Synthesis and Characterization of New Biocompatible Amino Amphiphilic Compounds Derived from Oleic Acid as Nanovectors for Drug Delivery †

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Abstract: Amphiphilic molecules have been actively explored as promising materials in the field of bio and nanotechnology. These molecules are constituted by a polar head and a lipophilic tail and in an aqueous medium are self-assemble to form different types of macromolecular structures such as micelles, monolayer vesicles, bars, sheets and tubes. In this work, a convergent synthetic approach for the synthesis of two new amphiphilic compounds based on a versatile amino polar head, a tetraethylene glycol spacer and a lipophilic tail derived from oleic acid has been developed. Subsequently, after a self-assembly process in aqueous medium, nanostructures as micelles have been obtained and characterized. Finally, a procedure for the inclusion of the highly lipophilic drug Dexamethasone has been carried out in order to study the ability of these micelles to act as nanovectors for drug delivery.

Keywords: amphiphilic compounds; self-assembly; nanovectors; drug delivery

1. Introduction

In the last years, amphiphilic molecules have been highly used in the development of nanostructures, representing a great promise for targeted delivery, improved bioavailability, and drugs controlled release [1–7]. These molecules consist of a polar head and a lipophilic tail that are distributed in an aqueous medium to form different types of structures such as micelles, monolayer vesicles, bars, sheets and tubes [8]. The cell membrane of living cells, formed by a bilayer self-organization is the most illustrative example of a complex nanosystem formed from units of phospholipid. Among the different types of structures formed by amphiphilic compounds, micelles have received growing scientific attention [9]. Micelles, in general, are self-assembled particles composed of amphiphilic compounds [10]. In an aqueous environment, these compounds are distributed with the hydrophobic tails in the centre of the micelle, and the polar heads towards the aqueous medium. In this way, they auto-assemble to form spheroidal structures with a hydrophilic shell and a hydrophobic core to minimize the contact of the hydrophobic segments with the aqueous environment by allowing a good grade of stability [11]. CMC, critical micelle concentration, is a relatively small range of concentrations separating the limit below which virtually no micelles are detected and the limit above which virtually all additional amphiphilic molecules form micelles.
Micelles have a particle size between 10 and 100 nm that is important to allow a high stability and a high bioavailability. This size makes it possible to inject these micelles into systemic circulation without risk of blood vessel blockage. The fate in vivo of micelles depends on their sizes, particles under 200 nm are less phagocytosed by macrophages after the opsonization, compared to those with larger dimensions [12].

One of the advantages of using micelles in drug delivery is their ability to transport drugs with different degrees of polarity thanks to their structure consisting in a hydrophilic shell and a hydrophobic core.

Drugs will be distributed differently by chemical conjugation, physical entrapment or ionic interactions depending on the nature of the drug and the amphiphilic compound properties: Hydrophilic drugs will bind to the surface (case 1), those with different hydrophilicity and lipophilicity ratios will be between the polar part and the lipophilic part of the nanosystem (case 2), and finally very lipophilic drugs will be distributed inside the micelle core (case 3) (Figure 1) [9].

Figure 1. Possible pattern of drug association with a micelle [9].

It is known that about 90% of drugs are lipophilic and are characterized by a low solubility in water, this causing a difficult distribution and accumulation in fatty tissues leading to a delayed release of the drug and an increase of side effects. Micelles are therefore used for the transport of highly lipophilic drugs, increasing the solubility of drugs from 10 to 8400 times and consequently their bioavailability [13,14].

One of the most successful examples of micellar formulation as alternative solubilizing agents is the formulation of paclitaxel (PTX) in a poly (D, L-lactide) MePEG diblock copolymer which increases the solubilized PTX levels in water around 5000 times [15].

Micelles can also be used in active targeting, directing the drug towards the specific cell-tissue-organ. Ligands such as carbohydrates [16], folic acid [17], antibodies [18], proteins [19], peptides [20] and aptamers [21,22] have been used.

In summary, the main advantages of micelles in drug delivery are the following:

i) high dynamic stability that permits their application in vivo
ii) the hydrophobic core of micelles confers them the ability to transport highly lipophilic drugs
iii) the hydrophilic shell of micelles increases their solubility in water, resulting in greater bioavailability and lower toxicity for poorly water-soluble drugs.
iv) the possibility of modifying their surface with specific ligands confers the ability to direct drugs to specific targets.

