel is formed and a signal corresponding to a diameter of more than 5 µm appears.

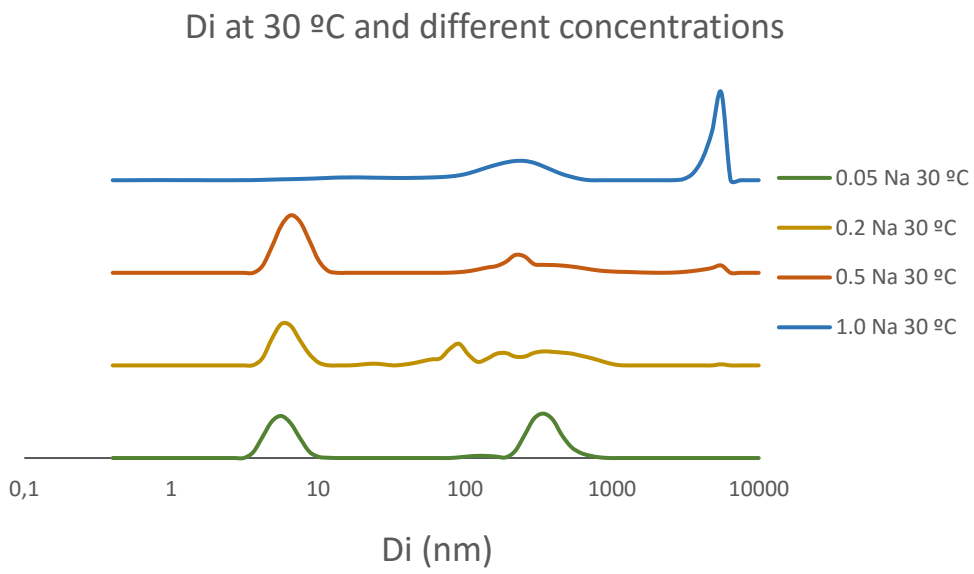


Figure 3.14 Averaged diameter by the intensity at 70 ºC and different concentrations of Na^+. [SucValDoc] = 5.2mM.

3.2.5 Gels of SucValDoc carboxylate as stimuli responsive materials

As an application of the results obtained above, the following pictures reveal how the gels are quite sensitive to the presence of cations, which can trigger gel formation/disassembly. In Figure 3.15, it is shown that switching from [K^+] of 0.6 M to 0.4 M results in gel disassembly, a property that could be used for controlled release of entrapped substances in response to changes in ionic strength. Similar behavior is detected for gel formation in the presence of Li^+ but at a considerably lower concentration of SucValDoc (26 mM vs. 5mM).

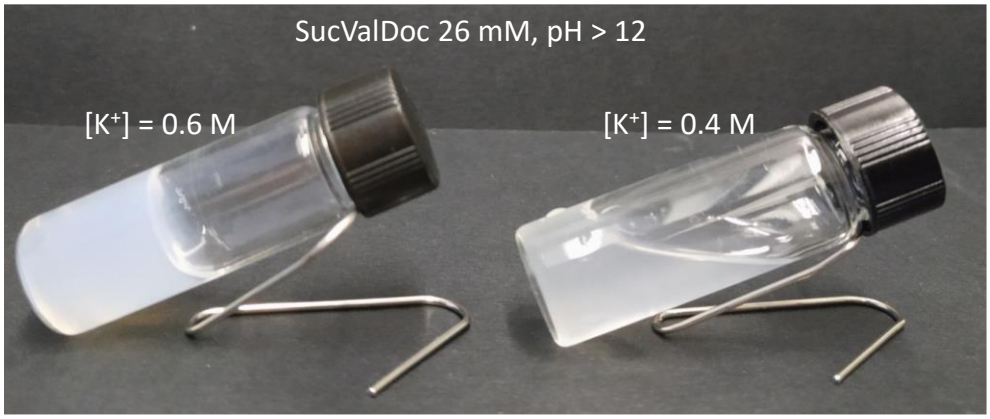


Figure 3.15 Picture of vials showing the influence of alkaline cation concentration on gel formation.

