5 SLN AND NLC BASED SEMI-SOLID FORMULATIONS

The pre-formulation studies for the development of a topical and dermatological dosage form need to focus on the physical and chemical behaviour of the drug and the many other components used for the preparation of the semi-solid formulation. Many of the criteria which are used to assess the physicochemical stability of those formulations are common to many pharmaceutical dosage forms, but some standards are particularly relevant concerning a topical semi-solid. These are related to the stability of a particular formulation in a specific container, to remain within its physical, chemical, therapeutic, and toxicological specifications. The first difficulty arises in attempts to assess the chemical stability of the drug in its complex vehicle, together with the stability of any potentially labile excipient. A general methodology for predicting chemical stability uses an accelerated stability test which subjects the material to elevated temperatures and uses the Arrhenius relationships to establish a shelf life. However, in a multiphase system such as cream for example, heat may alter the phase distribution and may even crack the emulsion. Thus, it might be limited to assess the preparation over a long time at the storage temperature. A related problem is that, because of the complexity of the vehicle, it may also be difficult to separate the drug or the labile adjuvant from the base in order to be analysed. In addition to performing specific analytical tests, any qualitative change in the semi-solid product during storage time needs to be carefully monitored. These changes are often apparent on visual inspection.

Many topical and dermatological formulations contain water or other volatile solvents, and batches may lose a proportion of the solvent either through the walls of unsuitable plastic containers or through faulty seams or ill-fitting caps. The product may lose weight and may shrink away from the container wall becoming puffy and stiff so that its application properties are jeoperdised. These semi-solid formulations are heterogeneous systems, being therefore susceptible to various changes if stored incorrectly. Emulsions may cream and crack, suspensions can agglomerate and cake, and ointments and gels may bleed as their matrices contract to squeeze out mobile constituents. High temperatures can produce or accelerate such adjustments. For milky white microemulsion creams or lotions it is difficult to determine if the final product requires additional mixing time to properly disperse or solubilize the drug.

To overcome such problems of these multiphase systems, a new approach would be the preparation of lipid nanoparticles (SLN and NLC) for drug entrapment and further incorporation into a monophasic system such as hydrogels. Concerning aqueous SLN and

NLC dispersions, once composed of physiological lipids they might be less comedogenic than either creams or lotions. It will therefore be possible to develop systems that are non-toxic, non-irritating, non-comedogenic and non-sensitizing and formulating pharmaceutically and/or cosmetically elegant topical formulations based on SLN and NLC.

Drug delivery from colloidal systems such as SLN and NLC dispersed in a hydrogel appears to be unique when compared to the delivery from traditional topical and dermatological formulations. The mobility of the drug within the lipid nanoparticles, through the semi-solid vehicle (hydrogel), and the mobility of vehicle components that enhance percutaneous transport must be considered. Lipid matrices with a brick wall structure where the drug is molecularly dispersed such as SLN of matrix model or of drug enriched core model (chapter 2, Fig. 2.6), may exhibit sustained release properties. Alternatively, the presence of a liquid core where the drug molecules have more freedom to move may accelerate the release rate. Therefore, SLN- and NLC-based semi-solid systems if properly formulated can provide optimum drug delivery profiles.

For SLN- and NLC-based semi-solid systems the phase behaviour should be adequately characterized to ensure that SLN and NLC are suitable carriers for a particular drug, as well as to determine the effect of the hydrogel in the physicochemical and thermodynamic stability of such colloidal systems.

Regarding the use of polymer gels, these have found extensive applications in the pharmaceutical industry. More specifically, gels have been successfully used for the local delivery of therapeutic agents. Another advantage of the use of hydrogels is the fact that their network can resist the physiological stress caused by body or skin movement and can adopt the shape of the application area [284]. Moreover, the drug release rate is extremely sensitive to the rheological behaviour of the topical gel formulation.

Carbomers (polyacrylates) have a potential wide range of applicability in the pharmaceutical and dermocosmetic fields. Upon neutralization, the anionic polymers expand in aqueous medium and form gel networks [284]. By employing organic amines, as neutralizing agents, it is possible to jellify carbomer networks in many semi-polar liquids or in mixtures of these liquids with water. Some advantages of using aqueous carbomer gels are their high viscosity at low concentrations (0.5 to 1.0%, m/v), wide viscosity interval and characteristic flow behaviour, compatibility with many active ingredients, bioadhesive properties, good thermal stability, as well as excellent organoleptic characteristics and good patient acceptance [284].

For the purpose of the present thesis, clotrimazole-loaded SLN and NLC developed in the previous chapter (section 4.1) have been used for the production of SLN- and NLC-based

semi-solid formulations intended for antifungal treatment. Carbopol®934 has been selected as the gel-forming polymer for the preparation of the semi-solid systems. The developed formulations have been stored at different temperatures (4ºC, 25ºC and 40ºC) for 3 months in an optimized package, i.e. laminate foil tubes. In the present chapter the physicochemical stability of SLN- and NLC-based semi-solid formulations will be assessed in terms of zeta potential and particle size analysis, polymorphic and phase behaviour of the lipid particles entrapped into carbomer gels, as well as in terms of encapsulation parameters, release profile and rheological and texture properties. Furthermore, physicochemical characterization of semi-solid formulations will be compared to commercially available creams containing clotrimazole.

5.1 Incorporation of SLN and NLC into carbomer hydrogels

For the present stage of the development of a product, freshly prepared aqueous SLN and NLC dispersions have been incorporated into polyacrylate-based hydrogels. Two different formulations have been developed and their composition is summarized in Table XXVII.

Table XXVII: Final composition of the developed SLN- and NLC-based semi-solid formulations.

Composition	SLN-based semi-solid formulation	NLC-based semi-solid formulation
Carbopol®934	0.50%	0.50%
Trizma®	0.15%	0.15%
Methyl paraben	0.05%	0.05%
Glycerol	5.00%	5.00%
Dynasan®116	9.00%	6.50%
Miglyol®812	-	2.50%
Clotrimazole	1.00%	1.00%
Tyloxapol®	2.50%	2.50%
Water ad	100%	100%

The developed semi-solid formulations have been stored at three different temperatures under appropriate packaging avoiding light exposure once it gradually decreases the consistency of Carbopol gels [218].

5.1.1 Zeta potential and particle size parameters

The prepared semi-solid systems showed a white appearance after dispersing the lipid nanoparticles in the hydrogels, which was maintained during the storage time at three different temperatures. The data depicted in Table XXVIII revealed that the values of the ZP of clotrimazole-loaded SLN and NLC increased significantly. Particles remained negatively charged after their entrapment in the gel network a least for three months of storage at different temperatures. Only NLC-based semi-solid formulations stored at 4ºC revealed a lower ZP value than SLN-based semi-solid formulations. The decrease of the ZP values during storage time was not significantly relevant for affecting the physical stability of lipid nanoparticles. In fact, no particle aggregation has been obtained, as observed by the particle size results. The mean particle size obtained by PCS remained lower than 260 nm and by LD it can be observed that 90% of the particles were lower than 3 μm. The PI remained lower than 0.550 after 3 months of storage.

These results are in agreement with the theory, which says that increased ZP provides increased stability by electrostatic repulsion. Thus, no size increase should occur. The increase of ZP values can be explained by adsorption of negatively charged Carbopol molecules onto the surface of the lipid nanoparticles.

Advantages of the use of nanoparticles in comparison to microparticles is the increase of surface area which allows therefore, the increase in contact area between the skin and the system and the increase of drug partition between both phases and bioavailability. For long term stability studies the particle size distribution of SLN and NLC entrapped in hydrogels needs to be monitored and notify any changes on storage. The particle size analysis may often detect a potentially unstable formulation long before any other parameter changes markedly. Lipid nanoparticles may grow or suffer aggregation as a gel network breaks down on storage, thus allowing Brownian motion to bring particles into contact so that they aggregate.

The apparent pH of a topical product may also alter during shelf life as chemicals change. Although it cannot be assigned a fundamental meaning to a pH measurement in a complex dermatological vehicle, it must be however monitored this parameter using a simple pH electrode to assess possible variations as the formulation ages. For the developed semi-solid formulations pH values between 5 and 6 have been recorded during the shelf life for all systems. This interval is suitable for administration onto the skin (pH of normal skin is 5.5 [353]), revealing no major changes in the formulations on storage.

Table XXVIII: Zeta potential and particle size parameters of SLN- and NLC-based semi-solids after one day (D1) and three months (M3) of storage at 4ºC, 25ºC and 40ºC.

Size parameters	Age	ºC	SLN-based semi-solid formulations	NLC-based semi-solid formulations
ZP (mV)	D1	4ºC	-31.9 ± 0.7	-24.5 ± 1.2
		25ºC	-30.2 ± 0.5	-32.6 ± 3.2
		40ºC	-25.6 ± 0.6	-30.7 ± 0.9
	M3	4ºC	-26.3 ± 0.8	-19.8 ± 2.1
		25ºC	-24.8 ± 0.3	-27.1 ± 1.4
		40ºC	-20.8 ± 0.7	-24.9 ± 1.1
PCS (nm)	D1	4ºC	243.5 ± 19.0	231.4 ± 10.1
		25ºC	183.1 ± 6.6	211.9 ± 9.1
		40ºC	269.7 ± 8.3	206.8 ± 8.5
	M3	4ºC	241.2 ± 27.2	243.8 ± 27.7
		25ºC	176.8 ± 9.3	206.1 ± 14.7
		40ºC	258.4 ± 15.9	196.8 ± 13.3
PI	D1	4ºC	0.321 ± 0.082	0.441 ± 0.013
		25ºC	0.461 ± 0.074	0.445 ± 0.027
		40ºC	0.210 ± 0.095	0.455 ± 0.027
	M3	4ºC	0.350 ± 0.108	0.403 ± 0.104
		25ºC	0.533 ± 0.032	0.428 ± 0.032
		40ºC	0.264 ± 0.087	0.390 ± 0.049
LD (50%)	D1	4ºC	0.199 ± 0.047	0.306 ± 0.009
		25ºC	0.240 ± 0.011	0.371 ± 0.010
		40ºC	0.307 ± 0.009	0.373 ± 0.016
	M3	4ºC	0.164 ± 0.001	0.272 ± 0.001
		25ºC	0.215 ± 0.004	0.359 ± 0.002
		40ºC	0.300 ± 0.001	0.354 ± 0.001
LD (90%)	D1	4ºC	1.599 ± 1.991	1.608 ± 0.078
		25ºC	3.184 ± 0.144	2.181 ± 0.088
		40ºC	0.519 ± 0.002	3.444 ± 0.192
	M3	4ºC	0.434 ± 0.001	0.910 ± 0.020
		25ºC	3.052 ± 0.013	2.025 ± 0.025
		40ºC	0.515 ± 0.000	3.160 ± 0.028

5.1.2 Polymorphic behaviour of SLN and NLC entrapped into hydrogels

Once SLN and NLC are composed of crystalline material, the crystallized fatty acid molecules that figure in their lipid matrix may enlarge, or change their habit, or revert to a more stable polymorphic form. Such changes in the crystal form may affect the therapeutic activity of the formulation.

Regarding SLN- and NLC-based semi-solid systems, they are composed of a crystalline phase (lipid matrix) which is dispersed and entrapped in a lamellar gel phase retaining water (hydrogel). A dynamic equilibrium is maintained between the internal phase (lipid nanoparticles) and the water interlamellarly inserted into the hydrophilic gel network. The latter is mainly fixed mechanically by the hydrophilic gel phase. A differentiation between lipid nanoparticles and hydrogel network is possible by means of DSC analysis once there is a sufficient high difference in the free energy between the two types of materials.

