Hindawi
International Journal of Polymer Science
Volume 2019, Article ID 8690145, 10 pages
https://doi.org/10.1155/2019/8690145

*Research Article*

# Solid Lipid Nanoparticles Based on L-Cysteine for Progesterone Intravaginal Delivery

**Roberta Cassano and Sonia Trombino**

*Department of Pharmacy, Health and Nutritional Sciences, Università della Calabria, Arcavacata di Rende, Cosenza, Italy*

Correspondence should be addressed to Sonia Trombino; sonia.trombino@unical.it

Received 28 September 2018; Revised 14 January 2019; Accepted 20 February 2019; Published 28 March 2019

Academic Editor: Christopher Batich



The present work has as its purpose the synthesis and characterization of a novel lipid material to be used in the preparation of solid lipid nanoparticles (SLNs) for the potential sustained release of progesterone in the vagina. For this reason, a material capable of ensuring the permanence of the formulation in the administration site for the time needed to guarantee the transmucosal absorption of the steroid was synthetized in order to reduce the number of administrations and to ensure an effective concentration of drug at the site of action. To this end, an ester, 2,3-dihydroxypropanoate of octadecyl (stearyl glycerine), containing two hydroxyl groups was initially synthesized. In particular, the hydroxyl group less sterically encumbered was functionalized with a thiol group, in a coupling reaction, with the amino acid L-cysteine. The obtained compound was characterized by FT-IR spectrometry and $^{1}$H-NMR. The functionalized lipid with L-cysteine was then used for the preparation of solid lipid nanoparticles that were loaded with progesterone. Finally, the release of progesterone from the lipid matrix based on newly synthesized ester, under conditions that simulate the vaginal physiological environment, was evaluated. All the obtained results suggest that the prepared nanoparticles could be used for the administration of progesterone, when its integration is essential, for example, in cases of threats of abortion or to increase fertility.

## 1. Introduction

The vagina, due to its anatomical position, the large surface area, the rich supply of blood, the relatively high permeability to many drugs, not only topically, is a highly effective site for the administration of drugs [1]. In particular, the vagina is an important alternative to the oral route for those systemic drugs that are badly absorbed or are rapidly metabolized by the liver [2]. However, the use of the vaginal route as a route of administration of drugs with systemic action is marginal, because the vagina is a gender organ and subject to cyclic variations. Furthermore, traditional commercial vaginal dosage formulations, such as creams, foams, gels, irrigations, and tablets, have the problem of residing in the vaginal cavity for a relatively short period due to the self-cleaning action of the vaginal tract and, usually, require more daily doses to guarantee the desired therapeutic effect [3, 4]. In this context, several approaches have been tested in the last years to develop novel vaginal drug delivery system (DDS) able to ensure controlled release to obtain long-term therapeutic drug concentration after a single dose, combining the therapeutic needs and increasing, at the same time, the compliance of the patient. Therefore, some polymers play a key role in determining the mucoadhesive ability of a dosage form [5, 6]. In particular, excellent mucoadhesive properties are typical for hydrophilic polymers possessing charged groups and/or nonionic functional groups such as polyacrylate synthesis, polycarbophil, chitosan, cellulose derivatives, hyaluronic acid derivatives, and pectin that become adhesive once activated by moistening capable of forming hydrogen bonds with mucosal surfaces [7]. Intense research has been made to increase the adhesive properties of the polymers existing and to identify new materials in order to obtain a specific release based on mucoadhesion [8].

The polymers belonging to this new generation of mucoadhesive polymers have been defined as thiomers. They are capable of forming covalent bonds with the subdomains of the cysteine-rich glycoproteins of the mucus through

the exchange reactions sulfhydryl/disulfide and/or a simple oxidation reaction [9, 10]. The strength of these covalent bonds exceeds the strength of noncovalent interactions between the polymer and the anionic substructures of the mucus layer [11].

Solid lipid nanoparticles (SLNs) are colloidal drug delivery systems able to transport lipophilic or hydrophilic molecules and can be obtained using different preparation approaches [12–21]. They are an alternative drug delivery system to other colloidal carriers, e.g., emulsions, liposomes, and polymeric nanoparticles, and very useful for the encapsulation, in particular, of drugs with poor water solubility such as progesterone [22]. This last is a lipophilic drug used to control the habitual abortion and to suppress or synchronize oestrus that can be administered orally, vaginally, rectally, or intramuscularly [23]. Its oral administration is characterized by a low bioavailability due to the first-pass effect of the liver. In fact, progesterone is absorbed in the gastrointestinal tract and undergoes rapid hepatic inactivation, which dramatically reduces the amount of progesterone pharmacologically active. Hence, in order to maintain an effective concentration in the blood serum, the multiple runs of oral dose are required [24]. To overcome this limitation, an extended and intimate contact of nanocarriers with the vaginal mucous is required; such contact can be successfully achieved using mucoadhesive polymers [25]. Therefore, the aim of the present investigation was the realization of solid lipid nanoparticles (SLNs) to favor the vaginal prolonged administration of progesterone. In particular, for the preparation of these particles, the amino acid L-cysteine and the glyceric acid, as starting substrates, were chosen. L-Cysteine has important properties; it works as a scavenger of free radicals that cause cellular damage through oxidative stress; in particular, it improves antioxidant capacity through the preservation of glutathione in inhibiting the inflammation process [26]. It also has been reported that this amino acid can regulate immune system activity [27]. But its choice is due, above all, to the presence of the thiol group -SH, that is, able to confer mucoadhesive properties to L-cysteine. In fact, literature data highlighted the improvement of mucoadhesivity of materials covalently linked to cysteine [28, 29]. Furthermore, thiolation has been found to impart other beneficial properties such as extended drug release and permeation-enhancing effect [30]. Another constituent used for the realization of SLN matrix is glyceric acid, a derivative of glycerine, a polyol compound, which has a significant positive effect on the properties of some soluble and insoluble substances [31]. It is an outstanding, versatile, and nontoxic material, with a good compatibility with many other substances. In this work, glyceric acid has been linked to 1-octadecanol (stearyl alcohol), the classic ingredient of lipid nanoparticles, and to L-cysteine. In particular, an ester, the octadecyl 2,3-dihydroxypropanoate (stearyl glycerine), containing two hydroxyl groups, was first synthesized. The hydroxyl group in position 3, less encumbered sterically than the group in position 2, was then functionalized with a thiol group by a coupling reaction with the amino acid L-cysteine to confer mucoadhesive properties to SLNs. The synthesis, involving the introduction of this amino acid, provided both protection and deprotection reactions in order to drive the coupling reaction between the protected amino acid and the synthesized ester. The obtained ester from the final synthesis was used as the lipid for the preparation of SLNs [16–22] useful as carriers of progesterone. All obtained compounds have been characterized by FT-IR and $^1$H-NMR spectrophotometry. The SLNs with and without progesterone were prepared by microemulsion technique. Finally, the release of progesterone from the newly synthesized lipid matrix has been evaluated under conditions that simulate the physiological vaginal environment.