Figure 3.16 Picture of vials showing the influence of alkaline cation concentration on gel formation.

Conclusions

Conclusions

- The carboxylate form of SucValDoc is capable of forming gels in the presence of relatively high to moderate concentrations of alkaline cations Li^+, $Na^{+,}$ or K^+.

- Gel formation is favored to a different degree depending on the nature of the cation, being the order of capability of gel formation induction $Li^+ > Na^+ > K^+$.

- The gels are formed by entangled fibrillary networks observed by electron microscopy (TEM).

- The results seem to indicate that micelles, and probably cylindrical micelles, are precursors of the gel fibers. Micelles have been visualized by electron microscopy of dried samples. On the other hand, dynamic light scattering shows the existence of objects with a diameter compatible with micelles (4-6 nm) and larger entities that could potentially be associated with cylindrical micelles.

- Overall, gels are obtained, which show a strong dependence on the nature and concentration of alkaline cations, as well as on the temperature. Therefore, these systems could constitute smart, stimuli-responsive, soft materials of potential use in controlled release applications, among other areas.

Experimental Section

Experimental Section

5.1 General methods

$^{1}H/^{13}C$ NMR spectra were recorded on a Varian Unity of 500 MHz and 400 MHz in the indicated solvent at 30 ºC. Signals of the deuterated solvent ($DMSOd_6$ or $CDCl_3$) were taken as the reference in DMSO-*d6*, the singlet at 2.50 and the quadruplet centered at 39.52 ppm for ^{1}H and ^{13}C NMR, respectively, and the reference in $CDCl_3$, the singlet at 7.26 and singlet at 77.16 ppm for ^{1}H and ^{13}C NMR. ^{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, dd which means singlet, doublet, triplet, quadruplet, quintet, multiplet, broad and doublet of doublets.

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.

5.2 Transmission Electron Microscopy (TEM).

Transmission electron micrographs were obtained using a JEOL 2100 microscope with a thermionic gun LaB6 200 kV equipped with a Gatan Orius high resolution CCD camera. TEM samples were prepared over a Formvar/Carbon film on 200 mesh copper grids. Fresh gels were applied directly onto the grid and the expelled solvent was carefully removed by capillary action with paper. The grids were immediately stained with one drop of 1% aqueous phosphotungstic acid for 2 min and the liquid was subsequently removed by capillary action.

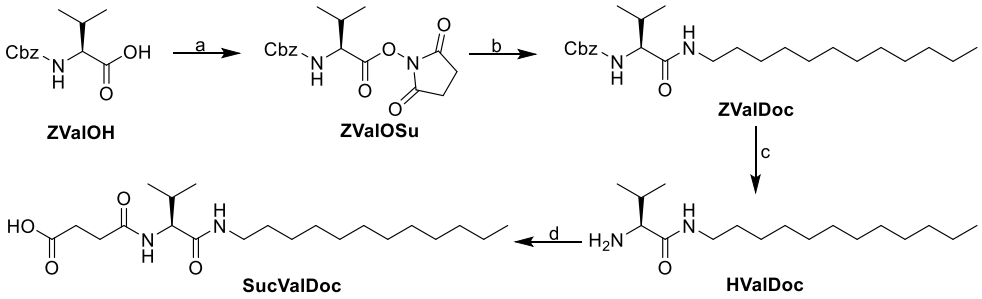
5.3 Wide-angle X-ray diffraction

Data collection was performed at room temperature with a Bruker D4 Endeavor X-ray powder diffractometer by using Cu-α radiation. A sample of the respective freeze-dried powder was placed on a sample holder and data were collected for 2θ values between 2 and 40° with a step size of 0.03° and a time step of 10 s.

5.4 Dynamic light scattering (DLS)

Size measurements of nanogel particles were performed by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern). Analyses were carried out using a He–Ne laser (633 nm) at a fixed scattering angle of 173°. Automatic optimization of beam focusing and attenuation was applied for each sample. The measurements were performed at 30°C, 40°C, 60°C and 70°C in 3 mL disposable PMMA cuvettes (10 mm optical path length). The particle size was reported as the average of three measurements.