To assess the polymorphic behaviour of SLN and NLC entrapped into hydrogels, both DSC and X-ray diffraction analyses have been performed for all samples stored at three different temperatures. Tables XXIX and XXX show the evaluated DSC parameters for SLN- and NLC-based formulations, respectively.

Table XXIX: DSC parameters of developed SLN-based semi-solid formulations obtained after seven days and three months of storage at three different temperatures.

DSC parameters	Day 7			Month 3		
	4ºC	25ºC	40ºC	4ºC	25ºC	40ºC
mp (ºC)	59.39	59.13	60.20	59.33	59.68	60.50
Integral (mJ)	370.20	359.04	202.04	322.2	291.23	249.65
Enthalpy (J/g)	15.04	13.94	9.45	13.67	12.86	8.50
Onset (ºC)	55.39	55.72	57.69	55.35	56.21	58.00
RI (%)	77.75	73.03	48.85	70.67	66.48	43.94

All SLN-based semi-solid formulations have shown a single sharp endothermic peak between 59ºC and 61ºC with a total enthalpy changes lower than 15 J/g depending on the storage temperature of semi-solid formulations, as well as on the nature of the lipid phase dispersed in the gel network. The RI values slightly decreased during 3 months of shelf life, being lower than 50% in the samples stored at 40ºC. In comparison to aqueous SLN dispersions (Table XI, section 4.1.4.3.1), which shows RI values not lower than 80%, the entrapment into hydrogels

changed the polymorphic form of the solid lipid in the dispersions. However, the melting event has been recorded in the same temperature range.

Table XXX: DSC parameters of developed NLC-based semi-solid formulations obtained after seven days and three months of storage at three different temperatures.

DSC parameters	Day 7			Month 3		
	4ºC	25ºC	40ºC	4ºC	25ºC	40ºC
mp (ºC)	57.31	61.23	61.86	57.55	61.56	61.70
Integral (mJ)	383.34	35.80	36.85	289.13	39.71	23.46
Enthalpy (J/g)	11.50	0.85	1.12	11.41	1.15	0.88
Onset (ºC)	52.40	58.70	59.81	53.34	59.09	60.05
RI (%)	59.45	4.39	5.79	58.99	5.95	4.55

Major changes have been detected in the DSC parameters of NLC-based semi-solid formulations. Very low enthalpies have been recorded particularly for samples stored at 25ºC and at 40ºC, having RI values lower than 6%. On the contrary, samples maintained at 4ºC could evidence RI values of approximately 60%. During storage the melting point slightly increased. Comparing these semi-solid formulations with aqueous NLC dispersions before incorporation into hydrogels (Table XII, section 4.1.4.3.1), it can be emphasized as well that hydrogel also changed the polymorphic form of the solid lipid in the dispersions in a higher extent than for SLN systems. NLC systems are more sensitive towards mechanical stress and therefore the liquid core might partition into the hydrophilic phase of the hydrogel, which has been observed with the increase of the melting point. However, the melting peak became smaller, as emphasized by the recorded melting enthalpy values.

The X-ray diffraction patterns of developed SLN- and NLC-based semi-solid formulations are shown in Figs. 5.1 and 5.2, respectively.

The obtained patterns are in agreement with the DSC results. For the SLN-based systems, the lipid nanoparticles show the typical polymorphic modifications (α, β', β), nevertheless, of decreased intensity in samples stored at 40ºC (Fig. 5.1, lower). The peak of highest intensity occurs at the interplanar distance of approximately 0.385 nm, corresponding to the β'/β_i polymorphic form. At 0.46 nm and 0.37 nm occur, respectively, the typical β and β'/β_i forms of triacylglycerols (Fig. 5.1, upper and middle). The sample stored at 40ºC does not reveal the characteristic peak of the α form. These curves can be compared to the one recorded for the bulk Dynasan®116 (section 4.1.4.1, Fig. 4.7 upper).

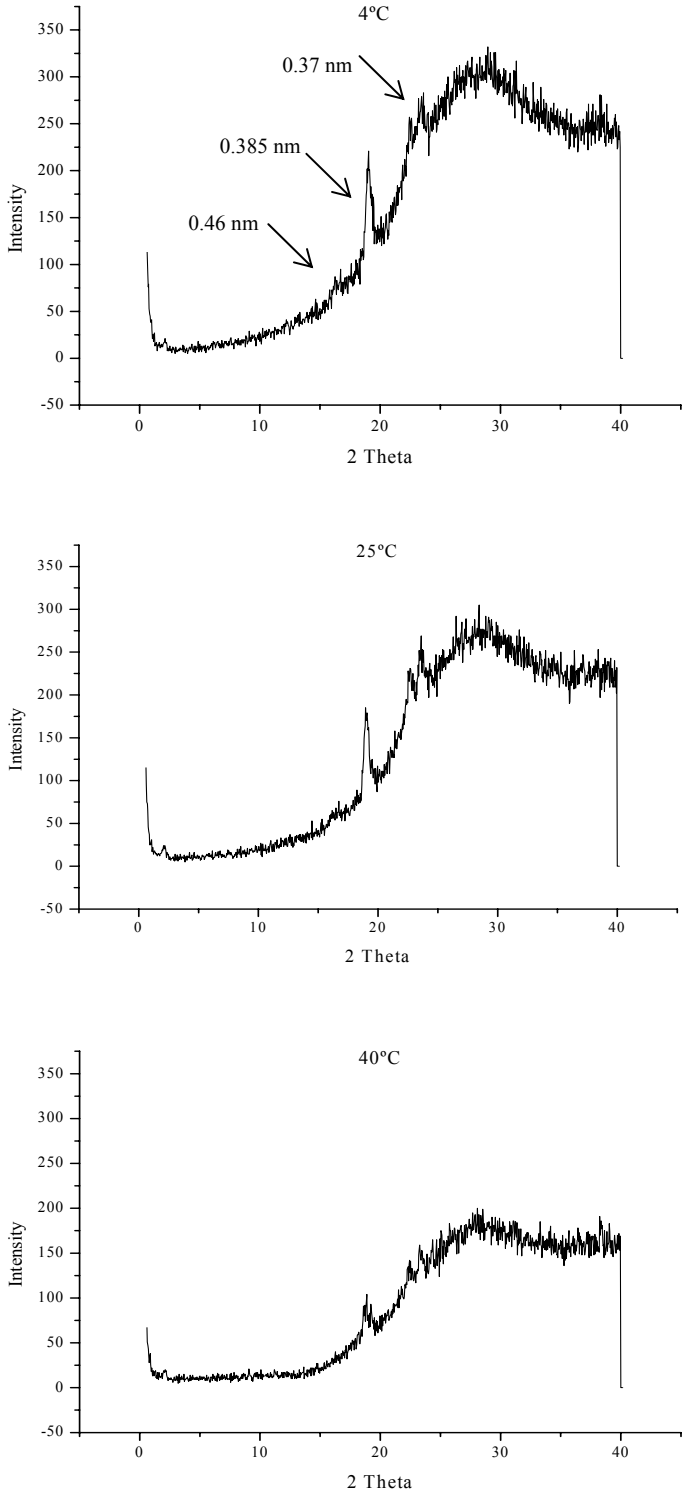

Fig. 5.1: X-ray diffraction patterns of SLN-based semi-solid formulations.

For the NLC-based systems, those stored at 25ºC and at 40ºC (Fig. 5.2, middle and lower) remained in their amorphous status, while those stored at 4ºC (Fig. 5.2, upper) a significant increase in peaks intensity could be recorded, in agreement to the higher RI values obtained by DSC analysis.

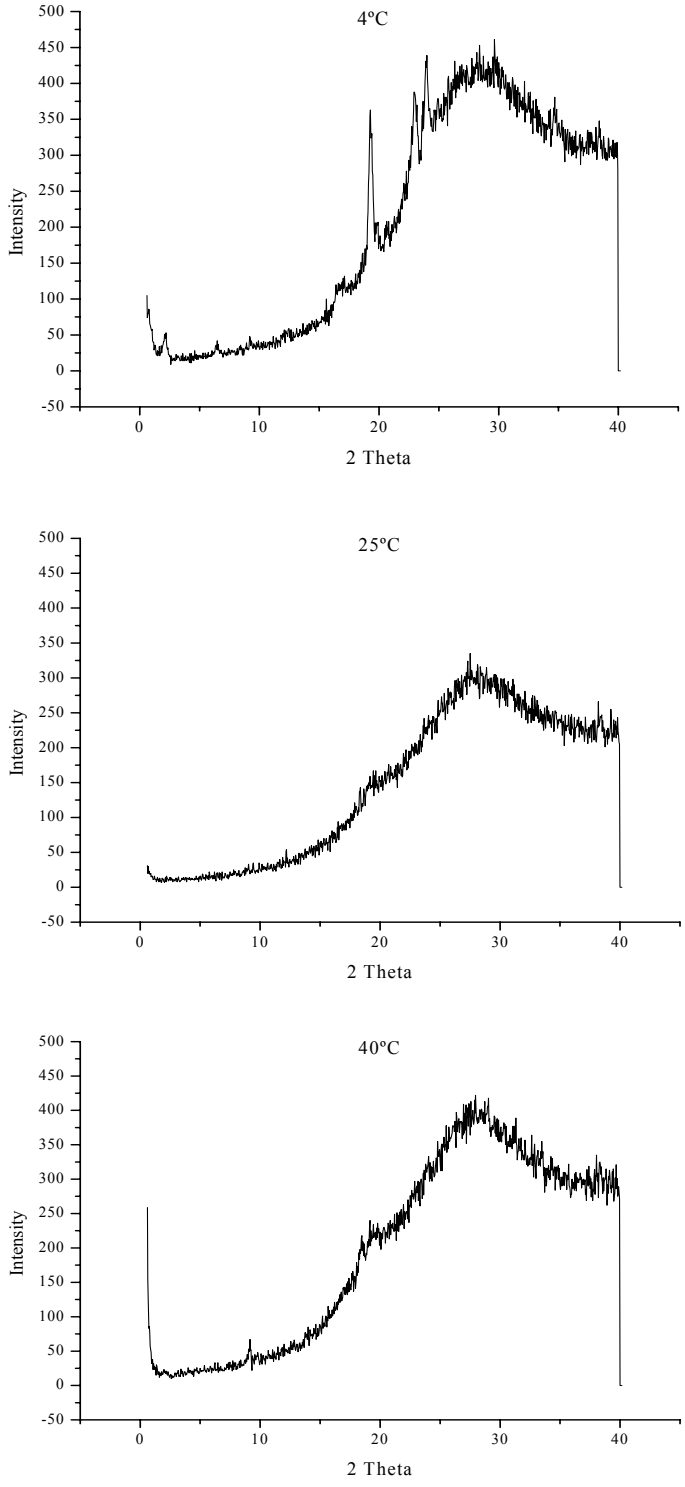

Fig. 5.2: X-ray diffraction patterns of NLC-based semi-solid formulations.

5.1.3 Mechanical characterization of semi-solid formulations

An important field of research is the one that studies the mechanical properties of a dermatological formulation, such as its viscoelastic properties, adhesiveness, consistency and gel strength. Those properties influence the manufacturing processes such as mixing, milling, pumping, stirring, extrusion, filling and sterilization, and might also affect the release of a drug and subsequent percutaneous absorption (bioavailability of the medicament) [218].
As reported previously, hydrogels are transparent to opaque semi-solid containing a high ratio of water to jelling agent. When dispersed in water, the jelling agent merges or entangles to form a three-dimensional colloidal network structure. This network limits the fluid flow by entrapment and immobilization of the water molecules. The network structure is also responsible for the resistance of the gel to deformation and, therefore for its viscoelastic properties. The hydrogel network is prepared through successive increases in the jelling agent concentration. This results in a reduction of the interparticle distances, which subsequently leads to chain entanglement and the establishment of cross-links. As the number of cross-links increases, the chains lock, the water mobility is reduced, and a gel network is formed. The continued polymer addiction strengthens the gel network and results in polymeric interactions. The integrity of the gel will be determined by the nature of the polymer-water molecules affinity.
For the characterization of the mechanical properties of the freshly prepared hydrogel, as well as of the developed semi-solid formulations, rheological and texture analyses have been performed.