All the obtained results suggest that the prepared nanoparticles based on L-cysteine could be used for the administration of progesterone, in all the cases in which its integration is essential.

## 2. Materials and Methods

*2.1. Reagents.* All solvents, of analytical grade, were purchased from Carlo Erba Reagents (Milan, Italy): tetrahydrofuran (THF), chloroform ($CHCl_3$), dichloromethane (DCM), n-hexane, methanol, ethanol, 1-butanol, concentrated sulfuric acid (96% *w*/*w*), and dimethyl sulfoxide (DMSO). Dry chloroform and tetrahydrofuran were obtained by distillation. 1-Octadecanol (MW: 270.49 g/mol), di-*tert*-butyl dicarbonate ($BOC_2O$) (MW: 218.25 g/mol), D-glycerate of hemicalcium monohydrate (MW: 286.25 g/mol), L-cysteine (MW: 121.16 g/mol), sodium hydrogen carbonate ($NaHCO_3$) (MW: 84.01 g/mol), dicicloesilcarbodimmide (DCC) (MW: 206.33 g/mol), dimethylaminopyridine, (DMAP) (MW: 122.17 g/mol), trifluoroacetic acid (TFA), sodium sulfate, taurodeoxycholic acid (sodium salt) (MW: 521.69 g/mol), Tween 20 (MW: 1227.54 g/mol, $d = 1.1$ g/ml), and citric acid were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA). The dialysis membranes Spectra/Por®, cutoff 12-14 kDa, manipulated before use according to the method of Fenton et al. [32], were purchased from Spectrum Laboratories Inc., Rancho Dominguez, CA, USA.

*2.2. Instruments.* The infrared spectra were carried out on KBr pellets, using a spectrometer FT-IR Perkin-Elmer 1720, in the range 4000-400 $cm^{-1}$. The dimensional analysis was performed by means of an instrument Brookhaven 90 plus particle size analyzer. The UV-Vis spectra were carried out using the JASCO-530 UV spectrophotometer. $^1$H-NMR spectra were processed by using a spectrometer Bruker VM30, and the chemical shifts are expressed in $\delta$ and referred to the solvent. The samples were freeze-dried using a "freezing-drying apparatus" Micro Modulyo, Edwards.

*2.3. Synthesis of Octadecyl 2,3-Dihydroxypropanoate.* The reaction was carried out according to the literature [33]. In a three-necked flask, equipped with the reflux condensator, dropping funnel, and magnetic stirrer, carefully flamed and maintained under an inert atmosphere, 50 ml of dry dichloromethane (DCM) was placed and added with 0.200 g ($1.887 \times 10^{-3}$ moles) of glyceric acid (2,3-dihydroxypropanoic acid), obtained by acidification of the corresponding salt of D-glyceric hemicalcium monohydrate, and 0.389 g of DCC

($1.887 \times 10^{-3}$ mol), an activating/condensing agent, which favors the formation of the ester by the elimination of a neutral molecule known as dicyclohexylurea (DCU), and 0.011 g of DMAP ($9.43 \times 10^{-5}$ moles, equivalent to 5% moles of the glyceric acid), a base that accelerates the esterification of activated DCC. The solution was left under stirring for 15 minutes at 0°C and then at room temperature until complete dissolution of DMAP and DCC. Subsequently, through the aid of the funnel dripper, 1.021 g ($3.774 \times 10^{-3}$ moles, equivalent to twice the moles of glyceric acid) of 1-octadecanol dissolved in 20 ml of dry DCM was added slowly. The reaction continued for 5 h at room temperature and under stirring. The progress of the reaction was monitored by silica TLC (thin layer chromatography) using $CHCl_3$ as eluent mixture. At the end of the reaction, the solution was left to rest for 12 hours in order to promote the precipitation of the waste product or of the dicyclohexylurea (DCU). This process has been facilitated by placing the flask containing the reaction mixture in ice. The precipitate was then removed by filtration at atmospheric pressure. The filtrate was subjected to evaporation at reduced pressure. The obtained product, octadecyl 2,3-dihydroxypropanoate (glyceryl stearate) (MW: 358.55 g/mol), was dried, weighed, and characterized by FT-IR analysis and $^1$H-NMR.

*2.4. Protection of the L-Cysteine Amine Group.* The reaction was carried out according to the reported procedure [34]. In a three-necked flask, equipped with the reflux condensator, dropping funnel, and magnetic stirrer, carefully flamed and maintained in an inert atmosphere, the mixture of solvents (40 ml) THF/$H_2O$ (1 : 1) was placed. After that, 1 g ($8.253 \times 10^{-3}$ moles) of L-cysteine and 2.8 g (0.0248 moles) of $NaHCO_3$ were added and placed under magnetic stirring. Subsequently, 2.162 g ($9.9 \times 10^{-3}$ moles) of di *tert*-butyl dicarbonate ($BOC_2O$) was added. The reaction was left under stirring for about 1 h at a temperature of 0°C using an ice bath and then overnight at room temperature. The crude reaction was treated with a 10% citric acid aqueous solution. The formation of a white precipitate which was isolated by filtration and subjected to drying with a mechanical pump was observed. The product was analyzed by FT-IR and $^1$H-NMR.

*2.5. Synthesis of (R)-2-Amine-3-mercaptopropanoate of Octadecyl 2,3-Dihydroxypropanoate.* In a three-necked flask, fitted with the reflux condensator, dropping funnel, and magnetic stirrer, thoroughly flamed and maintained in an inert atmosphere, dry THF (about 100 ml) was placed. Subsequently, the following reagents have been added: 0.185 g ($8.37 \times 10^{-4}$ mol) of L-cysteine bearing the BOC-protecting group on the amine function (PM 221,274 g/mol), 0.173 g ($8.37 \times 10^{-4}$ mol) of DCC, and 0.005 g of DMAP (5% compared to 2.3 moles of octadecyl 2,3-dihydroxypropanoate, $4.185 \times 10^{-5}$ mol). The reaction was stirred for 30 minutes at 0°C. Then, through a dropping funnel, 0.30 g of octadecyl 2,3-dihydroxypropanoate ($8.37 \times 10^{-4}$ moles), dissolved in approximately 30 ml of THF, was added. The reaction was left at room temperature for 12 hours under magnetic stirring, monitored by silica TLC and using THF as eluent mixture, until the absence of reagents in the crude reaction has been confirmed. The obtained product was subjected to evaporation at reduced pressure to remove the reaction solvent; it was dried, and then, it was washed with hot methanol to remove the DCU present. On the final product, obtained after evaporation of the solvent and drying with a mechanical pump, a FT-IR analysis and $^1$H-NMR were performed.