This work is placed in the field of nanotechnologies applied to drug delivery and specifically focused on the synthesis of amphiphilic compounds in order to obtain a new family of micelles as drug nanovectors within the organism.

Both of these amphiphilic compounds synthesized present a versatile amino polar head, a spacer and a lipophilic tail. The spacer in all cases is tetraethylene glycol, a polymer derived from polyethylene glycol which presents two important advantages: (i) an adequate hydrophilic-hydrophobic balance for the optimal formation of the micelle and (ii) the ability to avoid the activation of the immune system. It has been discovered that PEG derivatives are biocompatible and
they are not attacked by macrophages escaping the opsonization. Furthermore, the amphiphilic compounds synthesized present oleic acid as the lipophilic tail (Scheme 1).

![Scheme 1. General structure of the amphiphilic compounds.](image)

Micelles have been obtained in water by a self-assembly process of the amphiphilic compounds synthesized. After the characterization of micelles, the internalization of Dexamethasone, a synthetic highly lipophilic anti-inflammatory corticosteroid drug [23], has been studied in order to verify the advantages of the use of our micelles in drug delivery (Scheme 2).

![Scheme 2. Formation of micelles by supramolecular self-assembly of amphiphilic compounds and internalization of Dexamethasone.](image)

## 2. Method

### 2.1. Materials and Techniques

Unless otherwise stated, the starting materials, reagents, and solvents were purchased as high-grade commercial products from Sigma-Aldrich. THF, CH₂Cl₂, DMF and Toluene were dried using molecular sieves, and highest quality solvents were used. All non-aqueous reactions were performed under an argon atmosphere in oven-dried glassware.

**Analytical TLC** was run on silica gel plates supported by alluminio Alufram® Sil.G / V245 Merck di 0.25 nm. Plates eluted and dried with 5% of phosphomolybdic acid in ethanol.

**Flash chromatography** was performed on glass column using silica gel type 60 (particle size 230–400 mesh, Merck). The composition of the eluent used is different for each compound, as indicated.

**1H- and 13C- spectra** were recorded on a Bruker AMX-500 e Bruker Advance DRX-500 (500 MHz) instrument at rt at the centre of Research, Technology and Innovation of the University of
Seville’s NMR core facility. Chemical shifts (δ) are expressed in parts per million relative to the residual solvent peak for 1H and 13C nucleus (acetone-d6: δH = 2.05, δC = 29.84; CDCl3: δH = 7.26, δC = 77.16; DMSO-d6: δH = 2.50, δC = 39.52; methanol-d4: δH = 3.31, δC = 49.00); coupling constants (J) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), sext (sextuplet), m (multiplet), app (apparent), and br (broad).

High resolution mass spectrometry (HRMS) was carried out on a Kratos MS-80-RFA spectrometer and in a AutoSpec micro-mass spectrometer at the centre of Research, Technology and Innovation of the University of Seville.

2.2. Synthesis of the Amphiphilic Derivatives

Synthesis of the First Amphiphilic Derivative

1,11-mesyl-3,6,9-trioxaundecane (1)

To a solution of tetraethylene glycol (8.90 mL, 15.49 mmol) in dry THF (200 mL) under argon atmosphere, and Et3N (17.9 mL, 128.71 mmol) was added MsCl (9.96 mL, 128.71 mmol) drop by drop at 0°C and was stirred for 1 h. After this time the mixture was allowed to warm slowly at room temperature. Then the solvent was evaporated and the mixture was dissolved in CH2Cl2 (50 mL) and washed 3 times with NH4Cl (3 × 15 mL), afterwards was neutralized with NaHCO3 (15 mL) and washed with brine (15 mL). The organic extract was dried over anhydrous Na2SO4, filtered, and evaporated to obtain the product 1 as a yellowish oil (5.42 g, 15.48 mmol, 99.97%).