5.1.3.1 Rheological analysis

Dispersions of lipid nanoparticles under shear flows experience different types of forces such as hydrodynamic forces (including the viscous drag force and particle-particle interaction through flow field induced by the neighbouring particles), colloidal chemical forces (including electrostatic, steric, and London-van der Waals attractive forces), and forces due to gravitational, inertial, electro-viscous, and thermal or molecular collision effects. For the evaluation of these properties the applied stress, as well as the duration of the stress application, must be previously defined in order to avoid the destruction of the structure, so that measurements can provide information of the inter-molecular and inter-particle forces in the material [308].

Small-angle cone and plate rheometers are used to determine the shear stress versus shear rate for a variety of formulations [354]. This device has the advantage that experimental data, in form of measured torque and normal force at different rotational speeds, can be converted directly into shear stress and first normal stress functions, using very simple algebraic expressions that are independent on the rheological constructive equation. These expressions are based on the small-angle approximation which means that the shear rate and consequently the shear and normal stresses are essentially uniform and known throughout the fluid under test.

Knowledge of the rheological properties is very important because the microstructural environmental or mobility, which is responsible for drug diffusion and compatibility, can be indirectly probed using these measurements. Extensive study may lead to the possible employment of rheological parameters and models to optimize topical drug delivery from dermatological formulations. Due to their importance, the rheological properties of carbomer gels (Carbopols) have extensively been investigated as a function of concentration, pH, and cross-link density [271, 355, 356]. Most of the studies use water as the solvent with a few reports about hydroalcoholic systems. The choice of the solvent is important because solvents like glycerine and propylene glycol can modify hydrogen bonding characteristics between water, solvent and polymer, thereby affecting the swelling and viscoelastic properties of the polymers [357].

Therefore, exhaustive characterization of the flow behaviour of these systems as a function of neutralization and polymer concentration is essential to evaluate the ability of Carbopol polymers to jellify for a range of pH values and their potential use as dermatological bases. The aim of the present study was to assess the rheological behaviour of SLN- and NLC-based semi-solid formulations prepared with Carbopol®934 as jellifying agent.

To gain some insight into the influence of storage temperature and nature of lipid matrix, the shear stress variation was recorded at a pre-defined shear rate from 0 s^{-1} to 100 s^{-1} for semi-solid systems stored at three different temperatures. The rheological properties of carbomer gels have been characterized in several studies [358, 359], therefore, they are not reported here. The focus of the present investigation was the rheological behaviour of such gels when lipid nanoparticles are entrapped into their network. According to this, analysis has been performed for SLN- and NLC-based formulations and Figs. 5.3 and 5.4 show, respectively, the obtained results recorded after one week of storage at 4ºC, 25ºC and at 40ºC. Table XXXI summarises their complex viscosity values evaluated during the same experiment.

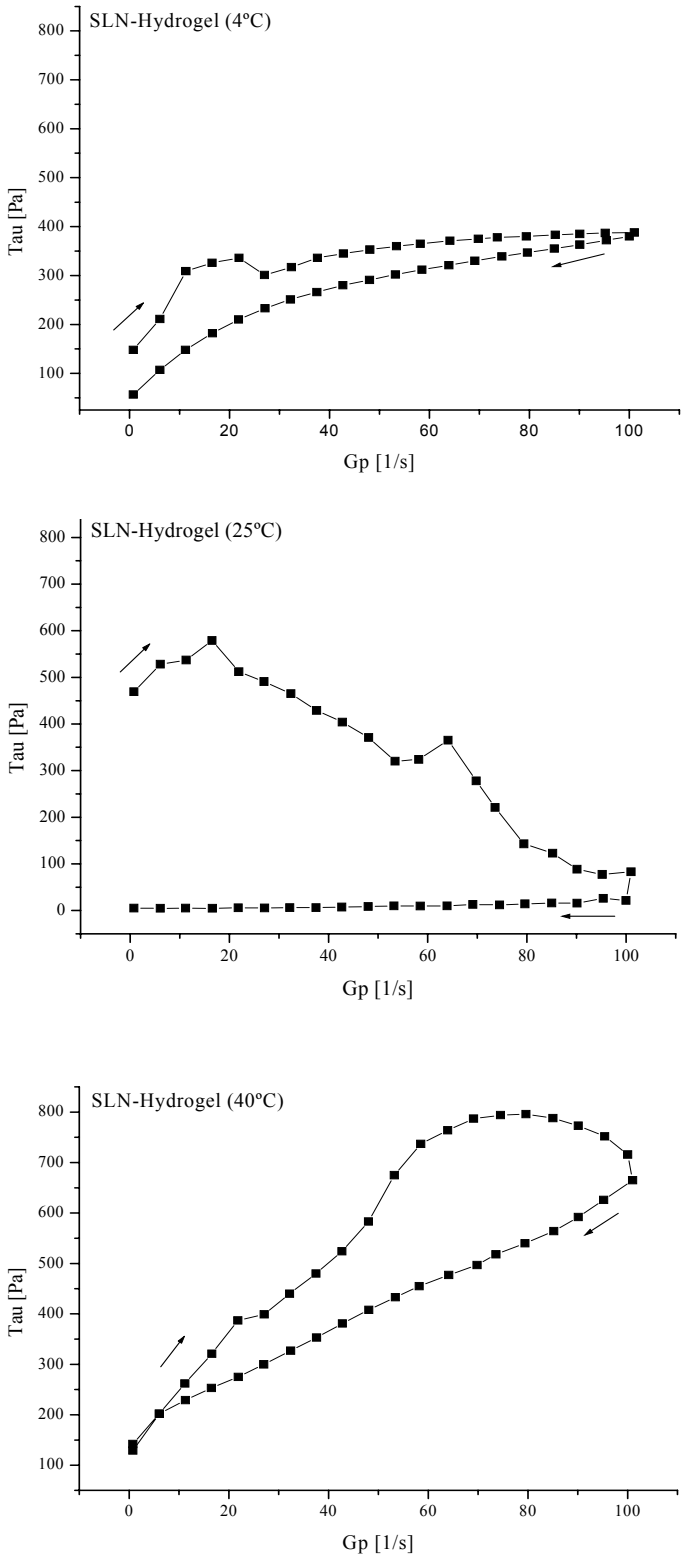

Fig. 5.3: Shear rate [1/s] versus shear stress [Pa] of SLN-based semi-solid formulations obtained after one week of storage at three different temperatures.

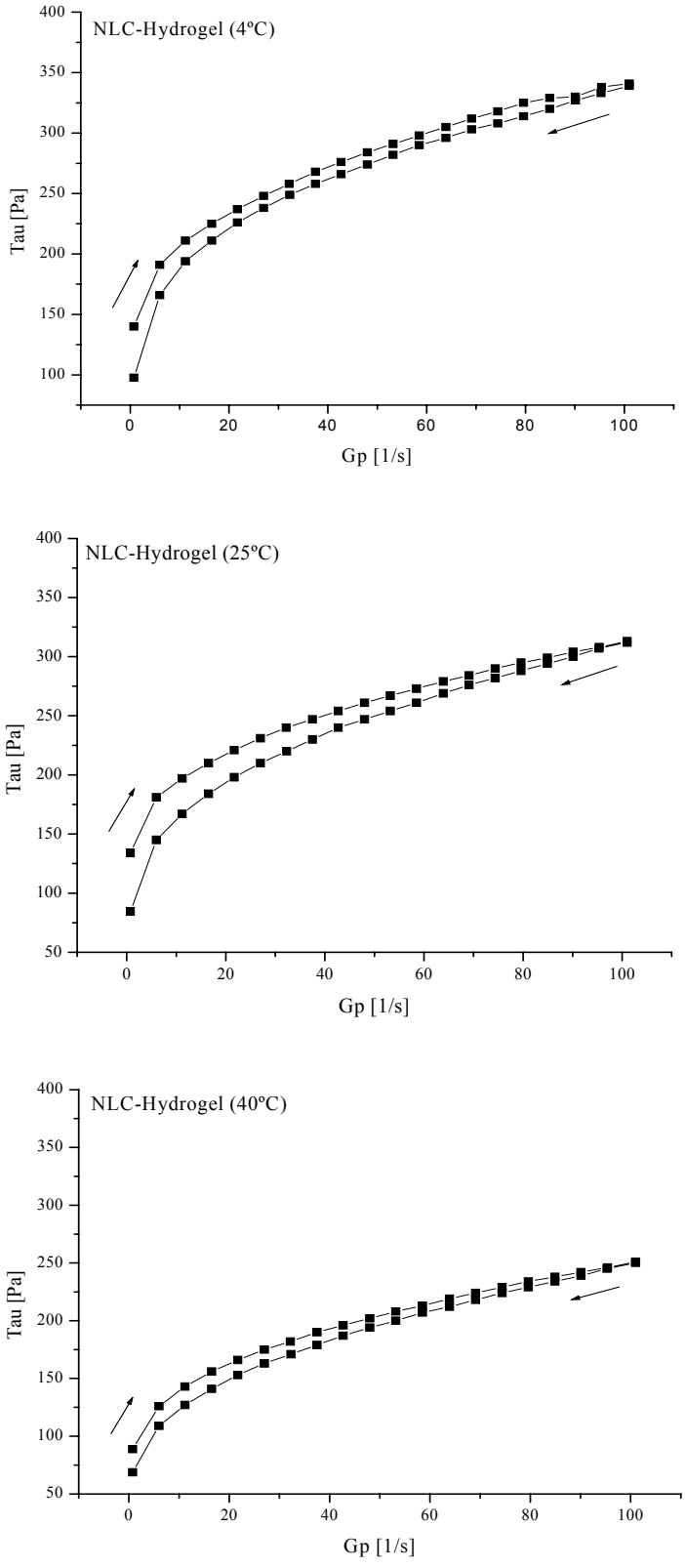

Fig. 5.4: Shear rate [1/s] versus shear stress [Pa] of NLC-based semi-solid formulations obtained after one week of storage at three different temperatures.

In the range of shear rates studied in this work, the shear stress was not proportional to the shear rates in both types of systems (SLN and NLC). The characteristic concavity of the rheogram toward the shear rate axis indicates that all developed formulations exhibited pseudoplastic flow. This pseudoplasticity results from a colloidal network structure that aligns itself in the direction of shear, thereby decreasing the viscosity as the shear rate increases (Table XXXI).

Table XXXI: Complex viscosity variation of SLN- and NLC-based semi-solid formulations stored at three different temperatures recorded during a shear rate interval from 0 s^{-1} to 100 s^{-1}.

Semi-solid formulation	Shear rate variation	Storage temperature/Complex viscosity [mPa.s]		
		4º	25º	40ºC
SLN-based hydrogels	0 s^{-1}	197000	625000	172000
	100 s^{-1}	3850	823	6610
	0 s^{-1}	75500	6980	189000
NLC-based hydrogels	0 s^{-1}	187000	179000	118000
	100 s^{-1}	3390	3110	2500
	0 s^{-1}	130000	113000	91700

During all experiments, the temperature has been accurately maintained at 20±0.1ºC using a thermostated water bath. It is important that the temperature does not change during the rheological determination to avoid obtaining false positive results in the test for thixotropy.