*2.6. Deprotection of the L-Cysteine Amine Group.* The reaction was performed according to the literature [34]. 0.363 g ($6.46 \times 10^{-4}$ moles) of (R)-2-amine-3-mercaptopropanoate of octadecyl 2,3-dihydroxypropanoate (MW: 561.8 g/mol) was subjected to deprotection reaction using trifluoroacetic acid (TFA) in dichloromethane (DCM) 1 : 1. The reaction was carried out at room temperature for about 2 hours. The mixture was evaporated at reduced pressure. The crude product was partitioned between an aqueous solution of $NaHCO_3$ and ethyl acetate. The organic solution was then dried at reduced pressure.

*2.7. Preparation of Solid Lipid Nanoparticles (SLNs).* The SLNs have been obtained by the technique of the microemulsion [16–22, 35–37]. The obtained ester, in the presence or in the absence of progesterone, was melted in a beaker at a temperature of about 70°C (temperature value higher than the melting temperature of the lipid). Meanwhile, separately and at the same temperature, a mixture consisting of Tween 20 ($d = 1.1$ g/ml), biliar salt taurodeoxycholic acid, 1-butanol ($d = 0.81$ g/ml), and distilled water was melted, to obtain a clear solution. The lipid melt was added to the clear solution to obtain a microemulsion O/A which was maintained at a temperature to avoid solidification of the lipid. This preemulsion was quickly poured into a flask containing distilled water maintained at 2-3°C using an ice bath (100 volumes/ml of the microemulsion). A dispersion was then formed which was mechanically stirred for 30 minutes. The obtained product was submitted to diafiltration, to remove unreacted components, centrifugation (10,000 rpm for 30 minutes), characterization by dimensional analysis, and freeze-drying.

Table 1 shows the amount of reagents used for the preparation of SLNs based on amino-3-mercaptopropanoate of octadecyl 2,3-dihydroxypropanoate in the absence (not loaded Cys-SLNs) or in the presence (Cys-loaded SLNs) of progesterone.

*2.7.1. Transmission Electron Microscopy (TEM).* The morphology of the SLN dispersions was examined using TEM. A drop of dispersion was applied to a carbon-coated copper grid and left for 1 min to allow some of the particles to adhere to the carbon substrate. The excess of dispersion was removed by adsorbing the drop with a piece of a filter paper. A drop of 1% phosphotungstic acid solution was applied; again, excess of solution was removed by adsorbing the liquid with the tip of a filter paper, and the sample was air-dried. The sample was then observed under a ZEISS EM 900 electron microscope at an accelerating voltage of 80 kV [16].

*2.7.2. Entrapment Efficiency Determination.* The entrapment efficiency (EE) (%) is the percentage of active substance encapsulated in SLNs expressed referring to the initial drug amount used.

Table 1: Amount of reagents used for SLN preparation.

| Reagents | Not loaded CYS-SLNS | Loaded CYS-SLNS |
|---|---|---|
| Ester (g; moles) | 0.03 g; $6.5 \times 10^{-5}$ mol | 0.03 g; $6.5 \times 10^{-5}$ mol |
| Tween 20 (g; moles) | 0.024 g; $1.95 \times 10^{-5}$ mol | 0.024 g; $1.95 \times 10^{-5}$ mol |
| 1-Butanol (g; moles) | $5.78 \times 10^{-3}$ g; $7.8 \times 10^{-5}$ mol | $5.78 \times 10^{-3}$ g; $7.8 \times 10^{-5}$ mol |
| Biliar salt (g; moles) | 0.01 g; $1.95 \times 10^{-5}$ mol | 0.01 g; $1.95 \times 10^{-5}$ mol |
| Water (g; moles) | 0.0702 g; $3.9 \times 10^{-3}$ mol | 0.0702 g; $3.9 \times 10^{-3}$ mol |
| Progesterone (g; moles) | — | 0.002 g; $6.5 \times 10^{-6}$ mol |

The EE of SLNs was calculated through a spectrophotometer UV-Vis. Briefly, the amount of unencapsulated drug in the SLNs was removed by centrifugation (at 8000 rpm for 30 min) and filtration. Successively, the obtained samples were diluted in methanol (1 : 9) and analyzed. The sample absorbance was measured using quartz cells with a thickness of 1 cm and operating at specific wavelengths of progesterone ($\lambda = 254$ nm). EE% has been calculated as follows:

$$\text{EE\%} = \frac{\text{gf}}{\text{gi}} \cdot 100, \quad (1)$$

where gi indicates the grams of progesterone initially used and gf indicates the final amount effectively entrapped into nanoparticles.

*2.8. Evaluation of Progesterone Release from SLNs.* The drug release from SLNs was assessed by placing an aliquot (1 ml) of nanoparticles containing the drug within a dialysis membrane, which, in turn, was placed in a beaker containing 20 ml of buffer solution at pH 4. The system was maintained under stirring for a predetermined time, after which the content of the membrane was analyzed by UV-Vis spectrophotometry, after being subjected to breakage in 10 ml of methanol and dissolution in 5 ml of ethanol. It was thus possible to derive the concentration of drug still present within the SLNs and to calculate, by difference, the concentration of drug released. Therefore, in order to evaluate the release profile, the content of 8 distinct membranes was analyzed; each of which has been maintained under stirring for a different number of hours (1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, and 72 h).

*2.9. In Vitro Bioadhesion Study.* The bioadhesive potential of the SLNs was evaluated by an *in vitro* method according to Nakamura et al. [38]. Briefly, an agar plate (1% *w*/*w*) was prepared in pH 4.5 citrate phosphate buffer. SLN dispersion of 50 mg was placed at the center of the plate. After 5 min, the agar plate was attached to a US Pharmacopeia disintegration test apparatus and moved up and down in pH 4.5 citrate phosphate buffer at 37 ± 1°C. The sample on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the test samples on the plate was then evaluated.

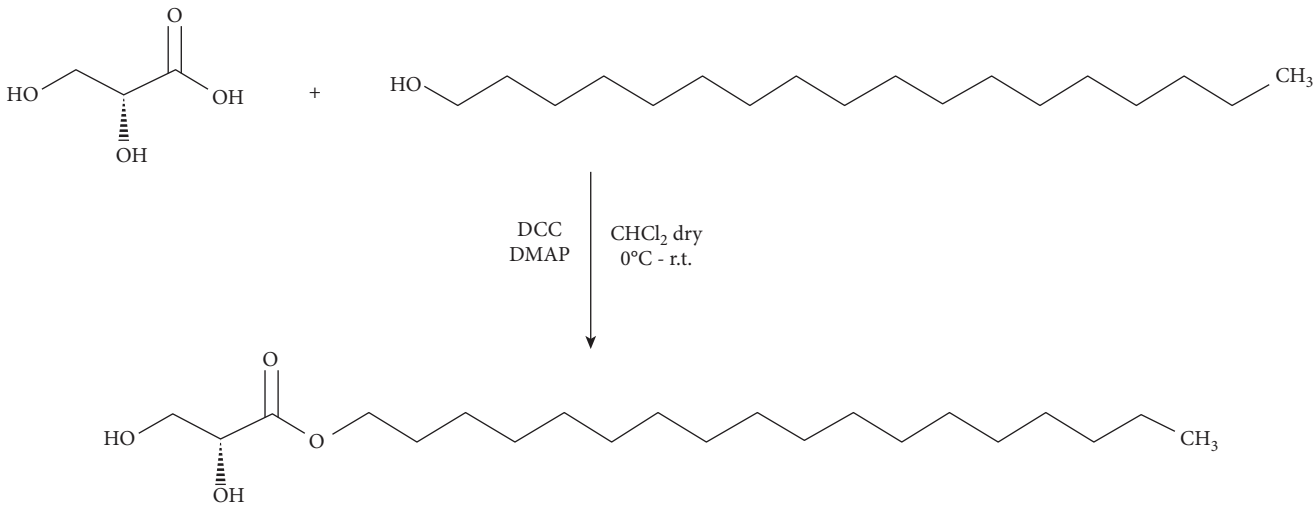
*2.10. Stability Studies.* The stability of SLNs was assessed for a period equal to 20 days. In particular, a water dispersion, containing our particles, was exposed to light and maintained at room temperature. After this period, the particles were submitted to dimensional analysis and their diameter variation was evaluated.