Rf (CH2Cl2/MeOH 9:1): 0.62
1H NMR (500 MHz, CDCl3): δ 4.383–4.365 (m, 4H, CH2CH2OMs), 3.776–3.677 (m, 4H, CH2CH2OMs), 3.671–3.633 (m, 8H, OCH2CH2O), 3.066 (s, 6H, CH3).
13C NMR (125.7 MHz, CDCl3): δ 70.752, 70.620 (CH2O), 69.331, 69.128 (CH2CH2OSO2), 52.689 (CH2OSO2), 37.769 (OSO2CH3).
HRMS calcd for C10H22O9S2 [M + H]+: 351.0778; found 351.0778.

1,11-diazido-3,6,9-trioxaundecane (2)

To a solution of 1 (18.80 g, 53.66 mmol) in dry EtOH (35.8 mL) was added sodium azide (8.72 g, 134.15 mmol). The mixture was allowed to reflux during 24 h, under argon atmosphere and after this time, was added NaCl (50 mL) to deactivate the sodium azide. Then the solvent (EtOH) was removed by rotary evaporation. Successively the mixture was extracted with CH2Cl2 in order to obtain the product in the organic phase, and after all, was evaporated the solvent. Then the crude product was purified by flash chromatography column on silica gel with AcOEt: Hexane (1:2), to yield 2 as a yellowish oil (11.9 g, 48.71 mmol, 99%).

Rf (AcOEt/Hexane 3:1): 0.58
1H NMR (500 MHz, CDCl3): δ 3.694–3.654 (m, 4H, CH2CH2N3 and CH2CH2O), 3.386 (t, J = 5 Hz, 4H, CH2N3).
13C NMR (125.7 MHz, CDCl3): δ 70.856 (CH2O), 70.162 (CH2CH2N3), 50.846 (CH2CH2OSO2), 37.769 (OSO2CH3).
HRMS calcd for C8H16N6O3Na [M + Na]+: 267.1176; found 267.1179

11-azido-3,6,9-trioxaundecan-1-amine (3)

To a solution of 2 (18.80 g, 53.66 mmol) in dry EtOH (35.8 mL) was added sodium azide (8.72 g, 134.15 mmol). The mixture was allowed to reflux during 24 h, under argon atmosphere and after this time, was added NaCl (50 mL) to deactivate the sodium azide. Then the solvent (EtOH) was removed by rotary evaporation. Successively the mixture was extracted with CH2Cl2 in order to obtain the product in the organic phase, and after all, was evaporated the solvent. Then the crude product was purified by flash chromatography column on silica gel with AcOEt: Hexane (1:2), to yield 2 as a yellowish oil (11.9 g, 48.71 mmol, 99%).

Rf (AcOEt/Hexane 3:1): 0.58
1H NMR (500 MHz, CDCl3): δ 3.694–3.654 (m, 12H, OCH2CH2O and CH2CH2N3), 3.386 (t, J = 5 Hz, 4H, CH2N3).
13C NMR (125.7 MHz, CDCl3): δ 70.856 (CH2O), 70.162 (CH2CH2N3), 50.846 (CH2CH2N3).
HRMS calcd for C8H16N6O3Na [M + Na]+: 267.1176; found 267.1179
purified by a flash column chromatography on silica gel eluting with AcOEt: Hexane (1:1), to obtain product 3 (4.70 g, 21.52 mmol, 72%), as a colorless oil.

Rf (AcOEt/Hexane 1:1): 0

^1^H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 3.687–3.619 (m, 10H, OCH\textsubscript{2}CH\textsubscript{2}O and CH\textsubscript{2}CH\textsubscript{2}N\textsubscript{3}), 3.515 (t, \(J = 5\) Hz, 2H, CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{2}), 3.388 (t, \(J = 5\) Hz, 2H, CH\textsubscript{2}N\textsubscript{3}), 2.881–2.861 (m, 2H, CH\textsubscript{2}NH\textsubscript{2}), 1.806 (s, 2H, NH\textsubscript{2}).

^1^C NMR (125.7 MHz, CDCl\textsubscript{3}): \(\delta\) 73.387 (CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{2}), 70.851, 70.801, 70.772, 70.422, 70.162 (CH\textsubscript{2}O), 50.841 (CH\textsubscript{2}N\textsubscript{3}), 41.857 (CH\textsubscript{2}NH\textsubscript{2}).