From Figs. 5.3 and 5.4 it can be stated that all systems show thixotropy, which may be defined as an isothermal and comparatively slow recovery, on standing of a material, of a consistency lost through shearing. In complex systems such as SLN- and NLC-loaded hydrogels in which a loose network connects together the sample, thixotropy proceeds from structural breakdown and re-aggregation. At a steady-state or at very low shear rates, the three-dimensional structure provides the system with some rigidity and the system behaves as a gel. Once the device starts stressing the sample, the structure begins to disrupt as the points of contact break and the particles start to align. The sample starts flowing, and its consistency decreases progressively with time as the shear stress and shear rate increase. Thus, the system suffers transformation from a gel-network to a sol-network. When the applied stress is decreased or removed, the internal structure of the system starts to reform but with a time lag, as the particles which build the network need a period in which to contact each other.

Therefore, the shapes of rheograms which are obtained for thixotropic systems are highly depend on the rate at which the shear conditions are changed and the time during which the sample is under the applied shear rate. Furthermore, the recorded flow curve will also depend on the previous shear history of the sample, including the way in which the viscometer has been loaded with the test sample. Using a rotational viscometer, in conjunction with an X-Y plotter, the shear rate has been steadily increased to obtain readings for the up curve. At a shear rate of 100 s^{-1} (pre-set maximum value achieved after a set sweep time) the shear rate has been steadily decreased to obtain the down curve.

Regarding the SLN-loaded hydrogels (Fig. 5.3), the hysteresis loops of the rheograms were significantly different depending on the storage temperature of the hydrogels. Samples stored at 25ºC and at 40ºC showed the highest areas of which measures the extent of thixotropy in the body under the conditions of the test. This means that there is a higher structural breakdown in those samples. At 4ºC the sample shows smaller differences between shear stress values at low shear rates, indicating a more ordered structure of particles entrapped in the gel network. A stress overshoot typical of nonlinear viscoelastic behaviour was also observed in all storage temperatures at low shear rates.

Concerning the NLC-loaded hydrogels (Fig. 5.4), also up curve does not coincide with the down curve, meaning that the samples also show thixotropy, however with lower structural breakdown than SLN-loaded hydrogels, once the areas are much smaller. The observed differences of the registered shear stress values at low shear rates indicate that forces between entrapped nanoparticles, and also the type of the interactions (electrostatic versus steric) between them, control the flow behaviour of semi-solid systems at low shear rates.

The differences on the rheological behaviour of both developed semi-solid systems (SLN- and NLC-loaded hydrogels) can be explained based on the lipid content of the formulations. According to Krieger [360], the increase of lipid content in a formulation increases its viscosity described by the equation 27:

$$\eta = \eta_0 \left(1 - \frac{\varphi}{p}\right)^{-[\eta]p} \tag{27}$$

where η is the viscosity of the dispersion, η_0 is the viscosity of the dispersion medium, $[\eta]$ is the intrinsic viscosity, φ is the volume fraction of the disperse phase and p is the volume fraction of disperse phase at most dense packing. It is clear that SLN-based systems have higher viscosity than NLC-based systems, once the former have higher solid lipid content. The parameters p and $[\eta]$ strongly depend on the shape of the dispersed nanoparticles. Since cold-stored nanocrystals of saturated, monoacid triacylglycerols generally exhibit a platelet-

like shape [256, 342], p can be assumed to have a constant value of approximately $\pi/4$ (value for all flat cylindrical particles). The intrinsic viscosity $[\eta]$ equals to 5/2 in the case of perfectly spherical particles and becomes larger with increasing particle anisometry, i.e. with increasing ratio between the diameter D and the thickness h. Therefore, the observed increase of viscosity η of equally concentrated SLN and NLC can only be due to an increase in $[\eta]$ and, hence, it is a clear indication for a successively increasing anisometry of nanocrystal shape. This result is in good agreement with earlier X-ray studies showing a decrease of the average nanocrystals thickness (Figs. 5.1 and 5.2) and a simultaneous increase in particle size (Table XXVIII), in comparison to non-entrapped lipid nanoparticles.

The semi-solid systems behave quite different according to the nature of the lipid matrix dispersed in the hydrogel. Although both systems show thixotropy (Fig. 5.3 and 5.4), the behaviour of SLN-based semi-solid systems was more dependent on the storage temperature than NLC-based semi-solid systems.

In SLN-based semi-solid formulation, when the shear rate increased, an increase of shear stress was also observed for samples stored at 4ºC and at 40ºC. The strongest behaviour was observed for those stored at 25ºC, where the down curve creates a higher hysteresis loop, probably due to the more severe shear deformation promoted. It is possible that for a more complex flow history, in which both the magnitude and the sign of the shear rate changes, the systems may lose their efficiency by their partial removal from the interface between the gel network and the particle matrix, which in turns allows coalescence among particles. In a similar fashion, the high shear generated during the shear rate tests may have caused a redistribution of the particles around the dispersion phase and even a partial removal from the interface, leading to coalescence, once the total strain caused by the shearing is much greater than those applied.

In dispersed systems, thixotropy arises when shear stress values measured by progressively increasing the shear rate are higher from those measured when one progressively decreases it. Thixotropy is a kind of viscoelasticity that has a very long relaxation time caused by flow induced changes in structure that are generally erased after hours of quiescence [284]. Figs. 5.3 and 5.4 show that the developed formulations have thixotropy at relatively low shear rates. However, the amount of thixotropy is very high for SLN-based systems in comparison to NLC-based systems, as evidenced by the proximity of the curves shown by these latter systems. At low shear rates, measurements are quite reliable once it decreases the risk of edge fraction and/or sample expulsion owing to high centrifugal forces. When the topical gels experience high shear rates, the network structure between neighbouring microgel particles as

well as the entanglements between long polymer chains segments break down. Therefore, the shear stresses or viscosities in decreasing shear rate curve are lower.

5.1.3.2 Texture analysis

The efficiency of the topical and dermatological therapy depends on the way in which the patient spreads the formulation in even layers to administer a standard dose. Spreadability is therefore an important characteristic of these formulations and it is responsible for correct dosage delivery to the target site.

In general, the incorporation of aqueous SLN and NLC dispersions into Carbopol hydrogels affect their rheological behaviour, as exposed in the section 5.1.3.1 of the present chapter. The structure of Carbopol (network extent) has implications on the facility in which the initial deformation occurs and in the flow properties of each formulation. Therefore, it is important to take into account the decrease of gel viscosity observed by the consistency of semi-solid formulations after the incorporation of the colloidal systems. The present section will evaluate the texture of the developed formulations in terms of adhesiveness, consistency and gel strength.

5.1.3.2.1 Adhesiveness

Adhesiveness is the work necessary to overcome the attractive forces between the sample and the probe with which the sample comes into contact [309]. Once the test has been performed applying the same force during 10 sec, the lower the distance is the higher is, the adhesiveness between the two surfaces.

The adhesiveness of freshly prepared pure Carbopol®934 hydrogels has been compared to SLN- and NLC-based semi-solid formulations (Fig.5.5). The analysis of the hydrogels texture shows the decrease of adhesive properties of polyacrylate hydrogels with the presence of lipid nanoparticles.

Once the particle size of SLN and NLC is similar (Table XXVIII), both hydrogels could be compared concerning their surface stickiness and stringiness properties. SLN-based formulations have shown to be more adhesive than NLC-based formulations (Fig. 5.5). This observation is in good agreement with the assumption that swelling is not interrupted by the water insoluble lipid nanoparticles dispersed in the gel network.

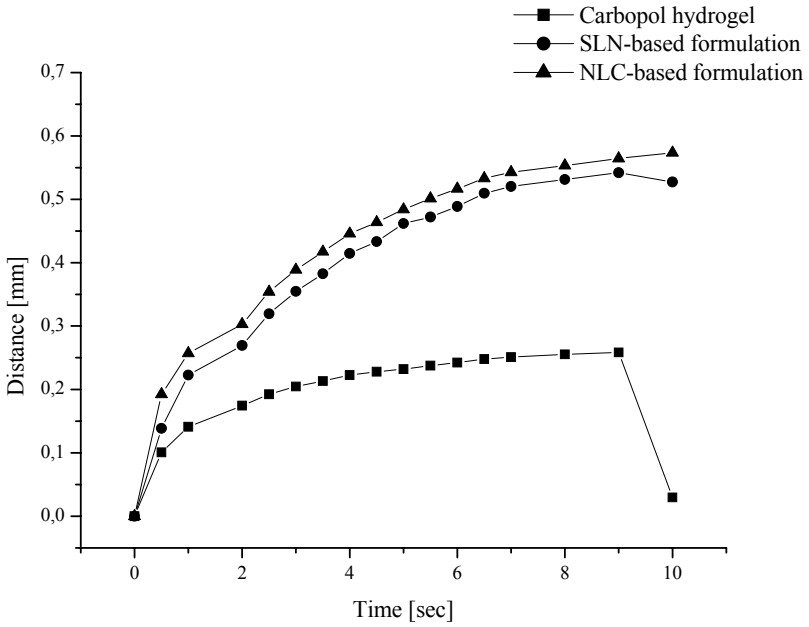

Fig. 5.5: Adhesiveness patterns of Carbopol®934 gel obtained on day 0, in comparison to freshly prepared SLN- and NLC-based semi-solid formulations.

The adhesiveness was also evaluated according to the storage temperatures (4ºC, 25ºC and 40ºC) of both SLN- and NLC-based semi-solid formulations. Figs. 5.6 and 5.7 show the obtained results after one week of storage.

For both systems, the adhesiveness patterns obtained at three different temperatures were very similar. Formulations stored at 25ºC were shown to be slightly less adhesive than those at higher and lower temperatures. SLN-based semi-solid formulations stored at 40ºC were more adhesive than those stored at 4ºC (Fig. 5.6). The opposite has been observed for NLC-based semi-solid formulations (Fig. 5.7).

As reported previously (section 5.1.3.1), the SLN- and NLC-based semi-solid formulations stored at 25ºC showed the highest hysteresis loop, i.e. area of thixotropy, in the flow curves (Figs. 5.3 and 5.4, middle). This means that under these conditions the systems are more sensitive to shear deformation, which might be related to the lowest adhesive properties in comparison to the ones stored at higher (40ºC) and lower (4ºC) temperatures.

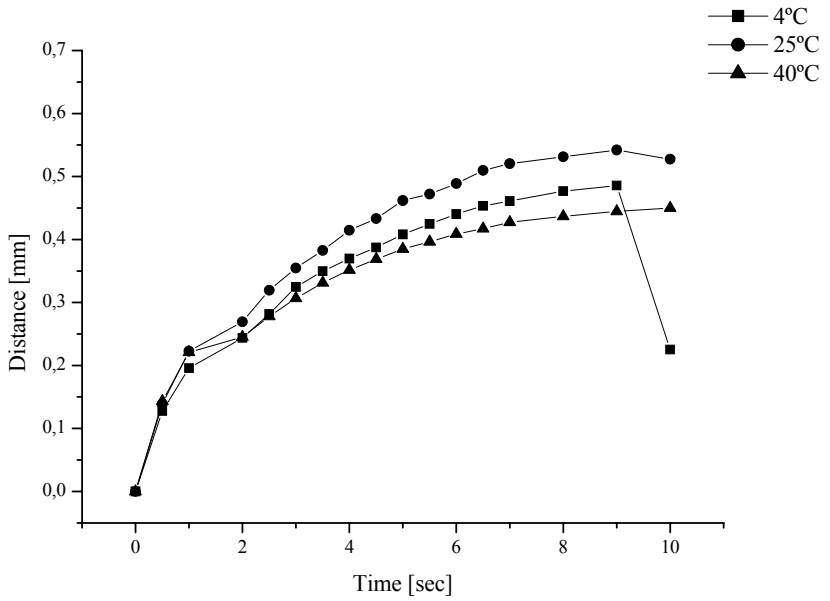

Fig. 5.6: Adhesiveness patterns of SLN-based semi-solid formulations obtained after one week of storage at three different temperatures.