## 3. Results and Discussion

*3.1. Synthesis of Octadecyl 2,3-Dihydroxypropanoate.* The first objective was to synthesize a compound containing lipophilic character functions derivatized. To this end, an esterification was performed at room temperature in dry DCM between the 2,3-dihydroxypropanoic acid (glyceric acid) and 1-octadecanol (stearyl alcohol or octadecyl alcohol) in the presence of DCC as a condensing agent and N,N-dimethylaminopyridine (DMAP), which acts as a catalyst (Scheme 1).

The reaction mechanism involves the activation of 2,3-dihydroxypropanoic acid with DCC to give the O-acylisourea, a very reactive species in the acylation reaction because the dicyclohexylurea (DCU) is having a good leaving group. However, due to the rearrangement of the same O-acylisourea, it can form N-acylurea, a stable species that does not react with the alcohol. For this reaction, the addition, in catalytic amount of DMAP, which acts as a transfer agent, catalyzes the reaction by promoting the acylation of 1-octadecanol with the formation of the octadecyl 2,3-dihydroxypropanoate (stearyl glycerine). 0.558 g of the product with a yield of 82.54% was obtained, in line with expectations for this type of esterification. The ester formation was confirmed by analysis of the product by the common spectroscopic techniques. FT-IR (KBr) $\nu$ ($cm^{-1}$): 3330, 3228, 2930, 2854, and 1739. $^1$H-NMR ($CD_2Cl_2$) $\delta$ (ppm): 4.463 (1H, t), 4.174 (2H, t), 3.524 (2H, d), 1.730 (2H, tdd), 1.380 (1H, tt), 1380 (1H, tt), 1.282 (2H, tt), 1.277 (2H, hex), 1.239 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.231 (2H, tt), 1.231 (2H, tt), 1.231 (2H, quint), 1.229 (2H, quint), and 0.865 (3H, t).

*3.2. Protection of the Amine Group of L-Cysteine.* L-Cysteine or (R)-2-amino-3-mercaptopropanoic acid is a polar amino acid bearing at the side chain of a thiol group. Then after the oxidation of two thiol groups, there is the formation




Scheme 1

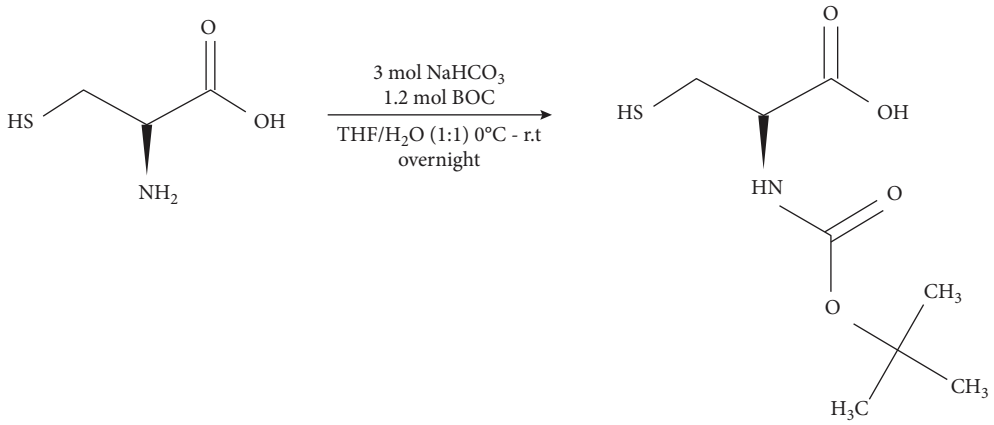
of a covalent bond, the disulfide bridge. Therefore, the L-cysteine is very convenient in the synthesis of the lipid matrix to be used as material for the preparation of SLNs and to increase the potential mucoadhesive properties of the compound. Indeed, the thiol groups of L-cysteine can form covalent bonds with the subdomains rich of the cysteine of glycoproteins of the mucus through the sulfhydryl/disulfide exchange reactions and/or through a simple oxidation reaction [5]. The strength of these covalent bonds exceeds the strength of noncovalent interactions between the material and the ionic substructures of the mucus layer. To this end, it was decided to perform an esterification reaction between the ester previously synthesized, the octadecyl 2,3-dihydroxypropanoate, and L-cysteine. However, in order to proceed to the synthesis of this compound, it was necessary to perform a reaction of the amino group protection placed in $\alpha$ to the carbonyl acid, with the aim to inhibit the reactivity and to obtain our product. Indeed, the nucleophilicity of the amino group could cause the formation of secondary compounds, lowering the yield of the reaction. For this reason, the -$NH_2$ group was protected with *tert*-butyl dicarbonate ($BOC_2O$), a protecting group, capable of silencing the amino group in $\alpha$ to the carbonyl and transforming it into an amide (Scheme 2). In this way, the carboxyl group has been made reactive for the subsequent esterification reaction with the hydroxyl group in $\beta$ to the carbonyl of the octadecyl 2,3-dihydroxypropanoate. The product was obtained following the procedure described in the literature [29]. The workup was carried out by acidification, treating the crude reaction with 10% citric acid. This allowed reprotonating the thiol group and the carboxylic acid, thus favoring the precipitation and isolation of BOC-L-cysteine produced. The obtained product was dried and weighed. 1.17 g of L-cysteine BOC-protected was obtained. The reaction yield was of 63.93%. The product was analyzed by FT-IR and $^1$H-NMR. FT-IR (KBr) $\nu$ ($cm^{-1}$): 3498, 3396, 2982, 2931, 2660, 1718, and 1688. $^1$H-NMR ($D_2O$) $\delta$ (ppm): 3.003 (2H, d), 4.518 (1H, t), 1.427 (3H), 1.427 (3H), and 1.427 (3H).