5.5 Experimental procedure for synthesis of SucValDoc



*Scheme 5.2 Reagents and conditions in the synthesis of the SucValDoc: a) DCC, N-hydroxisuccinimide, DME, 1 h., 80%; b) n-*dodecylamine*, DME, 16h., 90%; c) Pd/C, H_2, MeOH, 4h., 100%; d) Succinic anhydride, K_2CO_3, THF, 16 h., 86%.*

5.5.1 Synthesis of ZValOSu

A solution of commercial available carbobenzyloxy-*L*-valine acid (**ZValOH**) (20 mmol) and N-hydroxysuccinimide (20 mmol, 1.0 eq.) in dry DME (50 mL) was added dropwise under N_2 at 0 ºC with a dropping funnel to a solution of *N,N'*-dicyclohexylcarbodiimide (20.2 mmol, 1.01 eq.) in dry DME (20 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 a white solid was obtained (yield 80%); the NMR spectra were consistent with those described in the literature.[14]

5.5.2 Synthesis of ZValDoc

A solution of carbobenzyloxy-*L*-valine ester activated (**ZValOSu**) (8.6 mmol) in DME (50 mL) was added dropwise under N_2 at room temperature with a dropping funnel to a solution of commercial available *n*-dodecylamine (8.6 mmol, 1 eq.) in DME (120 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 for 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 (**ZValDoc**): A white solid was obtained (yield 90%).

^{1}H NMR (300 MHz, DMSO-*d*$_6$): 7.84 (t, *J* = 5.1 Hz, 1H), 7.43 – 7.26 (m, 5H), 7.15, (d, *J* = 8.7 Hz, 1H), 5.03 (s, 2H), 3.79 (t, *J* = 7.8 Hz, 1H), 3.16 – 2.91 (m, 2H), 1.92 (sext, *J* = 6.6, 13.2 Hz, 1H), 1.45 – 1.33 (m, 2H), 1.32 – 1.17 (m, 20H), 0.84 (d, overlapped, *J* = 6.6 Hz, 9H).

^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 170.8, 156.0 (C=O), 137.1, 128.2 (x2), 127.6, 127.5 (x2) (CH), 65.3 (CH_2), 60.3 (CH), 38.3, 31.3 (CH_2), 30.2 (CH), 29.0 (x4) 28.9 (x2), 28.7, 26.3, 22.0 (CH_2), 19.1, 18.2, 13.9 (CH_3).

HR ESMS: *m/z*: calcd for $C_{25}H_{42}N_2O_3$: 441.3093; found: 441.3089 [M + Na^+].

5.5.3 Synthesis of HValDoc

Palladium catalyst (20% w/w) was suspended in MeOH (100 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of carbobenzyloxy amino compound (**ZValDoc**) in MeOH (50 mL) was added via syringe, followed by stirring under H_2 at room temperature for 4 h. The reaction mixture was then

[14] Burguete, M. I., López-Diago, L., García-España, E., Galindo, F., Luis, S. V., Miravet, J. F., & Sroczynski, D. (2003). New Efficient Procedure for the Use of Diethoxyphosphoryl as a Protecting Group in the Synthesis of Polyazamacrocycles. Preparation of Polyazacyclophanes Derived from Resorcinol. Journal of Organic Chemistry, 68(26), 10169–10171.

filtered through Celite, and the solvent was removed under reduced pressure to yield respective amine. A white solid was obtained (yield 100%).

(*S*)-2-amino-*N*-dodecyl-3-methylbutanamide (**HValDoc**) was used in crude form for the next reaction.