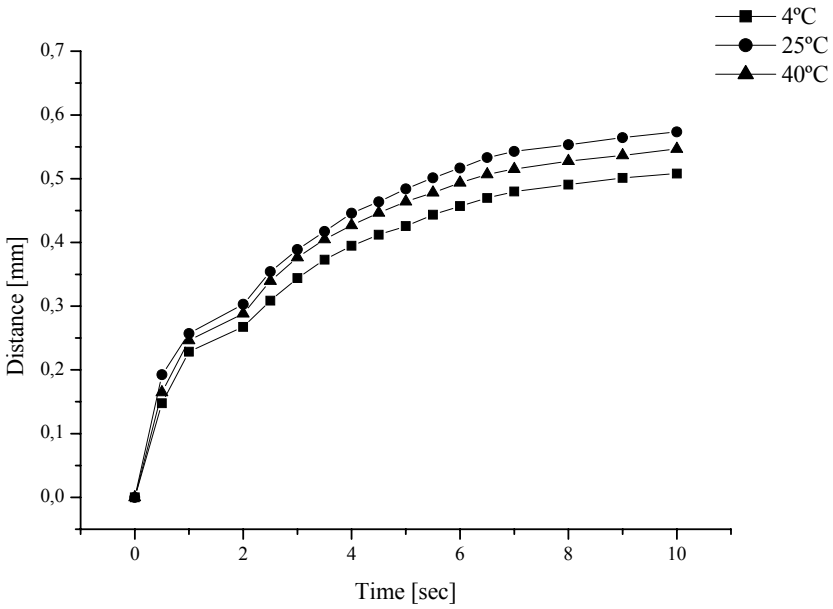

Fig. 5.7: Adhesiveness patterns of NLC-based semi-solid formulations obtained after one week of storage at three different temperatures.

5.1.3.2.2 Consistency

A test for measuring the consistency of SLN- and NLC-based semi-solid formulations has been developed by adapting a texture-profile analyser to pull the test sample placed on the base of the instrument upwards from 0 mm to 2 mm, and downwards from 2 mm to 0 mm. The force (N) needed to lift the sample probe to the pre-set distance has been recorded. Table XXXII shows the obtained results of the consistency test of the SLN- and NLC-based semi-solid formulations stored at three different temperatures for one week.

Table XXXII: Recorded force (N) during the consistency test of the SLN- and NLC-based semi-solid formulations stored at three different temperatures for one week.

Formulations		**Recorded force (N) at different distances (mm)**		
Sample ID	**Storage temperature**	**0 mm**	**2 mm**	**0 mm**
Carbopol®934	4ºC	0.0246	0.1538	-0.0454
	25ºC	0.0238	0.3715	-0.0637
	40ºC	0.0239	0.1387	-0.0222
SLN-based semi-solid formulations	4ºC	0.0238	0.0784	-0.0143
	25ºC	0.0242	0.1605	-0.0146
	40ºC	0.0234	0.0837	-0.0148
NLC-based semi-solid formulations	4ºC	0.0237	0.0584	-0.0050
	25ºC	0.0241	0.0761	-0.0310
	40ºC	0.0241	0.0551	-0.0043

Once the peak (or the maximum force) is taken as a measurement of consistency, i.e. the firmness of the sample, it can be stated that the higher the value the firmer will be the formulation.

At a distance of 2 mm, SLN-based formulations stored at 25ºC recorded a force of 0.1605 N, whereas at the same distance and storage temperature for NLC-based formulations that value was only 0.0761 N. This result emphasizes the higher consistency of SLN-based formulations in comparison to the NLC-based formulations. Comparing those values with the consistency of pure Carbopol hydrogels, i.e. without lipid nanoparticles (0.3715 N), it confirms that both consistency and adhesiveness of hydrogel decreases with the incorporation of lipid nanoparticles. Those properties are lower for NLC-based semi-solid formulations, especially

those stored at higher temperatures (40ºC). For these latter also the rheological properties revealed lower viscosity in comparison to SLN-based semi-solid formulations (Table XXXI, section 5.1.3.1).

5.1.3.2.3 Gel strength

Gel strength analysis has been previously performed in the Carbopol®934 gels without lipid nanoparticles once stored at three different temperatures. The penetration force (N) needed to break the sample placed in the base of the instrument to a pre-set depth of 2 mm has been recorded and translated as the gel strength. Fig. 5.8 shows the obtained patterns after one week of storage.

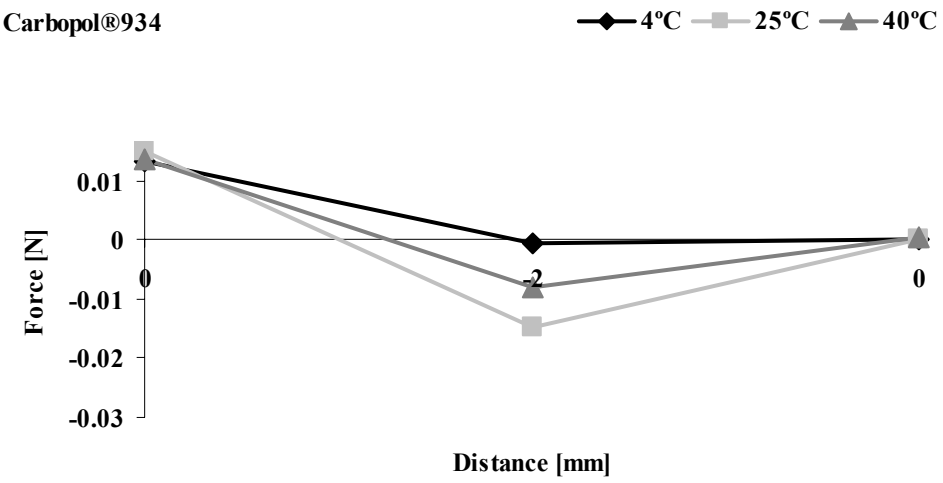

Fig. 5.8: Gel strength of Carbopol®934 gels stored at 4ºC, 25ºC and at 40ºC for one week.

The higher value recorded for the penetration force was obtained for the Carbopol®934 gels stored at 4ºC, followed by those stored at 40ºC and the lowest was observed for gels stored at room temperature. This means that the lowest gel strength was measured in the samples stored at 25ºC, those which showed lower adhesiveness after incorporation of aqueous SLN and NLC dispersions (Figs. 5.6 and 5.7, section 5.1.3.2.1).

Figs. 5.9 and 5.10 depict the obtained results for the SLN- and NLC-based semi-solid formulations, respectively. Table XXXIII shows the areas (N.sec) of the curves obtained in Figs. 5.9 and 5.10 for comparison to the ones obtained for Carbopol®934 gels (Fig. 5.8), applying the same test.

From the patterns presented in Figs. 5.9 and 5.10, it is clear that the presence of SLN requires higher force than the presence of NLC. Comparing the obtained areas at 25ºC, SLN-based

formulations showed higher, while NLC-based formulations showed lower gel strength values than Carbopol®934 gels. At 4ºC and at 40ºC the values were not significantly different.

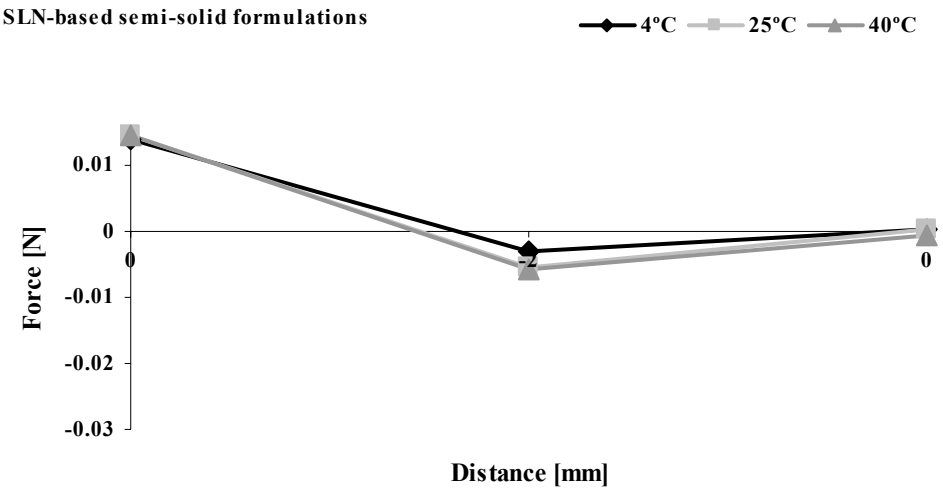

Fig. 5.9: Gel strength of SLN-based semi-solid formulations stored at 4ºC, 25ºC and at 40ºC for one week.

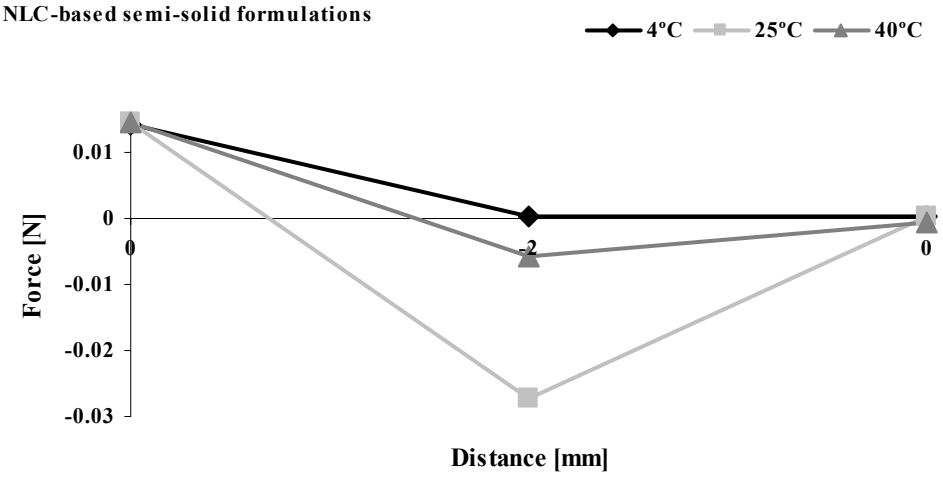

Fig. 5.10: Gel strength of NLC-based semi-solid formulations stored at 4ºC, 25ºC and at 40ºC for one week.

Table XXXIII: Areas (N.sec) obtained from the curves shown in Figs. 5.8, 5.9 and 5.10.

Storage temperature	Carbopol®934	SLN-based semi-solid formulations	NLC-based semi-solid formulations
4ºC	0.062	0.201	0.062
25ºC	0.914	0.279	1.700
40ºC	0.271	0.255	0.255

These results emphasize the fact that gel strength is not directly related to adhesiveness and consistency of hydrogels containing lipid nanoparticles.

5.1.3.3 Correlation between rheological and texture properties

As stated in the chapter 3 of the present thesis, the non-Newtonian behaviour of topical and dermatological formulations based on colloidal carriers is reflected by the Power Law index (*n*) [361].