*3.3. Synthesis of (R)-2-Amino-3-mercaptopropanoate of Octadecyl 2,3-Dihydroxypropanoate.* To obtain the ester to be used for the preparation of the nanoparticles, the reaction of esterification of the carboxyl group of L-cysteine, previously protected with BOC on the amino group (BOC-L-Cys), with the hydroxyl group in $\beta$ to the carbonyl of octadecyl 2,3-dihydroxypropanoate, was carried out.

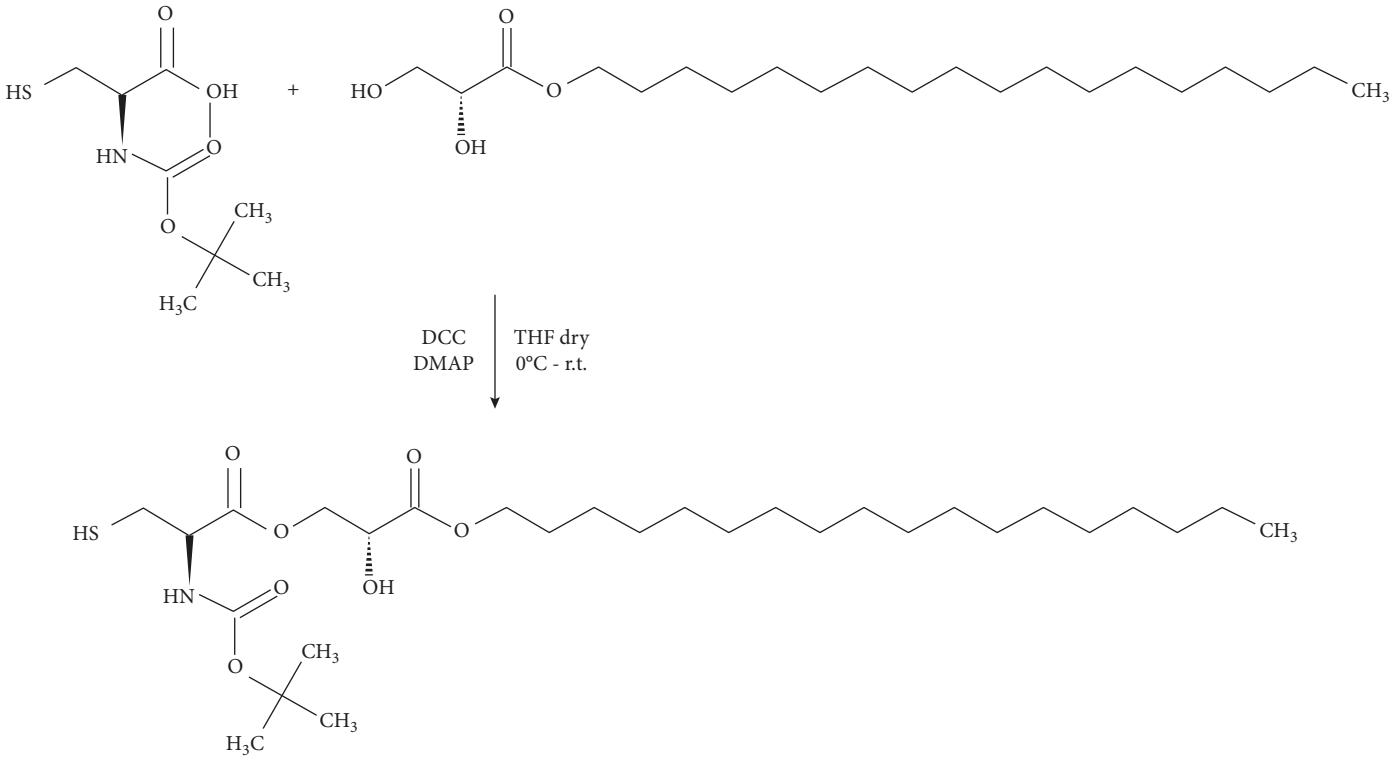
The reaction was carried out in an inert atmosphere, at reflux for 12 hours, in THF, maintaining a stoichiometric ratio of BOC-L-Cys/2,3-dihydroxypropanoate of octadecyl 1/1 (Scheme 3). To facilitate the formation of the new ester bond, the DCC was used, a condensing agent, which, interacting with the carboxyl group of BOC-L-Cys, increases the electrophilicity of the carbonyl carbon. Moreover, the addition of DMAP (5% in mol) accelerates the reaction. In fact, it acts as a nucleophilic catalyst, deprotonate the hydroxyl group of the ester, and/or the transfer agent acyl group. The nucleophilic attack of the alkoxide ion to the ester carbonyl activated, causes the release of a highly stable group, the DCU, which is removed by washing with hot methanol. After this, reaction of 0.363 g of the product was obtained with a yield of 77.23%. The final product was analyzed by FT-IR and $^1$H-NMR.

FT-IR (KBr) $\nu$ ($cm^{-1}$): 3525-3231, 2954, 2850, 2666, 1771, 1760, and 1720. $^1$HNMR ($D_2O$) $\delta$ (ppm): 4.615 (1H, t), 4.551 (1H, t), 4.503 (2H, d), 2.989 (2H, d), 2.431 (2H, t), 1.537 (2H, tt), 1.425 (3H), 1.425 (3H), 1.425 (3H), 1.277 (2H, hex), 1262 (2H, tt), 1.259 (2H, quint), 1.239 (2H, quint), 1.232 (2H, tt), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.231 (2H, quint), 1231 (2H, tt), 1.229 (2H, quint), and 0.865 (3H, t).

*3.4. Deprotection of the Amine Group.* In order to get the final ester or an ester with functionalized long chain with L-cysteine, it was necessary to deprotect the amine group in $\alpha$ to the carbonyl of the cysteine (Scheme 4). The BOC fleeting in acid medium was removed with trifluoroacetic acid (TFA) in