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 7.75 (t, *J* = 4.5 Hz, 1H), 3.13 – 2.97 (m, 2H), 2.89 (d, *J* = 5.0, 1H), 1.83 (sext, *J* = 6.0, 13.0, 19.5, 1H), 1.44 – 1.32 (m, 2H), 1.31 – 1.15 (m, 18H), 0.91 – 0.81 (m, 6H), 0.77 (d, *J* = 6.5 Hz, 3H). The amine's signals (NH_2) are very broad and cannot distinguish in the spectrum.

^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 174.2 (C=O), 60.0 (CH), 38.2, 31.6 (CH_2), 31.3 (CH), 29.2, 28.9 (x2), 28.7 (x3), 26.4, 22.1 (x2) (CH_2), 19.5, 17.1, 13.9 (CH_3).

HR ESMS: *m/z*: calcd for $C_{17}H_{36}N_2O$: 258.2906; found: 258.2908 [M + H^+].

5.5.4 Synthesis of final compound (SucValDoc)

A solution of **HValDoc** (7.7 mmol) in THF (250 mL) was treated at 0 ºC under N_2 with solid K_2CO_3 (29.4 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 (15.5 mmol, 2.0 eq.) in THF (100 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 = 2. 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.

A (S)-4-((1-(dodecylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**SucValDoc**) was obtained liked a white solid (yield 86%).

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 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). The acid signal (-COOH) is very broad and cannot distinguish in the spectrum.

^{13}C NMR (125 MHz, DMSO-*d*$_6$): 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^+].

5.6 Experimental method for determination of MGC

A stock dissolution of MOH 0.1 M (M= Li, Na or K) was prepared; the concentration of cation (M^+) was adjusted by the addition of solid MCl.

In a typical experiment, 4mg of SucValDoc and 1 mL of stock dissolution were introduced into a cylindrical screw-capped glass vial (8 mL, diameter =1.5 cm). The system was heated up with heat air to 100ºC with a heat gun. Once the solid was dissolved, the system was cooled by immersion into a water bath at 25ºC for 30 minutes. Gel formation was checked with the inverted vial test. All determinations were done by triplicate.

5.7 T_{gel} determination.

The gel to sol transition temperature was determined using cylindrical screw-capped glass vials (8 mL, diameter =1.5 cm). To control the temperature, a Digital Dry Bath with Advanced Microprocessor Control (Thermo Scientific) was used. The measures were obtained checking gel stability towards vial inversion after stabilization at the corresponding temperature for 15 min. The temperature started to 25 ºC and was incremented each step by 2 ºC. All determinations were done by triplicate.