There are several models, which may be used to establish the index flow (*n*) in different non-Newtonian systems [362]. The most adequate model for a viscoplastic fluid depends on the fluid response to deformation and how well the experimental data fit the model. The developed semi-solid formulations have been analysed using the most common models, which are the following:

Bingham: $\tau = \tau_0 + \eta.\gamma$

Casson: $\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5}.\gamma^{0.5}$

Ostwald: $\tau = k.\gamma^n$

Herschel-Bulkley: $\tau = \tau_0 + k.\gamma^n$

The τ_0 is the yield stress, η is the viscosity, *n* is the flow index, k is the consistency index, τ is the shear stress and γ is the shear rate [362]. For this particular study, continuous shear investigations were performed in order to evaluate the shear rate [1/s] as a function of shear stress [Pa]. This study started applying 0 Pa up to a maximum shear stress of 50 Pa and the resulting shear rate was measured. Tables XXXIV and XXXV show, respectively, the results of SLN- and NLC-based formulations adjusted to the above mentioned models. Once none of the developed formulations revealed a yield value in the applied shear stress range the Herschel-Bulkley was not considered.

Table XXXIV: Correlation coefficient (R^2) of SLN-based semi-solid formulations stored at different temperatures for various flow models in shear rate/shear stress curves.

Formulations	Bingham	Casson	Ostwald
4ºC	1.0000	0.9155	0.9078
25ºC	1.0000	0.9305	0.9845
40ºC	1.0000	0.9305	0.9422

Table XXXV: Correlation coefficient (R^2) of NLC-based semi-solid formulations stored at different temperatures for various flow models in shear rate/shear stress curves.

Formulations	Bingham	Casson	Ostwald
4ºC	1.0000	0.9305	0.5232
25ºC	1.0000	0.9154	0.1971
40ºC	1.0000	0.9305	0.9060

According to the obtained R^2 values, the rheograms of this study were better adjusted to the Bingham's model. The results showed acceptable regression coefficients, providing that the Potency Law reproduced properly the rheological behaviour of these systems. Shear thinning or pseudoplastic behaviour as described by the Bingham's model is very common in cosmetic and toiletry products [363]. During flow these materials may exhibit three distinct regions as shown in Fig. 5.11.

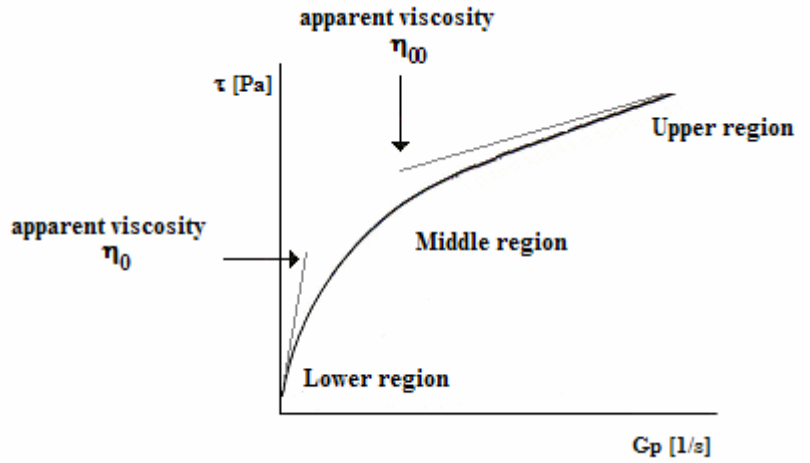

Fig. 5.11: Rheogram of an idealised shear-thinning or pseudoplastic behaviour (modified after Steffe [363]).

A lower Newtonian region can be identified, where the apparent viscosity, called the limiting viscosity at zero shear rate, is constant with changing shear rates. The middle region is the one where the apparent viscosity is changing, decreasing for shear-thinning materials with shear rate, and the power law equation is a suitable model for the phenomenon. The third region is identified by the upper Newtonian region where the slope of the curve, called the limiting viscosity at infinite shear rate, is constant with changing shear rates.

All formulations showed adequate characteristics to be applied topically. In the case of dermatological formulations plastic properties are preferred because the formulation flow resistance is low when it is applied under medium to high shear conditions. On the other hand, the flow is zero under stress caused by gravity [364].

As exposed previously, lipid nanoparticles have been dispersed after the preparation of the hydrogel therefore the former do not play any role in the production of the semi-solid system. SLN and NLC will be simply dispersed in the network. Fig. 5.12 shows the proposed three-dimensional model network for the entrapment of lipid nanoparticles into Carbopol hydrogels. The increase in the concentration of lipid nanoparticles will change the rheological behaviour as explained above.

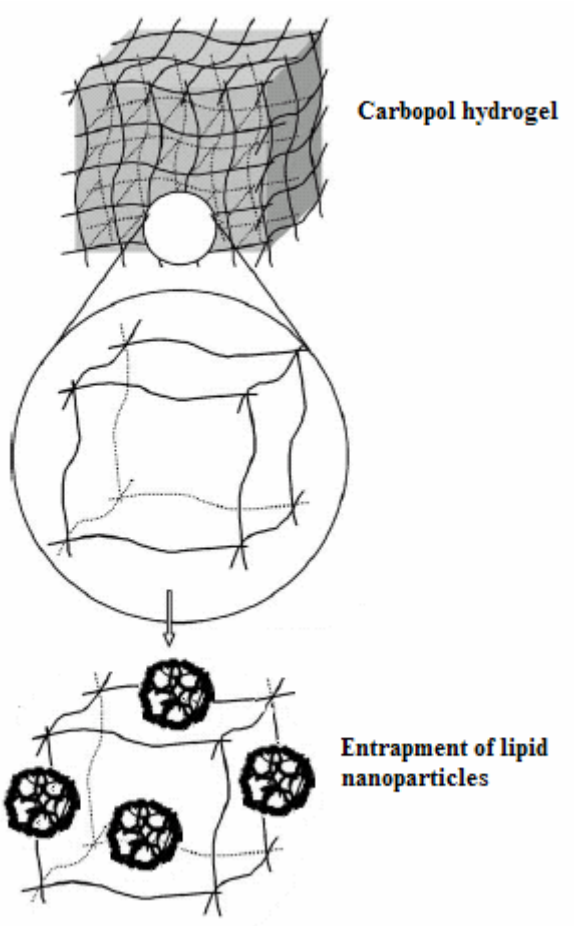

Fig. 5.12: Schematic representation of a proposed three-dimensional model network for the entrapment of lipid nanoparticles into Carbopol hydrogels.

In aqueous medium, an increase in pH causes repulsion among the carboxylic groups and swelling of the polymer to give an extensive gel-like structure [270]. This alteration can be explained by the increase of the consistency of the gel control and the possible rearrangement of the polymeric network during the storage period. The structure of Carbopol gels is determined by the level in which the macromolecules connect and form entanglement networks [361].

5.2 Comparison of developed semi-solid formulations with commercial creams

For the present study, a new semi-solid formulation has been developed based on the use of a hydrophilic cream (*unguentum emulsificans aquosum*) instead of freshlty prepared hydrogels. Aqueous SLN dispersions developed in the chapter 4 of the present thesis (D116SLN_Co, section 4.1) have been entrapped into a suitable hydrophilic cream and their particle size, as well as their rheological properties have been analysed and compared to commercially available creams. The release of clotrimazole from the aqueous dispersion D116SLN_Co has also been assessed in comparison to well known market products, i.e. Canesten® and Fungizid-ratiopharm® creams.

5.2.1 Particle size analysis

Table XXXVI shows the obtained particle size parameters of SLN before and after incorporation into hydrophilic cream. Prior to particle size analysis of SLN incorporated into a hydrophilic cream (20% SLN), samples were diluted with bidestilled water to weak opalescence, a procedure that did not alter the size distribution obtained as could be verified beforehand.

Table XXXVI: Particle size analysis of SLN before and after their entrapment into hydrophilic cream. The inner oil droplets of the o/w hydrophilic cream have been analysed by LD as control.

Evaluated parameters	Pure aqueous SLN dispersion	Hydrophilic cream	SLN-containing hydrophilic cream
PCS diameter (nm)	201.4 ± 4.7	-	-
PI	0.220 ± 0.022	-	-
LD50% (µm)	0.273 ± 0.007	1.312 ± 0.106	1.932 ± 0.039
LD90% (µm)	0.561 ± 0.003	3.891 ± 0.271	4.529 ± 0.161
LD95% (µm)	0.647 ± 0.005	4.754 ± 0.156	5.773 ± 0.186

As shown in Table XXXVI, a mean particle size of approximately 200 nm could be obtained for clotrimazole-loaded SLN prepared with 20% of lipid phase. LD measurements revealed that 95% of the nanoparticles were smaller than 650 nm, showing a considerable small

particle size and a narrow distribution (PI < 0.220). With regard to incorporation of SLN into hydrophilic cream, size analysis by LD led to a superposition of the size distribution of the emulsion droplets and the SLN. LD data of the SLN-containing o/w hydrophilic cream are also shown in Table XXXVI (right column). Due to the relatively weak scattering intensity of 200 nm particles versus 2-5 μm droplets ($I \approx R^3/r^6$), separate analysis of the SLN inside of the emulsion was not possible. In a previous study, a rather complex separation procedure was developed for a special cream system [203]. This cannot be transferred directly to any of these creams. Therefore, a simple approach was taken, i.e. transferring the microscopic analysis method for parenteral fat emulsions [365]. The samples were placed undiluted on a cover slip and analysed microscopically to screen for aggregates of approximately 1-5 μm. In polarised light irregularly shaped crystalline SLN aggregates can be differentiated from spherical oil droplets. No major aggregation was observed (i.e. less than 2 particles > 1 μm/microscopic field).

5.2.2 Clotrimazole release profile from semi-solid formulations

The release of active substances from the lipid matrix is influenced by the crystal structure of the lipid molecules. As described in the chapter 4 of the present thesis, Dynasan®116-based SLN show an orthorhombic lamellar lattice structure and the drug might be entrapped between the lipid lamellae. From SEM pictures no drug crystals have been observed on the surface of the SLN (Fig. 4.2, section 4.1.2), therefore the release profile obtained for D116SLN_Co might be only due to drug diffusion from the solid matrix of the lipid nanoparticles.

Drug penetration into certain layers of the skin can be achieved using SLN or NLC as a consequence of the creation of a supersaturated system [16, 17]. Supersaturation is a very useful method of enhancing the permeation of drugs across membranes such as skin, because unlike other methods, it does not interfere with the ultrastructure of the stratum corneum. Incorporation of lipid nanoparticles such as SLN and NLC into topical formulations (creams, ointments, emulsions, gels) can be performed to create supersaturated systems. The increase in saturation solubility will lead to an increased diffusion pressure of drug into the skin.

The developed semi-solid systems consist of a three-dimensional vehicle (hydrogel or hydrophilic cream) which encloses and it is interpenetrated by an aqueous SLN or NLC dispersion. During storage, the drug remains in the lipid matrix because these particles preserve their modification. After application onto the skin, the increase in temperature and

water loss observed lead to transformation to a more ordered lipid modification and are responsible for drug expulsion from the lipid matrix. The drug is expelled into the semi-solid vehicle (hydrogel or hydrophilic cream) already saturated with drug and thus leading to supersaturation. This phenomenon increases the thermoactivity and leads to drug penetration into the skin. A schematic representation of this theory is shown in Fig. 5.13.