HRMS calcd for C\textsubscript{8}H\textsubscript{18}N\textsubscript{4}O\textsubscript{3} \([M + H]^+\): 219.1452; found 219.1447

\textbf{N-(2-propin-1-yl) oleamide (4)}

A solution of propargylamine (0.15 mL, 2.34 mmol) and dimethylaminopyridine (0.066 g, 0.54 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (4 mL) was added to a solution of oleoyl chloride (0.50 g, 1.8 mmol) and diisopropylcarbodiimide (0.4 mL, 2.7 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (4 mL), under argon atmosphere and was stirred overnight. Afterwards the reaction was dissolved in CH\textsubscript{2}Cl\textsubscript{2} then was treated with HCl 4N (2 \times 7 mL), neutralized with NaHCO\textsubscript{3} (7 mL) and finally washed with brine (7 mL). The organic phase was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and the solvent evaporated to afford the crude product. To obtain the pure product 5 (0.505 g, 1.34 mmol, 57%), as a white solid, the crude was purified with column chromatography on silica gel using AcOEt: Hexane (1:5).

Rf (Hexane/AcOEt 3:1): 0.55

^1^H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 5.579 (bs, 1H, NHCO), 5.390–5.353 (m, 2H, CH=CH), 4.079 (m, 2H, HCCCH\textsubscript{2}NHCO), 2.249–2.230 (m, 1H, HCCCH\textsubscript{2}NHCO), 1.673–1.644 (m, 2H, HNCOCH\textsubscript{2}), 2.051–2.011 (m, 4H, CH\textsubscript{2}CH=CHCH\textsubscript{2}), 1.326–1.292 (m, 20H, CH\textsubscript{2}), 0.905 (t, 3H, \(J = 7\) Hz, CH\textsubscript{3}).

^1^C NMR (125.7 MHz, CDCl\textsubscript{3}): \(\delta\) 170.37 (COO), 155.67 (CONH), 79.967 (t-BuC\textsubscript{3}), 70.729, 70.683, 70.662, 70.049, 68.914, 64.361, 60.365, 53.401, 50.712, 42.249 (BocNHCH\textsubscript{2}COO), 28.313.

HRMS calcd for C\textsubscript{21}H\textsubscript{37}NONa \([M + Na]^+\): 342.2753, found 342.2767.

\textbf{[(Z)-4-Octadec-9-enoic-amidomethyl-1H-(1,2,3-Triazol-1-yl)]-3,6,9-trioxaundecan-amine (5)}

A solution of 3 (0.25 g, 0.71 mmol) and 4 (0.23 g, 0.71 mmol), in CH\textsubscript{2}Cl\textsubscript{2} (6 mL), was added to a solution of CuSO\textsubscript{4} (0.018 g, 0.11 mmol) and sodium ascorbate (0.059 g, 0.30 mmol) in water (8 mL). Afterwards, the reaction mixture was stirred vigorously over three days. Then the mixture was separated and the organic phase was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The CH\textsubscript{2}Cl\textsubscript{2} was removed by rotary evaporation to yield the crude product. The triazole derivative was isolated by flash column chromatography on silica gel, eluting with a CH\textsubscript{2}Cl\textsubscript{2}:MeOH (9:1) mixture. Thus, product 5 was obtained as a white solid (0.34 g, 0.51 mmol, 30%).

Rf (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 9:1): 0.08

^1^H NMR (500 MHz, MeOD): \(\delta\) 7.802 (s, 1H, H-triazol), 5.388–5.232 (m, 2H, CH\textsubscript{2}triazole), 4.473 (t, \(J = 6\) Hz, 2H, CH\textsubscript{2}Htriazole), 4.254 (s, 2H, NHCH\textsubscript{2}triazole), 3.796 (t, \(J = 5\) Hz, 2H, CH\textsubscript{3}triazol), 3.606–3.596 (m, 2H, CH\textsubscript{2}HN\textsubscript{3}), 3.550–3.503 (m, 8H, OCH\textsubscript{2}CH\textsubscript{2}O), 3.032 (s, 2H, CH\textsubscript{2}NH\textsubscript{2}), 2.121 (t, \(J = 7.5\) Hz, 2H, CH\textsubscript{2}CO), 1.934–1.922 (m, 4H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.7914–1.733 (m, 2H, NH\textsubscript{3}), 1.513 (m, 2H, CH\textsubscript{2}), 1.220–1.194 (m, 20H, CH\textsubscript{2} oleic acid), 0.799 (t, \(J = 7\) Hz, 3H, CH\textsubscript{3}).