Scheme 2

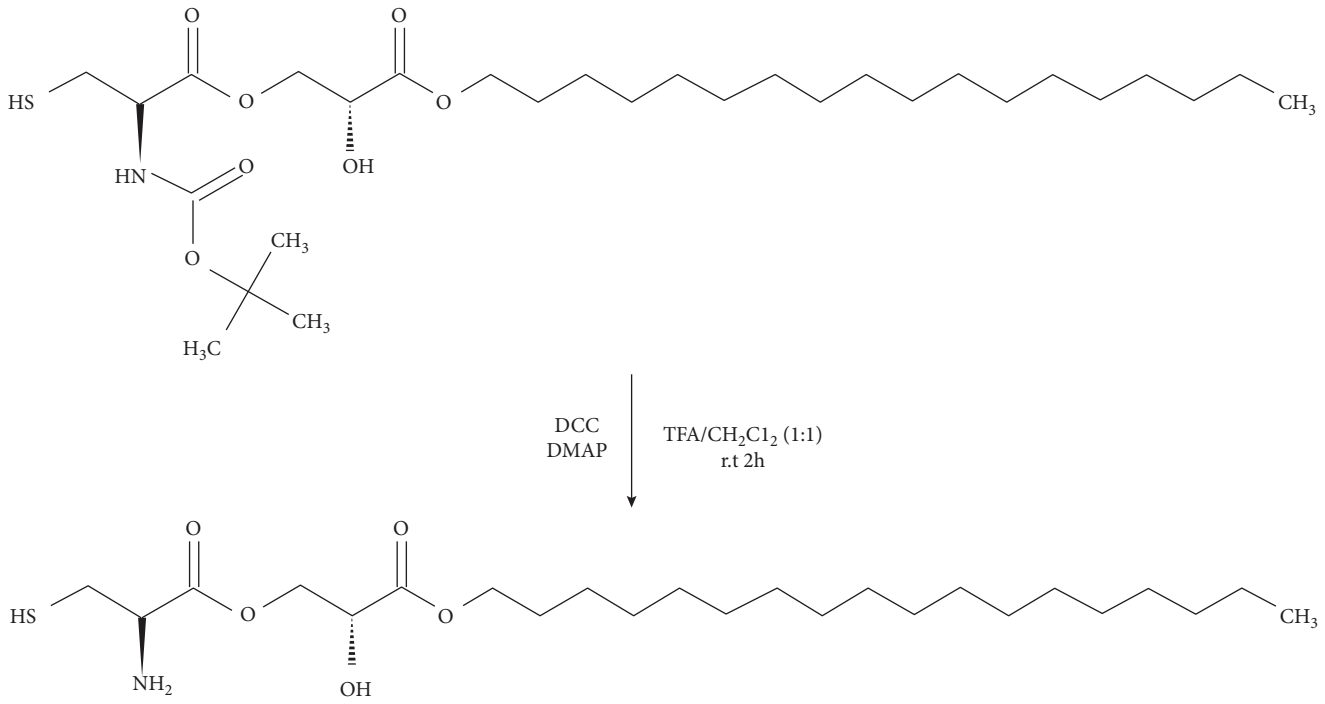



Scheme 3

dichloromethane (DCM) with ratio 1 : 1. Its lability in acidic environment depends on the fact that there are two good leaving groups: $CO_2$, which escapes as a gas equilibrium, and the *tert*-butyl cation that is relatively stable in an acidic environment. This, in a second time, can give an E1 elimination forming 2-methylpropene or can give a SN1 substitution with trifluoroacetic acid to form *tert*-butyl trifluoroacetate.

The final product was analyzed by common spectroscopic techniques: FT-IR (KBr) $\nu$ ($cm^{-1}$): 3335, 3238, 2967, 2849, 2665, 1780, and 1713. $^1$HNMR ($CD_2Cl_2$) $\delta$ (ppm): 4.680 (1H, t), 4.415 (2H, d), 4.175 (2H, t), 3.593 (1H, t), 2.708 (2H, d), 1.730 (2H, tt), 1.380 (2H, tt), 1.282 (2H, tt), 1.277 (2H, hex), 1.239 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.232 (2H, quint), 1.231 (2H, tt), 1.231 (2H, tt), 1.231 (2H, quint), 1.229 (2H, quint), and 0.865 (3H, t). The product of our interest (0.250 g) was obtained with a yield of 83.89%.

*3.5. Preparation of Solid Lipid Nanoparticles.* The SLNs based on 2-(R)-2,3-dihydroxypropanoate of octadecyl 2,3-dihydroxypropanoate in the presence or in the absence of progesterone have been prepared with success through the microemulsion technique in a temperature range between




Scheme 4

approximately 60°C and 80°C. The formation of the nanoparticles occurred due to the rapid dispersion of the preemulsion warmed in cold water (2°C) under mechanical stirring.

*3.6. Morphology.* Photomicrograph of not loaded Cys-SLNs, visualized by TEM, showed in Figure 1, revealed that the nanoparticles were spherical in shape and their size is ~743 nm.

*3.7. Determination of Entrapment Efficiency (EE%).* The progesterone entrapment efficiency (EE%) within the SLNs was calculated using the equation (1), after the SLN solubilization in ethanol and consequent spectrophotometric analysis of the filtered and not diafiltered sample.

Table 2 shows the value of the molar extinction coefficient ($\varepsilon$), relative to progesterone obtained through the construction of a calibration line of the same drug in ethanol, and the values of absorbance at 254 nm relative to SLN diafiltered and not.

The entrapment efficiency calculated is equal to 54.94%. This is a satisfactory result, in line with expectations. In fact, SLNs, due to their lipid nature, lend themselves well to the load of lipophilic active ingredients such as progesterone.

*3.8. Dimensional Analysis of the SLNs.* Dimensional analysis performed by dynamic light scattering has allowed determining the average diameter of the synthesized nanoparticles and their polydispersity index (PI), as shown in Table 3. In particular, for the SLNs based on 2-(R)-2,3-dihydroxypropanoate of octadecyl 2,3-dihydroxypropanoate, called "not loaded Cys-SLNs," the value of the diameter was equal to 743.8 nm, while for the Cys-SLNs loaded with

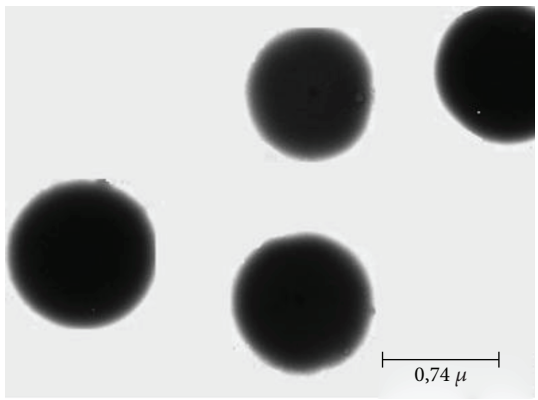



Figure 1: Photomicrography of Cys-SLNs.

Table 2: Absorbance values.

| Molar extinction coefficient $\varepsilon$ ($L \cdot mol^{-1} \cdot cm^{-1}$) | Absorbance (diafiltrate SLNs) | Absorbance (not diafiltrate SLNs) |
|---|---|---|
| 3948.6 | 0.400 | 0.728 |

Table 3: Dimensions and polydispersity index of the obtained Cys-SLNs.

| Formulation | Dimensions (nm) | Polydispersity index (PI) |
|---|---|---|
| Not loaded Cyst-SLNs | 743.8 ± 61.6 | 0.326 ± 0.026 |
| Loaded Cyst-SLNs | 642.05 ± 60.3 | 0.414 ± 0.045 |

progesterone was equal to 642.05 nm. The polydispersity index, as shown in the same table, was 0.326 for not loaded Cys-SLNs while 0.414 for loaded Cys-SLNs. These PI values are indicative of a good homogeneity in the distribution of particle sizes.

As can be seen from the data reported in Table 3, the loaded Cys-SLNs have a diameter less than the not loaded ones. This can be explained with the lipophilic nature of the progesterone, establishing hydrophobic interactions with the lipid matrix, and can have a firming effect on the nanoparticles, reducing their size.