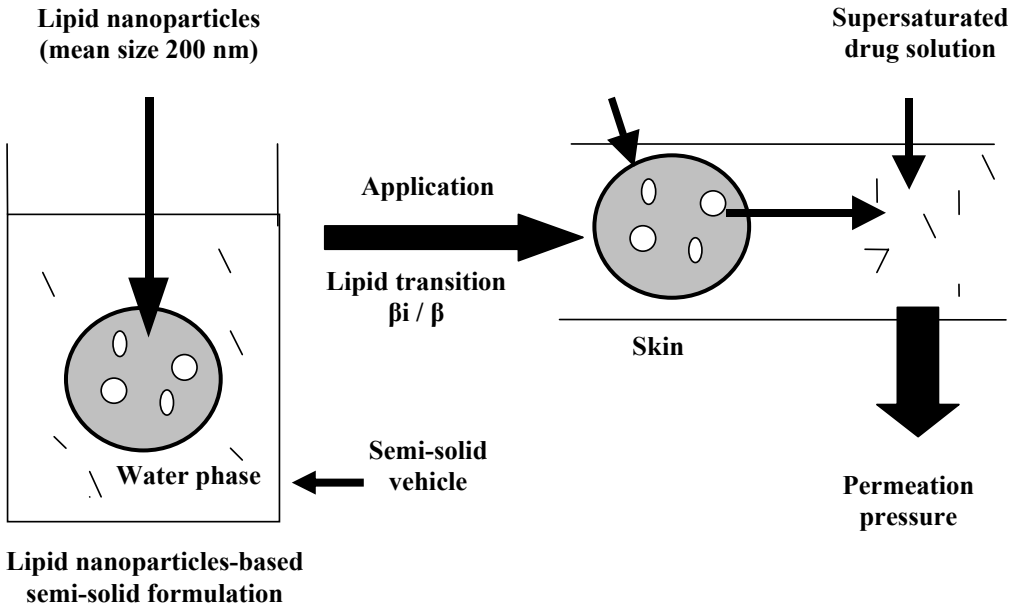

Fig. 5.13: Phenomenon of triggered drug release and supersaturation effect of lipid nanoparticles entrapped into a dermatological formulation, i.e. semi-solid vehicle (modified after Müller *et al.* [16]).

Concerning the release properties of the developed formulations, the jellifying agent can modify the observed diffusivity of the drug from lipid nanoparticles by either mechanically impeding its movement or by adsorbing the drug on the polymer surface. Since the actual pathway for diffusion in gels is through the fluid phase, the factors which affect diffusivity in pure liquid phases similarly control diffusion within the gels.

It the present work, it has only been concerned how the presence of a solid phase (lipid nanoparticles) further alters the diffusivity of the drug, concentrating first on situations in which the regions available for diffusion are very large compared to the hydrodynamic volume of the drug.

Concerning the drug release from nanoparticles, most published studies involve Franz-type cells [160, 188, 304, 366]. Therefore, for the study of clotrimazole release profile Franz diffusion cells were also used, separating the acceptor fluid and the tested formulations with a lipophilic membrane, i.e. cellulose nitrate membrane previously treated with isopropyl

myristate. The release profile was followed for 24 hr and the profiles are a mean of 3 independent tests, i.e. three Franz cells for each tested formulation.

The diffusion process through the membrane is most often lead by the thermodynamic activity c/c_s, showing linear increasing diffusion rates with increasing thermodynamic activities [367]. For a given vehicle the concentration c of a drug within the vehicle can be used instead of thermodynamic activity because the saturation c_s is constant. In the case of the tested different nanoparticles and commercial formulations the saturation solubility is not constant. Due to experimental difficulties the saturation concentration of clotrimazole in lipid nanoparticles cannot be measured directly. Instead, c_s has been calculated by addition of the solubilities in Miglyol®812 and in solid Dynasan®116. The solubility of clotrimazole in Miglyol®812 at room temperature was higher than 5% and in solid Dynasan®116 was roughly 10%. The amount of clotrimazole penetrated into the acceptor compartment was determined against an appropriate linear calibration curve at 243 nm. The assay was linear ($R^2 > 0.9991$) in the concentration range of 25 and 150 µg/ml (Fig. 3.13, section 3.2.8). According to the Fick's second law of diffusion, the total amount of clotrimazole (Qt) appearing in the acceptor solution in time t is expressed as follows [207]:

$$Q_t = AKLC_0\left[\left(\frac{D_t}{L^2}\right)-\left(\frac{1}{6}\right)-\left(\frac{2}{\pi^2}\right)-\sum\left(\frac{(-1)^n}{n^2}\right)\exp\left(\frac{D^n 2\pi^2 t}{L^2}\right)\right] \quad (28)$$

where A is the effective diffusion area, C_0 is the initial concentration of drug which remains constant in the semi-solid formulation, D is the diffusion coefficient, L is the thickness of the diffusion membrane and K is the partition coefficient of the drug between membrane and formulation. At steady-state, the previous equation can be simplified in the following equation:

$$\frac{Q_t}{A} = KLC_0\left[\left(\frac{D_t}{L^2}\right)-\left(\frac{1}{6}\right)\right] \quad (29)$$

The flux of drug through the diffusion membrane is calculated as the amount of diffused drug divided by A *vs* time. Therefore, from the latter equation the flux, J, can be determined as follows:

$$J = C_0\frac{KD}{L} = C_0K_p \quad (30)$$

where K_p is the permeability coefficient.

Fig. 5.14 shows the amount per cm^2 of clotrimazole released from the studied formulations as a function of time. It is clearly visible that clotrimazole was faster released from the tested commercial formulations than from the SLN dispersions. After 1 hr the cumulative amount of

clotrimazole was higher than 0.6 mg/cm^2 for both commercial creams, while at the same period aqueous SLN dispersions showed a value lower than 0.15 mg/cm^2. SLN could retard clotrimazole release by the fact that the drug molecules are entrapped in the lipid matrix. The recorded J value for aqueous SLN dispersion was 45.6±0.7 µg/cm^2/hr. For commercial creams, i.e. Canesten® and Fungizid-ratiopharm® cream, the obtained values were 86.3±0.6 µg/cm^2/hr and 89.5±0.7 µg/cm^2/hr, respectively. To sum up, the percentage of drug released determined after 24 hr was more than 50% for both commercial formulations and not higher than 30% for the developed SLN formulation.

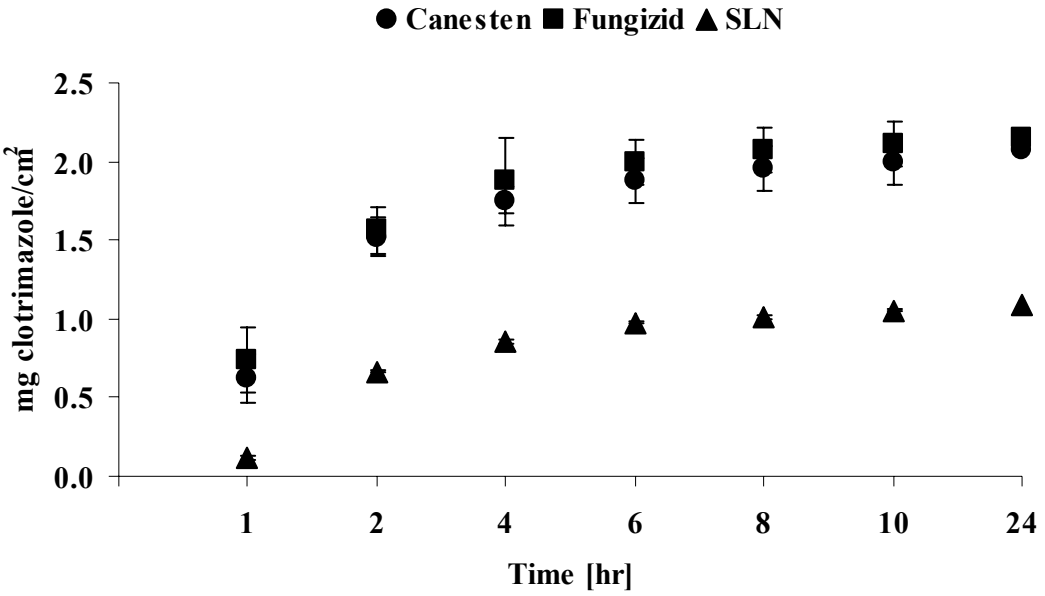

Fig. 5.14: *In vitro* release of clotrimazole from commercial formulations and aqueous SLN dispersion (20% SLN) in 100 mM acetate buffer, pH 6.0 with 35% (v/v) of dioxane, at 32ºC.

In order to improve the therapeutic efficacy of clotrimazole, a sustained release of this drug over a period of several hours might be highly beneficial. The release of incorporated clotrimazole should differ from the drug in the commercial creams because of the solid matrix of the former and subsequently drug immobilization [33]. Therefore, a modified release profile of the drug is guaranteed. This observation allows asserting that the selected excipients have different influences on drug release profiles, emphasizing the importance of SLN for the development of a new sustained delivery system for this antifungal agent.

Whenever a new solid dosage form is developed or produced, it is necessary to ensure that drug release occurs in an appropriate manner. The quantitative analysis of the values obtained in the release studies can easily be analysed using mathematical models that express the results as a function of some characteristic of the dosage form. The obtained profile for the

release of clotrimazole from aqueous SLN dispersion closely resembles to the ones obtained with commercial creams, however with lower J values, i.e. the curves show the same shape (Fig. 5.14). At periodic intervals, the concentration of clotrimazole has been determined. Table XXXVII shows the regression equations of *in vitro* release of clotrimazole from lipid nanoparticles, according to different release models.

Table XXXVII: The regression equations of *in vitro* release of clotrimazole from lipid nanoparticles, according to different release models.

Release model	Regression equation	R^2
Zero order	$Q_1=Q_0+K_0t$	0.01628
First order	$logQ_1=logQ_0+\frac{K_1}{2.303}t$	0.23323
Higuchi	$Q_t=K_H\sqrt{t}$	0.97527
Weibull	$log\left[-ln\left(1-\left(Q_t/Q_\infty\right)\right)\right]_1=b\times logt-log\,a$	0.93262

The most suitable model that describes the release profile of clotrimazole from lipid nanoparticles is the Higuchi model, which is in accordance to the literature [368, 369]. Table XXXVII shows for this model a R^2 close to 1 (approximately 0.9753). The Higuchi model has been adjusted to the clotrimazole release profile from aqueous SLN dispersion and is represented in Fig. 5.15.

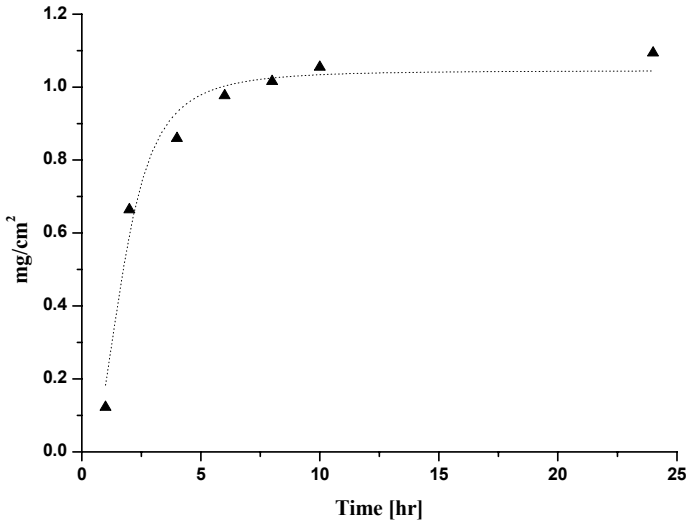

Fig. 5.15: Comparison between the release profile of clotrimazole obtained from SLN (▲) and the theoretical Higuchi model (···).

The Higuchi model describes the dissolution of drugs in suspension from ointment bases, but it is also in accordance to other dissolution profiles obtained from many other pharmaceutical dosage forms [370]. This model can be represented as shown in Fig. 5.16.