^1^C NMR (125.7 MHz, MeOD): \(\delta\) 176.191 (C=O), 130.871, 130.772 (C=C), 124.903, 71.496, 71.354, 71.342, 71.224, 70.373, 68.152, 51.376, 35.965, 35.543, 33.026, 30.807, 30.5272, 30.406, 30.330, 30.298, 30.216, 28.123, 28.104, 26.916, 23.702, 14.438.

HRMS calcd for C\textsubscript{29}H\textsubscript{55}N\textsubscript{5}O\textsubscript{4} \([M + H]^+\): 538.43; found 538.43.

\textbf{(Z)-(1-azido-3,6,9-trioxaundecan)-oleamide (6)}

To a solution of 3 (0.10 g, 0.46 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (0.58 mL) under argon atmosphere, Et\textsubscript{3}N (0.042 mL, 0.56 mmol) and oleoyl chloride (0.066 mL, 0.46 mmol) were added, and the reaction was vigorously stirred for 1 day at room temperature. Then, CH\textsubscript{2}Cl\textsubscript{2} (10 mL) was added and extracted
with HCl 1N (3 × 5 mL). The organic phase was washed with a saturated aqueous NaHCO₃ solution (5 mL). The organic phase was dried over anhydrous Na₂SO₄. The solvent was evaporated and the product was purified by flash column chromatography on silica gel with CH₂Cl₂:MeOH (15:1) to yield compound 6 (0.123 g, 57 %), as a white solid.

Rf (CH₂Cl₂/MeOH 9:1): 0.60

1H NMR (500 MHz, CDCl₃): δ 5.986 (s, 1H, NHCO), 5.353–5.328 (m, 2H, CH=CH), 3.690–3.60 (m, 10H, OCH₂CH₂O and CH₂CH₂N₃), 3.459 (t, J = 5 Hz, 2H, OCH₂CH₂NHCO), 3.470–3.449 (m, 2H, CH₂CH₂NHCO), 3.168–1.609 (m, 2H, CH₂CH₂CO), 1.300–1.253 (m, 20H, CH₂ oleic acid), 0.892 (t, J = 6.5 Hz, 3H, CH₃).

13C NMR (125.7 MHz, CDCl₃): 173.380 (C=O), 130.132, 129.905 (C=C), 70.886, 70.787, 70.738, 70.399, 70.226, 70.121, 50.836, 39.294, 36.889, 32.044, 29.913, 29.837, 29.865, 29.457, 29.314, 27.365, 27.336, 25.886, 22.820, 14.249.

HRMS calcd for C₂₆H₅₀N₄O₄Na [M + Na]⁺: 505.3724; found 505.3718

1-amino-3,6,9-trioxaundecan-(Z)-9-Octadecenamide (7)

To a solution of the azide 9 (0.423 g, 0.88 mmol) in dry THF (3.83 mL), under argon atmosphere, and cooled to 0 °C, 1.75 mL (1.75 mmol) of a 1M LAH solution in THF was added. After stirring at 0 °C for 1 h, the reaction mixture was quenched with saturated Na₂SO₄ aqueous solution (0.62 mL), and stirred for 30 min, at room temperature. The white precipitate (aluminium salts) formed, was filtered over celite and washed with ether (5 × 10 mL) and then with CH₂Cl₂ (10 mL). The combined organic phases were dried over anhydrous Na₂SO₄, filtered and concentrate. The residue was purified by flash column chromatography on silica gel using CH₂Cl₂: MeOH (15:1) to yield the product 7 (0.261 g, 0.57 mmol, 65%), as a colorless oil.