*3.9. Evaluation of Progesterone Release from SLNs.* The drug release from solid lipid nanoparticles was evaluated by spectrophotometry UV/Vis JASCOV-530 within 72 hours. The progesterone release was assessed by placing an aliquot (1 ml) of nanoparticles containing the drug within a dialysis membrane, which, in turn, was placed in a beaker containing 20 ml of buffer solution at pH 4 (47.0 ml 0.1 M acetic acid and 153.0 ml 0.1 M sodium acetate). The system was maintained under stirring for a predetermined time, after which the content of the membrane was analyzed by a UV-Vis spectrophotometer, after being subjected to breakage in 10 ml of methanol and dissolved in 5 ml of ethanol. It was thus possible to derive the concentration of drug still present within the SLN and to calculate, by difference, the concentration of drug released. Therefore, in order to evaluate the release profile, the content of 8 distinct membranes after being maintained under stirring for different times was analyzed: 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, and 72 h. After each period of time from the inside of the membrane, a quota of SLN was taken, to assess, after rupture and solubilization in ethanol (5 ml), the drug concentration of progesterone and calculate the difference of the concentration of drug released in the surrounding acidic environment to that time.

As can be seen from the graph in Figure 2, within the 2 h about 70% of the progesterone was released. The phenomenon is due to the burst release or to the initial release of the drug adsorbed to the superficial lipid matrix. Thereafter, the amount of encapsulated drug was slowly released. This establishes a sustained release over time; within 72 hours, 90% of the progesterone loaded into the carrier was released.

*3.10. In Vitro Bioadhesion Study.* The bioadhesive potential of the SLNs was *in vitro* evaluated. The results of the study showed a satisfactory retention time of our particles until 24 h. This indicated that the SLNs based on L-cysteine, having a good residence time in vagina, could permit the progesterone release for the necessary time to obtain a therapeutic effect.

*3.11. Stability Studies.* The stability of SLNs was assessed for a period equal to 20 days. In particular, a water dispersion, containing our particles, was exposed to light and maintained to room temperature. After this period, the particles were submitted to dimensional analysis, and their diameter value, being practically similar (~738 nm) to initial ones, indicated a very good stability of the particles.

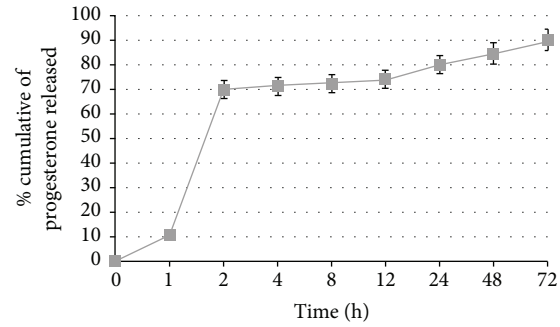



Figure 2: Progesterone release from Cys-SLNs evaluated within 72 hours.

## 4. Conclusion

The aim of this work was the preparation of solid lipid nanoparticles based on a new synthetized ester, 2(R)-2,3-dihydroxypropanoate of octadecyl 2,3-dihydroxypropanoate. This ester has been synthesized with high yields. The Cys-SLNs loaded with progesterone, prepared by the microemulsion technique, have been characterized by size, encapsulation efficiency, and the release capacity of the encapsulated progesterone.

The obtained particles showed dimensions suitable for topical administration. In particular, they could be potentially useful for the administration of drugs at the mucous level, thanks to the presence of thiol groups of cysteine that increase the residence time of the formulation at the site of administration/absorption, thus reducing the frequency of administration of the active ingredient.

Release studies in an acidic environment, typical of the vaginal cavity under physiological conditions (pH 3.5-4.5), have shown that 2-(R)-2,3-dihydroxypropanoate of octadecyl 2,3-dihydroxypropanoate-based SLNs entrapping progesterone is capable of implementing a prolonged drug release up to 72 hours. This could make these SLNs a viable alternative to the intramuscular administration of progesterone in women undergoing treatment of luteinic insufficiency, when there are threats of abortion or in the case of assisted reproduction treatments.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

[1] N. J. Alexander, E. Baker, M. Kaptein, U. Karck, L. Miller, and E. Zampaglione, "Why consider vaginal drug administration?," *Fertility and Sterility*, vol. 82, no. 1, pp. 1–12, 2004.

[2] R. M. Machado, A. Palmeira-de-Oliveira, C. Gaspar, J. Martinez-de-Oliveira, and R. Palmeira-de-Oliveira, "Studies

and methodologies on vaginal drug permeation," *Advanced Drug Delivery Reviews*, vol. 92, pp. 14–26, 2015.

[3] R. M. Machado, A. Palmeira-de-Oliveira, J. Martinez-de-Oliveira, and R. Palmeira-de-Oliveira, "Vaginal films for drug delivery," *Journal of Pharmaceutical Sciences*, vol. 102, no. 7, pp. 2069–2081, 2013.

[4] F. Acartürk, "Mucoadhesive vaginal drug delivery systems," *Recent Patents on Drug Delivery & Formulation*, vol. 3, no. 3, pp. 193–205, 2009.

[5] A. Gürsoy, I. Sohtorik, N. Uyanik, and N. A. Peppas, "Bioadhesive controlled release systems for vaginal delivery," *STP Pharma*, vol. 5, pp. 886–892, 1989.

[6] E. A. Kharenko, N. I. Larionova, and N. B. Demina, "Mucoadhesive drug delivery systems," *Pharmaceutical Chemistry Journal*, vol. 43, no. 4, pp. 200–208, 2009.

[7] V. V. Khutoryanskiy, "Advances in mucoadhesion and mucoadhesive polymers," *Macromolecular Bioscience*, vol. 11, no. 6, pp. 748–764, 2011.

[8] A. R. Mackie, F. M. Goycoolea, B. Menchicchi et al., "Innovative methods and applications in mucoadhesion research," *Macromolecular Bioscience*, vol. 17, no. 8, article 1600534, 2017.

[9] V. M. Leitner, G. F. Walker, and A. Bernkop-Schnürch, "Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 56, no. 2, pp. 207–214, 2003.

[10] H. E. Friedl, S. Dünnhaupt, C. Waldner, and A. Bernkop-Schnürch, "Preactivated thiomers for vaginal drug delivery vehicles," *Biomaterials*, vol. 34, no. 32, pp. 7811–7818, 2013.

[11] A. G. Mikos and N. A. Peppas, "Kinetics of mucus-polymer interactions," in *Bioadhesion–Possibilities and Future Trends*, R. Gurny and H. E. Junginger, Eds., pp. 65–85, Wissenschaftliche verlagsges, Stuttgart, Germany, 1990.

[12] C. Schwarz, W. Mehnert, J. S. Lucks, and R. H. Müller, "Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization," *International Journal of Pharmaceutics*, vol. 30, no. 1, pp. 83–96, 1994.

[13] K. Westesen, B. Siekmann, and M. H. J. Koch, "Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction," *International Journal of Pharmaceutics*, vol. 93, no. 1-3, pp. 189–199, 1993.