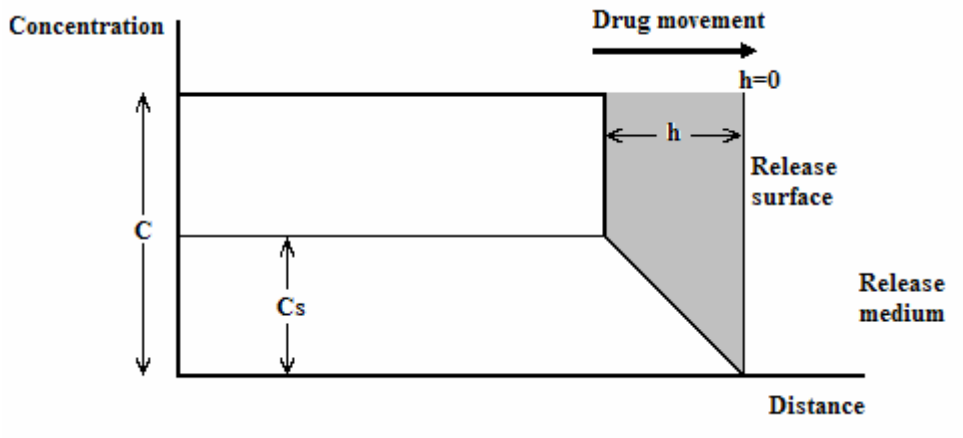

Fig. 5.16: Theoretical release profile from a matrix system in contact to a perfect sink release medium (modified after Costa and Sousa Lobo [370]).

The solid line represents the variation of drug concentration in the system, after time t, in the matrix layer normal to the release surface, being the drug rapidly diffused (perfect sink conditions). The total drug concentration would be expected to show a sharp discontinuity at distance h and no drug dissolution could occur until the concentration drops below the matrix drug solubility (Cs). To distances higher than h, the concentration gradient will be constant, provided C >> Cs. The linearity of the gradient over this distance follows the Fick's first law, as expected from the SLN theory (section 2.4.1). At time t the amount of drug released by the system corresponds to the shaded area in Fig. 5.16. Returning to Fig. 5.14, in the first 2 hr 17% of clotrimazole was measured, while in the following 8 hr the cumulative amount of drug released increased to 27% (Fig. 5.14). The longest release time was up to 10 hr. The results demonstrate a burst effect followed a slow and continuous release. The release of clotrimazole from lipid nanoparticles was faster in the first hours probably because the drug was adsorbed onto the nanoparticles surface rather than entrapped into the nanoparticles core. The cumulative clotrimazole release of the new SLN formulation is about 2-fold lower after 24 hr than from the commercial formulations. This is an indication for the excellent suitability of this vehicle for clotrimazole.

The advantage of the development of vehicles based on hydrogels (carbomer gels) is, on one hand, the transparent semi-solid preparation with high stability, and on the other hand, an additional 2-fold decrease in drug release compared to traditional formulations. In addition,

Carbopol®934 gels and the lipid used for the preparation of lipid nanoparticles (tripalmitin) have excellent skin properties. The first because of its thermal stability and optimum rheological properties, and the second because the lipid composition of the epidermis is mainly based on triacylglycerols (25%) [220]. To sum up, advantages of the new formulations based on hydrogels for skin are their suitable viscosity, transparency and high chemical stability of drug in the formulation, probably due to the special gel microstructure. Moreover, a lower drug release can be achieved. An additional advantage is the possibility of combining the drug-loaded SLN with the gel hydrophilic matrices, which results in an excellent adhering and constant releasing formulation. The resulting systems are well balanced formulations offering a wide field for topical treatment of antifungal diseases. The fact that more polar materials such as carbopol hydrogel matrices adhere excellently on skin may be partly due to the humidity of skin and due to the presence of hair follicles and sweat glands, which contains aqueous channels.

In conclusion, the developed systems are promising alternative drug carriers for topical pharmaceutics. Controlled release purposes have been accomplished by incorporating clotrimazole into the solid matrix of Dynasan®116-based lipid nanoparticles.

5.2.3 Mechanical characterization of semi-solid formulations

Creams, such as hydrophilic cream, are dispersions of two immiscible liquids, the oil of the internal phase and water (external or continuous phase). The presence of emulsifying molecules at the interface of oil and water decreases the interfacial free energy forming a homogeneous and stable cream. Incorporation of lipid nanoparticles might affect the stability or structure of the semi-solid system. Rheological behaviour of such systems provides qualitative and quantitative information of the internal structure of the cream. In addition, it relates also to the spreading, i.e. application behaviour, during administration to the skin (e.g. yield value should not be too low). Therefore, the prepared SLN-containing hydrophilic cream has been evaluated in comparison to commercial creams such as Canesten® and Fungizid-ratiopharm® cream. Fig. 5.17 represents the plots of shear rate as a function of shear stress for commercial creams selected for the present study, as well as for hydrophilic cream containing 20% (m/m) of aqueous SLN dispersion.

All formulations behaved as a dilatant system, revealing extremely low shear rates with the increase of the shear stress. The most linear shear rate under increasing shear stress was

Canesten® cream. The presence of 20% of SLN into hydrophilic cream slightly increased the recorded values of shear rate.

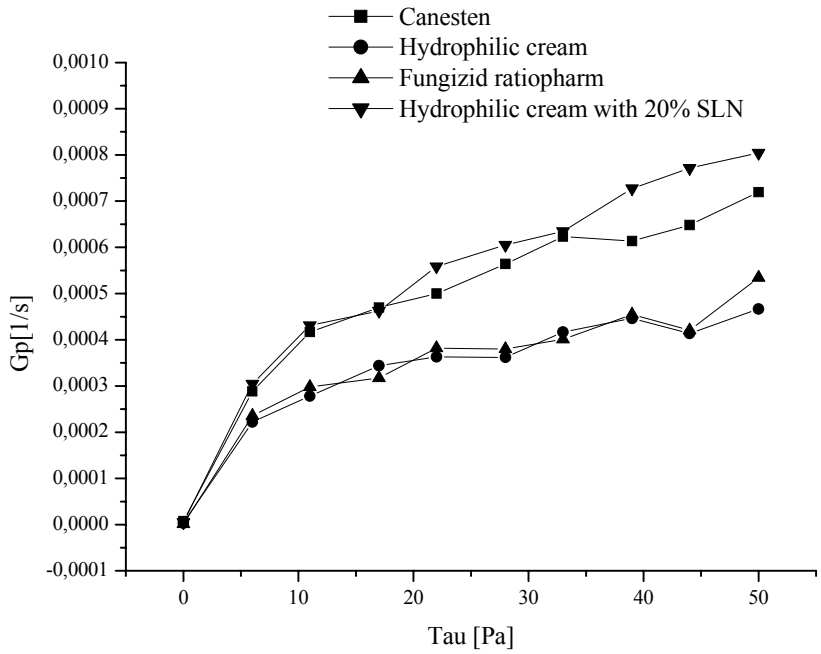

Fig. 5.17: Shear rate as a function of shear stress of Canesten® cream, Fungizid-ratiopharm® cream, hydrophilic cream and hydrophilic cream containing 20% (m/m) of aqueous SLN dispersion.

Fig. 5.18 shows the influence in the shear rate of the increase of SLN concentration into hydrophilic cream. Incorporation of 30% and 40% of aqueous SLN dispersion into hydrophilic cream resulted in shear-thinning and pseudoplastic semi-solid systems, revealing the presence of a yield value. The yield value of conventional creams is usually 9 Pa; however, the tested commercial creams did not show this typical behaviour (Fig. 5.17). Incorporation of 30% of SLN leaded to a yield value of 28 Pa and the presence of 40% of SLN increased this latter value to 39 Pa (Fig. 5.18). At steady-state, lipid nanoparticles confer some rigidity to the system but, as the flow starts the structure begins to break down and viscosity decreases. Viscoelastic and shear-thinning properties, as well as the presence of a yield value are optimal properties for predicting the stability of semi-solid systems such as creams, since these latter maintain their consistency during storage time and are afterwards easy to apply [371]. These results are probably due to the creation of a kind of gel network structures by SLN. Such a model was proposed for aqueous lipid particle dispersions with 30% to 50% of lipid content. The particles form a network similar to an Aerosil® gel, like

peals on a necklace [372]. The increase of SLN concentration had clearly the highest yield value point (Fig. 5.18). Commercial creams and SLN-free hydrophilic cream did not show yield points at the applied shear stress range.

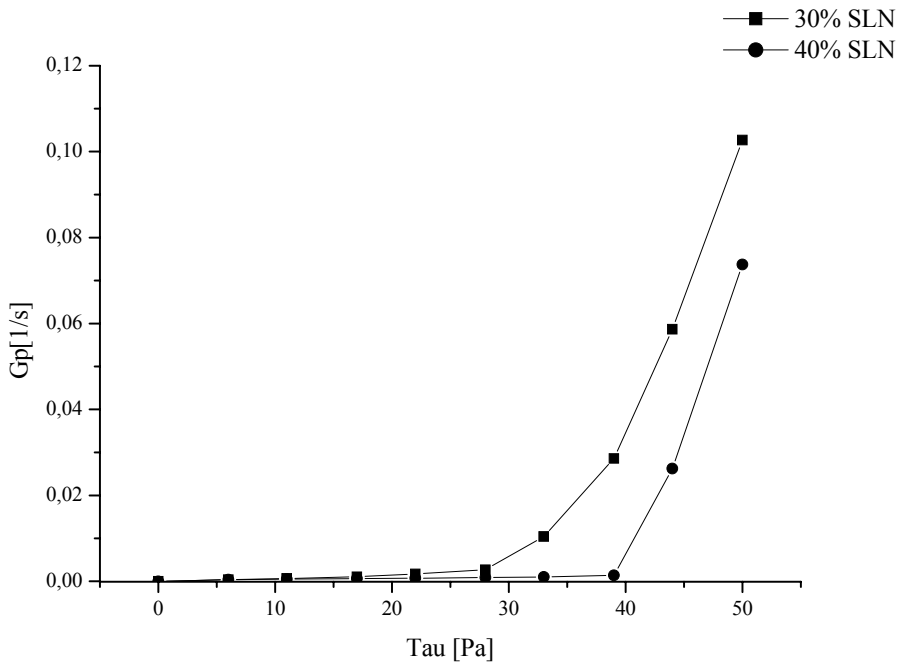

Fig. 5.18: Shear rate as a function of shear stress of hydrophilic cream containing 30% and 40% (m/m) of aqueous SLN dispersion.

Yield stress can be defined as the minimum stress that must be applied before the material really starts to flow [373]. This is an important parameter for topical gels. Yield stress of these materials should be high enough so that they do not flow out of their container due to their own weight if the container is placed in upside down position. It should not be too high to offer significant resistance during the application onto skin. The cross-linked microgel structure where individual particles are closely packed with their neighbours is responsible for the yield stress. The magnitude of the yield stress is a measure of the strength of the closed-pack structure that must be exceeded for the material to flow appreciably. The values of the yield stress of hydrophilic cream containing SLN dispersions were approximately 10 Pa and 35 Pa for 30% and 40% of dispersion, respectively (Fig. 5.18). The values obtained are much higher than the ones observed for the hydrophilic cream alone and also for the commercial creams applying the same evaluation method (Fig. 5.17). Moreover, hydrophilic cream could not even show yield values. The higher observed yield values in the current study are due to

the higher viscosity caused by the addition of aqueous SLN dispersion and higher attraction forces between dispersed particles, as well as between particles and oil droplets.

The observed differences between SLN-free and SLN-loaded formulations at low shear rates indicate that inter-particle forces, as well as the type of the interactions between lipid nanoparticles (i.e. electrostatic versus steric), control the flow behaviour of such systems. During shear flow investigations, SLN can come closer together and form pearl-like structures due to van der Waals attractive forces. Higher resistance to flow and higher degree of shear-thinning behaviour observed in Fig. 5.18 are due to breakdown of the structures with imposed shear stress. The presence of compact, organized and strong pearl-like network structures inside the hydrophilic cream can have significant beneficial effects on the stability and rheological properties of such topical formulations.