Rf (CH₂Cl₂/MeOH 15:1) = 0

1H NMR (500 MHz, CDCl₃): δ 6.632 (s, 1H, NHCO), 5.350–5.325 (m, 2H, CH=CH), 3.657–3.557 (m, 10H, OCH₂CH₂O and OCH₂CH₂NH₃), 3.482–3.436 (m, 2H, CH₂CH₂NHCO), 3.036–3.031 (m, 2H, CH₂NHCO), 1.985–1.957 (m, 2H, CH₂NH₂), 1.937–1.924 (m, 2H, NH₂), 2.204–2.174 (m, 2H, CH₂CO), 2.022–1.984 (m, 4H, CH₂CH=CHCH₃), 1.634–1.605 (m, 2H, CH₂CH₂CONH), 1.298–1.266 (m, 20H, CH₂ oleic acid), 0.878 (t, J = 7 Hz, 3H, CH₃).

13C NMR (125.7 MHz, CDCl₃): δ 173.682 (C=O), 130.118, 129.906 (C=C), 70.603, 70.560, 70.303, 70.248, 39.308, 36.808, 32.036, 29.908, 29.888, 29.834, 29.791, 29.657, 29.484, 29.467, 29.444, 29.333, 27.363, 27.340, 25.930, 22.811, 14.237.

HRMS calcd for C₂₆H₅₂N₂O₄ [M + H]⁺: 457.71; found 457.40

3. Results and Discussion Section

3.1. Synthesis of the Amphiphilic Compounds

The first synthesized amphiphilic compound, 5, has a tetraethylene glycol chain as a spacer, the versatile amine group as a polar head and the oleic acid fragment as a lipophilic tail. It was obtained through a five steps sequence (Scheme 3).
Mesylation of tetaethylene glycol with mesyl chloride gave the dimesylated derivative 1, which was transformed into diazide 2 by substitution with sodium azide, and then reduced with triphenylphosphine to yield compound 3. The linking of 3 to the lipidic part of the amphiphilic compound was carried out through a Cu (I) catalyzed Huisgen reaction. With this purpose, the corresponding alkynyl derivative of oleic acid 4 was previously prepared by amidation of oleic acid with propargylamine, and the Huisgen reaction was carried out with the corresponding azide 3, obtaining the final compound 5. The presence of the amine group in 5, as a versatile polar head, may constitute a binding site to other specific functional groups as a drug, a recognition ligand or an anionic group like phosphonate.

In the case of the second synthesized amphiphilic compound 7, with a structure similar to that of compound 5, the connection between the polar head and the lipophilic chain is an amide group instead of a triazole one (Scheme 4).

The reaction between compound 3 and oleoyl chloride yielded the amide 6, and the subsequent regioselective reduction of the azide group with LAH gave compound 7, without reducing the amide function.
3.2. Preparation and Characterization Of Micelles

In all cases, the range of CMC obtained was [0.01 mM–0.08 mM] using the pyrene method [24], very similar to the CMC values of polymeric micelles [25–27].

Micelles were formed in water solutions at a concentration of 1.25 mg/mL (0.02 M in the case of compound 7) much greater than CMC. Micelle formation process was previously optimized and consists on the dispersion of the amphiphilic compound in MilliQ water. Then, the sample was ultrasonicated by a sonic tip (Digital ultrasonic sonicator Q500 of 500 watts), for 30 min. After sonication, a microfiltration process was carried out with a 30 mm membrane filter (Interlab Ltd. Customables syringe filters) in order to eliminate suspended particles.

Micelles were then characterised by DLS and TEM. Figure 2 represents two electronic transmission microscope pictures with different magnification of micelles obtained from amphiphilic compound 5 (M5). As it can be seen, micelles are monodisperse, therefore aggregates are not present, and they have sizes in a range of 50–89 nm.

![Figure 2. TEM images of M5.](image)

In addition to microscopic analysis, the sample was analysed by DLS (Dynamic light scattering) in order to determine their hydrodynamic size. In the case of M5, it was of 99.80 nm (Figure 3).

![Figure 3. DLS Analysis of M5.](image)

As can be seen, both techniques determined similar micelles size.

Figure 4 represents a TEM photograph of micelles from compound 7 (M7) with diameters between 70–120 nm and DLS results with hydrodynamic size of 40 nm.
In this case, one more time, both techniques confirm the presence of micelles.

3.3. Inclusion of Dexamethasone in Micelles

Dexamethasone (Dexa) was introduced in the synthesized micelles, in order to verify the ability of these nanocarriers to contain a highly insoluble drug. This test was performed using synthesized micelles of compound 5 (M5) and 7 (M7) (Table 1).