ANNEX

Annex

6.1 NMR Spectra

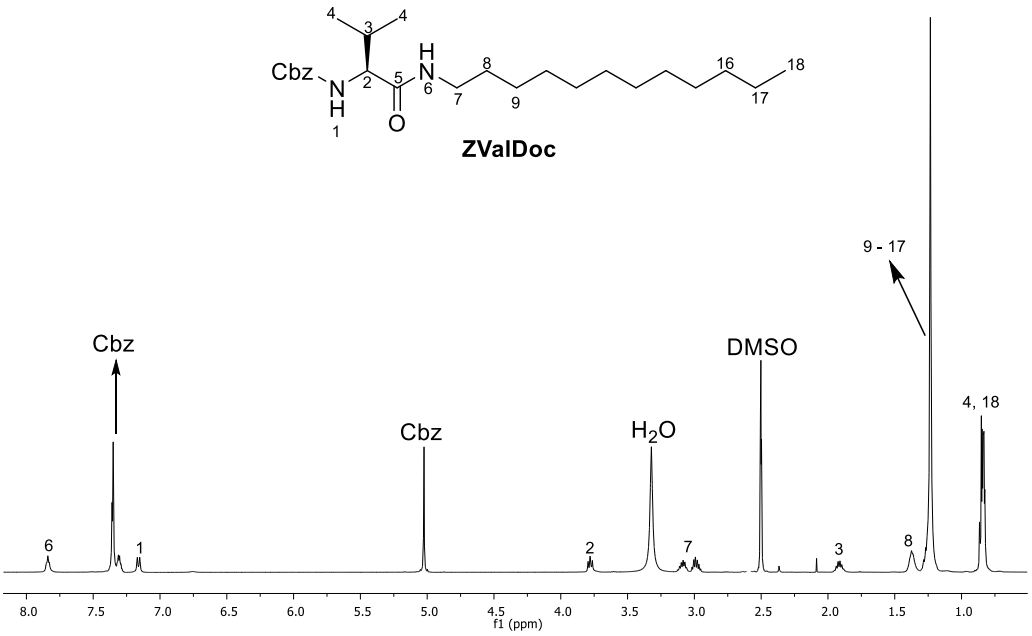


Figure 6-1. ^{1}H NMR of ZValDoc.

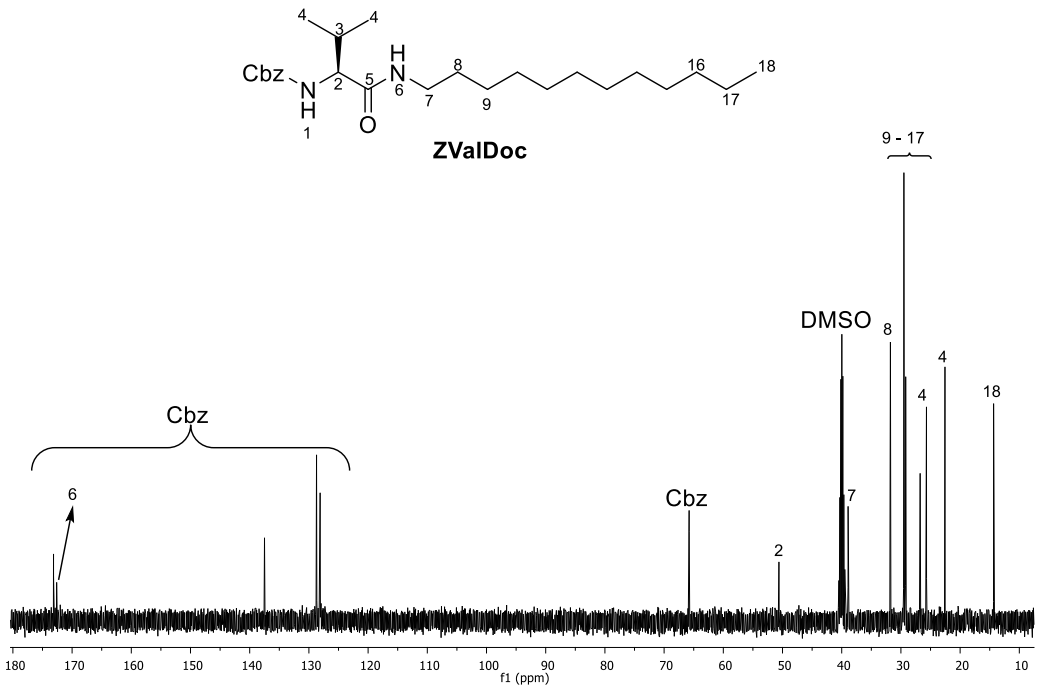


Figure 6-2. ^{13}C NMR of ZValDoc.

* The amine's signals (NH_2) are very broad and cannot distinguish in the spectrum.