[14] A. J. Domb, "Long acting injectable oxytetracycline-liposphere formulations," *International Journal of Pharmaceutics*, vol. 124, no. 2, pp. 271–278, 1995.

[15] R. Cavalli, O. Caputo, M. E. Carlotti, M. Trotta, C. Scarnecchia, and M. R. Gasco, "Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles," *International Journal of Pharmaceutics*, vol. 148, no. 1, pp. 47–54, 1997.

[16] S. Trombino, R. Cassano, R. Muzzalupo, A. Pingitore, E. Cione, and N. Picci, "Stearyl ferulate-based solid lipid nanoparticles for the encapsulation and stabilization of $\beta$-carotene and $\alpha$-tocopherol," *Colloids and Surfaces B: Biointerfaces*, vol. 72, no. 2, pp. 181–187, 2009.

[17] S. Trombino, R. Cassano, T. Ferrarelli, E. Barone, N. Picci, and C. Mancuso, "Trans-ferulic acid-based solid lipid nanoparticles and their antioxidant effect in rat brain microsomes," *Colloids and Surfaces B: Biointerfaces*, vol. 109, pp. 273–279, 2013.

[18] R. Cassano, S. Mellace, M. Marrelli, F. Conforti, and S. Trombino, "$\alpha$-Tocopheryl linolenate solid lipid nanoparticles for the encapsulation, protection, and release of the omega-3 polyunsaturated fatty acid: in vitro anti-melanoma activity evaluation," *Colloids and Surfaces B: Biointerfaces*, vol. 151, pp. 128–133, 2017.

[19] R. Cassano, T. Ferrarelli, M. V. Mauro, P. Cavalcanti, N. Picci, and S. Trombino, "Preparation, characterization and in vitro activities evaluation of solid lipid nanoparticles based on PEG-40 stearate for antifungal drugs vaginal delivery," *Drug Delivery*, vol. 23, no. 3, pp. 1047–1056, 2016.

[20] S. Serini, R. Cassano, P. Corsetto, A. Rizzo, G. Calviello, and S. Trombino, "Omega-3 PUFA loaded in resveratrol-based solid lipid nanoparticles: physicochemical properties and antineoplastic activities in human colorectal cancer cells in vitro," *International Journal of Molecular Sciences*, vol. 19, no. 2, pp. 19–38, 2018.

[21] S. Trombino, R. Russo, S. Mellace et al., "Solid lipid nanoparticles made of trehalose monooleate for cyclosporin-A topic release," *Journal of Drug Delivery Science and Technology*, vol. 49, pp. 563–569, 2019.

[22] R. Müller, "Lipid nanoparticles: recent advances," *Advanced Drug Delivery Reviews*, vol. 59, no. 6, pp. 375-376, 2007.

[23] A. Tavaniotou, J. Smitz, C. Bourgain, and P. Devroey, "Comparison between different routes of progesterone administration as luteal phase support in infertility treatments," *Human Reproduction Update*, vol. 6, no. 2, pp. 139–148, 2000.

[24] H. Yuan, L.-L. Wang, Y.-Z. du, J. You, F.-Q. Hu, and S. Zeng, "Preparation and characteristics of nanostructured lipid carriers for control-releasing progesterone by melt-emulsification," *Colloids and Surfaces B: Biointerfaces*, vol. 60, no. 2, pp. 174–179, 2007.

[25] Ž. Vanić and N. Škalko-Basnet, "Nanopharmaceuticals for improved topical vaginal therapy: can they deliver?," *European Journal of Pharmaceutical Sciences*, vol. 50, no. 1, pp. 29–41, 2013.

[26] M. F. McCarty and J. J. DiNicolantonio, "An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly," *Age*, vol. 37, no. 5, pp. 96–104, 2015.

[27] R. T. Stravitz, A. J. Sanyal, J. Reisch et al., "Effects of N-acetylcysteine on cytokines in non-acetaminophen acute liver failure: potential mechanism of improvement in transplant-free survival," *Liver International*, vol. 33, no. 9, pp. 1324–1331, 2013.

[28] V. M. Leitner, M. K. Marschütz, and A. Bernkop-Schnürch, "Mucoadhesive and cohesive properties of poly (acrylic acid)-cysteine conjugates with regard to their molecular mass," *European Journal of Pharmaceutical Sciences*, vol. 18, no. 1, pp. 89–96, 2003.

[29] A. Bernkop-Schnürch, C. E. Kast, and M. F. Richter, "Improvement in the mucoadhesive properties of alginate by the covalent attachment of cysteine," *Journal of Controlled Release*, vol. 71, no. 3, pp. 277–285, 2001.

[30] H. A. Nair, A. B. Jindal, and M. N. Wasnik, "Synthesis of thiolated alginate and evaluation of mucoadhesiveness, cytotoxicity and release retardant properties," *Indian Journal of Pharmaceutical Sciences*, vol. 72, no. 6, pp. 766–774, 2010.

[31] X. Jiang, Y. Zhao, and L. Hou, "The effect of glycerol on properties of chitosan/poly(vinyl alcohol) films with $AlCl_3{\cdot}6H_2O$ aqueous solution as the solvent for chitosan," *Carbohydrate Polymers*, vol. 135, pp. 191–198, 2016.

[32] R. R. Fenton, W. J. Easdale, H. M. Er et al., "Preparation, DNA binding, and *in vitro* cytotoxicity of a pair of enantiomeric

platinum (II) complexes, [(*R*)- and (*S*)-3-aminohexahydroazepine]dichloro-platinum (II) crystal structure of the s enantiomer," *Journal of Medicinal Chemistry*, vol. 40, no. 7, pp. 1090–1098, 1997.

[33] B. Neises and W. Steglich, "Simple method for the esterification of carboxylic acids," *Angewandte Chemie International Edition in English*, vol. 17, no. 7, pp. 522–524, 1978.

[34] D. M. Shendage, R. Fröhlich, and G. Haufe, "Highly efficient stereo conservative amidation and deamidation of $\alpha$-amino acids," *Organic Letters*, vol. 6, no. 21, pp. 3675–3678, 2004.

[35] M. R. Gasco, "Solid lipid nanospheres from warm micro-emulsions," *Pharmaceutical Technology European*, vol. 9, pp. 52–58, 1997.

[36] L. Boltri, T. Canal, P. A. Esposito, and F. Carli, "Lipid nanoparticles: evaluation of some critical formulation parameters," *Proceedings International Symposium Controlled Release of Bioactive Materials*, vol. 20, pp. 346-347, 1993.

[37] S. Morel, E. Terreno, E. Ugazio, S. Aime, and M. R. Gasco, "NMR relaxometric investigations of solid lipid nanoparticles (SLN) containing gadolinium(III) complexes," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 45, no. 2, pp. 157–163, 1998.

[38] F. Nakamura, R. Ohta, Y. Machida, and T. Nagai, "In vitro and in vivo nasal mucoadhesion of some water-soluble polymers," *International Journal of Pharmaceutics*, vol. 134, no. 1-2, pp. 173–181, 1996.