A procedure in 3 steps has been carried out:

(i) Addition of the solid drug (5.9 mg) directly to the previously prepared water solution of micelles and stirring 72 h at 50 °C. During this time, the sample was covered with an aluminum foil to prevent the degradation of the photosensitive drug Dexamethasone.

(ii) Centrifugation at 2000 rpm during 15 min, obtaining a precipitate, which represents the drug not included, and a solution containing the micelles with the drug inside.

(iii) Lyophilization for the elimination of water from the samples.

To confirm the inclusion of the drug in the micelle, the analysis of the samples by 1H-NMR using different deuterated solvents has been carried out and the results obtained are shown in Figure 5.
Figure 5. (a) $^1H$-NMR of dexamethasone in deuterated DMSO. (b) $^1H$-NMR of 5 in D$_2$O. (c) $^1H$-NMR of (M5) in D$_2$O. (d) $^1H$-NMR of M5 + dexamethasone in D$_2$O. (e) $^1H$-NMR of M5 + Dexamethasone in deuterated DMSO. (f) $^1H$-NMR of 7 in D$_2$O. (g) $^1H$-NMR of (M7) in D$_2$O. (h) $^1H$-NMR of M7 + dexamethasone in D$_2$O. (i) $^1H$-NMR of M7 + Dexamethasone deuterated DMSO.

$^1H$-RMN of micelles + Dexa in D$_2$O highlights only the signals corresponding to the protons of the amphiphilic compound, whereas $^1H$-NMR of micelles + Dexa in DMSO which is an organic solvent and causes the leakage of the drug, shows the proton signals corresponding to both, the amphiphilic compound and Dexamethasone. This analysis represents a further indication of the internalization of the drug (Scheme 5).

In conclusion, it is possible to considerate that the drug is located inside the hydrophobic cavity of the micelles. This is evidenced not only by a gravimetric method, but also by NMR which shows all the signals corresponding to the drug.
4. Conclusions

The goals of this experimental work were to synthesize a new family of amphiphilic compounds in order to obtain micelles as drug nanocarriers. For this purpose, we prepared two amphiphilic compounds 5 and 7, with an amine as a versatile polar head, which lead to the corresponding micelles, M5 and M7 respectively, characterized by DLS and TEM, which have different sizes and distribution in water.

In order to verify the ability of these micelles, characterized by an average size of about 100 nm, as drug transport agents, the inclusion of the highly lipophilic drug Dexamethasone into both micelles M5 and M7 was performed, containing about 54 % and 74% of the previously added drug, respectively. This represents a good percentage and demonstrates their ability to encapsulate a highly lipophilic drug in their core.

As a project in the near future, in vitro release of encapsulated Dexa studies will be performed and M5 and M7 micelles will be functionalized by exploiting the free amine groups of their amphiphilic monomers, in order to address them to a specific target.