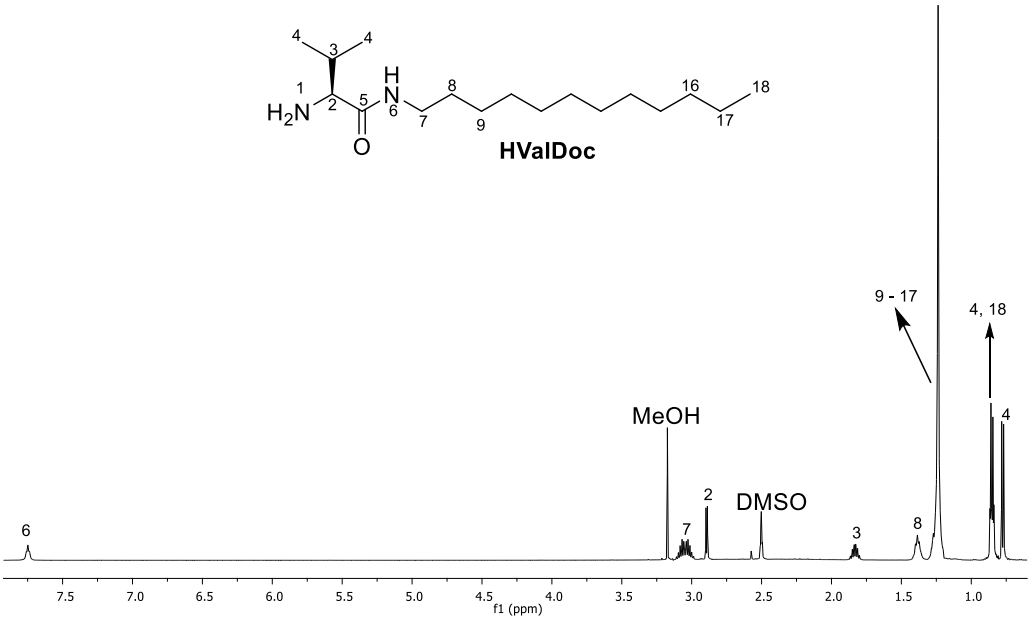


Figure 6-3. ^{1}H NMR of HValDoc.

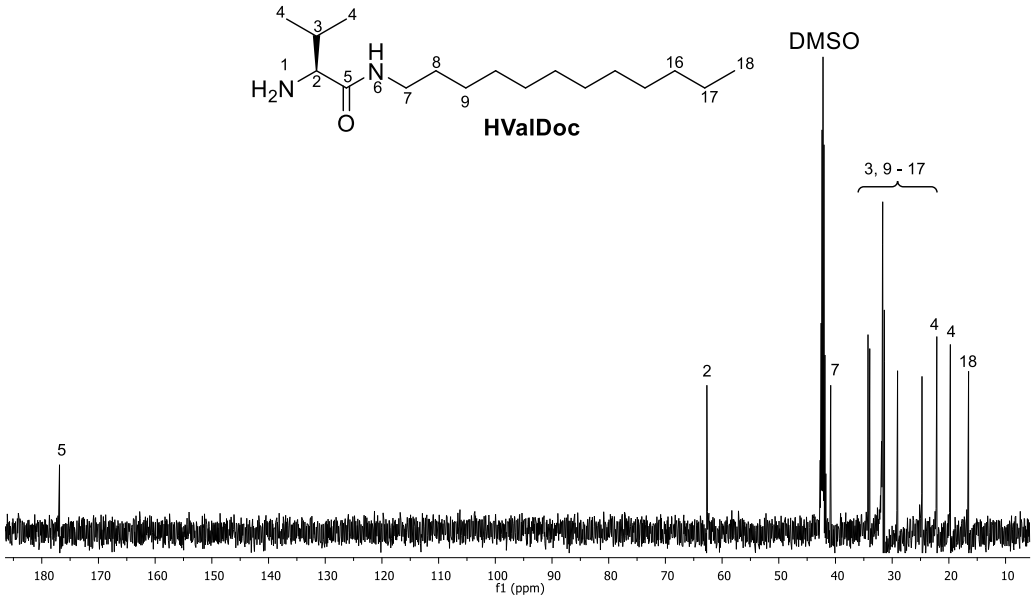


Figure 6-4. ^{13}C NMR of HValDoc.

* The acid signal (-COOH) is very broad and cannot distinguish in the spectrum.

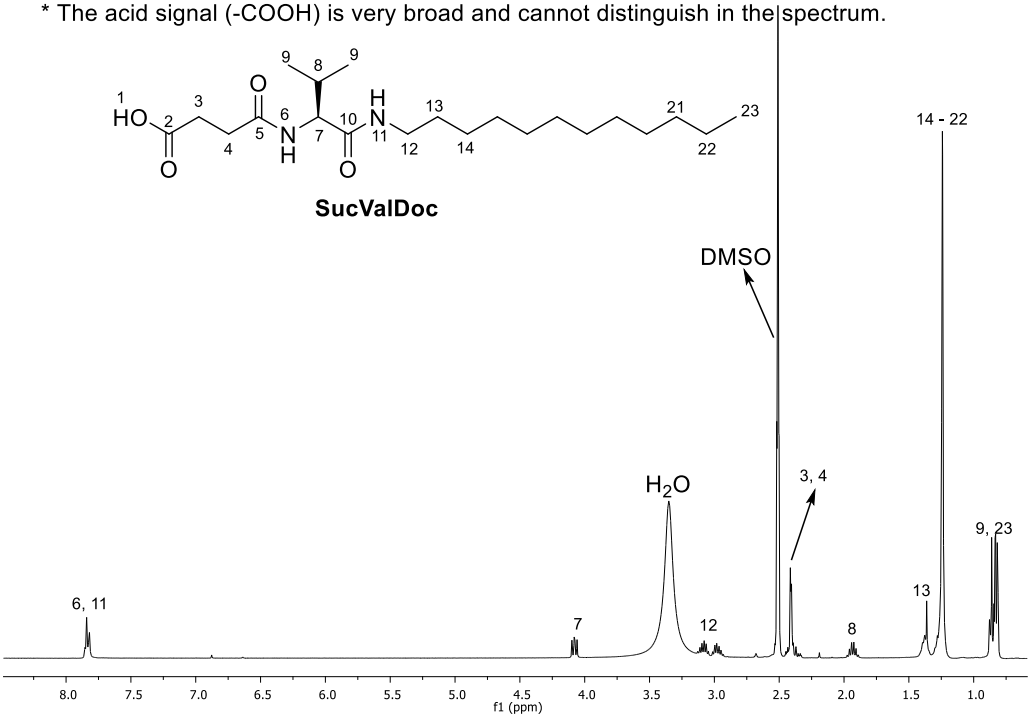
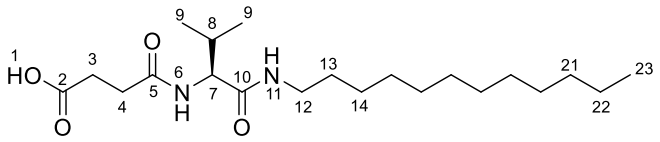


Figure 6-5. ^{1}H NMR of SucValDoc.



SucValDoc

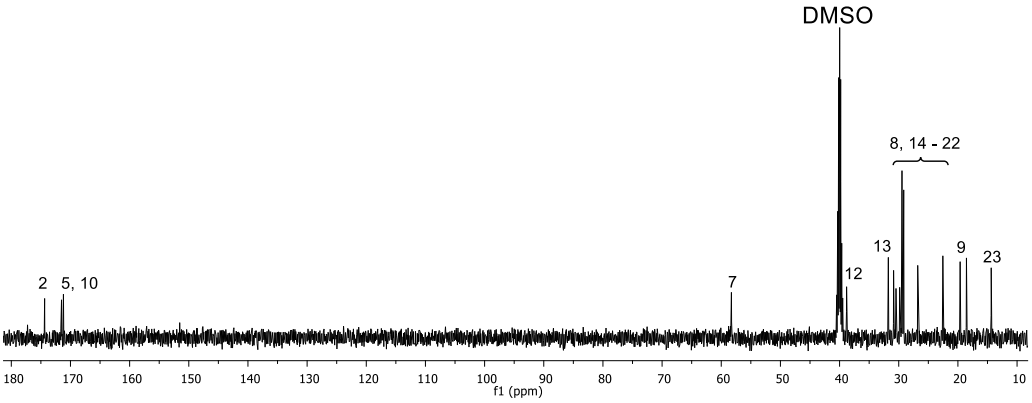


Figure 6-6.-. ^{13}C NMR of SucValDoc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