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References
1. Babu, A.; Templeton, A.K.; Munshi, A.; Ramesh, R. Nanodrug delivery systems: A promising technology for detection, diagnosis, and treatment of cancer. AAPS PharmSciTech 2013, 15, 709–721.
2. Kapse-Mistry, S.; Govender, T.; Srivastava, R.; Yergeri, M. Nanodrug delivery in reversing multidrug resistance in cancer cells. Front. Pharmacol. 2014, 5, 159, 1–22.
3. Renugalakshmi, A.; Vinothkumar, T.S.; Kandaswamy, D. Nanodrug delivery systems in dentistry: A review on current status and future perspectives. Curr. Drug Deliv. 2011, 8, 586–594.
4. Cihan, E.; Polat, M.; Polat, H. Designing of spherical chitosan nano-shells with micellar cores for solvation and safeguarded delivery of strongly lipophilic drugs. Colloids Surf. A 2017, 529, 815–823.
5. Li, R.; Xie, Y. Nanodrug delivery systems for targeting the endogenous tumor microenvironment and simultaneously overcoming multidrug resistance properties. J. Control. Release 2017, 251, 49–67.
6. Chen, P.; Zhang, H.; Cheng, S.; Zhai, G.; Shen, C. Development of curcumin loaded nanostructured lipid carrier based thermosensitive in situ gel for dermal delivery. Colloids Surf. A 2016, 506, 356–362.
7. Wim, H.; Jong, D.; Paul, J.A. Drug delivery and nanoparticle: Applications and hazards. Int. J. Nanomed. 2008, 3, 133–149.
8. Boullanger, P. Amphiphilic carbohydrates as a tool for molecular recognition in organized systems. In Glycoscience Synthesis of Substrate Analogs and Mimetics; Driguez, H., Thiem, J., Eds.; Springer Nature: Heidelberg, Germany, 1997; pp. 275–312.
9. Alberts, B.; Johnson, A.; Lewis. J. Molecular Biology of the Cell, 4th ed.; Taylor & Francis: San Francisco, CA, USA, 2002.
10. Dewars, T.; Paetzold, E.; Oehme, G. Reactions in micellar systems. Angew. Chem. Int. Ed. 2005, 44, 7174–7199.
11. Torchilin, V.P. Structure and design of polymeric surfactant-based drug delivery systems. J. Control Release. 2001, 73, 137–172.
12. Mavossaghian, S.; Merkel, M.O.; Torchilin, P.V. Applications of polymer micelles for imaging and drug delivery. WIREs Nanomed. Nanobiotechnol. 2015, 7, 691–707.
13. Savić, R.; Eisenberg, A.; Maysinger, D. Block copolymer micelles as delivery vehicles of hydrophobic drugs: Micelle-cell interactions. J. Drug Target 2006, 14, 343–355.
14. Xu, W.; Ling, P.; Zhang, T. Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. J. Drug Deliv. 2013, 2013, 340315.
15. Burt, H.M.; Zhang, X.; Toleikis, P.; Embree, L.; Hunter, W.I. Development of copolymers of poly(d,l-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. Colloids Surf. B. Biointerfaces 1999, 16, 161–171.
16. Lundquist, J.J.; Toone, E.J. The Cluster Glycoside Effect. Chem. Rev. 2002, 102, 555–578.
17. Yang, C.; Chen, H.; Zhao, J.; Pang, X.; Xi, Y.; Zhai, G. Development of a folate-modified curcumin loaded micelle delivery system for cancer targeting. Colloids Surf. B. Biointerfaces 2014, 121, 206–213.
18. Byrne, J.D.; Betancourt, T.; Brannon-Peppas, L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv. Drug Deliv. Rev. 2008, 60, 1615–1626.
19. Nag, M.; Gajbhiye, V.; Kesharwani, P.; Jain, N. Transferrin functionalized chitosan-PEG nanoparticles for targeted delivery of paclitaxel to cancer cells. Colloids Surf. B. Biointerfaces. 2016, 148, 363–370.
20. David, A. Peptide ligand-modified nanomedicines for targeting cells at the tumor microenvironment. Adv. Drug Deliv. Rev. 2017, 119, 120–142.
21. Alibolandi, M.; Ramezani, M.; Abnous, K.; Sadeghi, F.; Atyabi, A.M.; Ahmadi, A.; Hadizadeh, F. In vitro and in vivo evaluation of therapy targeting epithelial-cell adhesion-molecule aptamers for non-small cell lung cancer. J. Control Release. 2015, 209, 88–100.
22. Ashrafuzzaman, M. Aptamers as Both Drugs and Drug-Carriers. Biomed Res Int. 2014, 2014, 1–21.
23. Gessi, S.; Merighi, S.; Borea, P.A. Glucocorticoid’s pharmacology: Past, present and future. Curr. Pharm. Des. 2010, 16, 3540–3553.
24. Basu, R.G.; Chakraborty, I.; Moulik, S.P. Pyrene absorption can be a convenient method for probing critical micellar concentration (cmc) and indexing micellar polarity. J Colloid Interface Sci. 2006, 294, 248–254.
25. Kedar, U.; Phutane, P.; Shidhaye, S.; Kadam, V. Advances in polymeric micelles for drug delivery and tumor targeting. Nanomed.: Nanotechnol. Biol. Med. 2010, 6, 714–729.
26. Gothwal, A.; Khan, I.; Gupta, U. Polymeric micelles: Recent advancements in the delivery of anticancer drugs. Pharm. Res. 2016, 33, 18–39.
27. Peer, D.; Karp, J.M.; Hong, S.; Farokhzad, O.C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2007, 2, 751–760.