LANGMUIR

pubs.acs.org/Langmuir Article

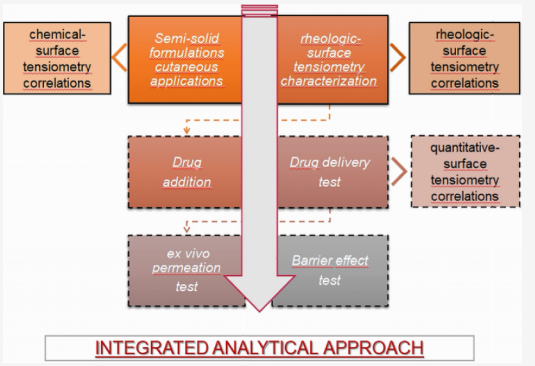
Characterization of Structure-Surface Correlations in Ointments Using Surface Tensiometry within the Concept of an Integrated Analytical Approach

Davide Rossi,* Giulia Lazzari, Erica Franceschinis, Elisa Vettorato, and Nicola Realdon

Cite This: https://doi.org/10.1021/acs.langmuir.4c01613 Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: In recent years, integrated data analysis has proven to be a valuable method for investigating complex systems, whether artificial or natural. The integrated analytical approach allows the simultaneous integration of data acquired from different analytical techniques employed for the same sample at the same time, leading to an expansion of the amount of information available. Surface tensiometry is a technique that was recently introduced in the Integrated Analytical Approach for investigating pharmaceutical dosage forms for topical application. Therefore, studies of rheological characterization, release, and skin permeation can be integrated with surface tensiometry measurements to develop chemical and rheological-surface correlation models, providing a new method for the quality control and process control of semisolid preparations. In this context, the aim of this research is to validate the utility of surface tensiometry measurements in the Integrated Analytical Approach and utilize these data to gain insights into the structure-surface correlation. The preparations chosen for this study were a PEG-gel, a lipogel, and an O/W cream containing carnitine as a model drug. The formulations were characterized using rheological measurements and evaluated for their carnitine release performance. Furthermore, surface tensiometry measurements were performed to rapidly and noninvasively assess the influence of carnitine on the surface properties of the semisolid preparations investigated. Our work demonstrated a close correlation between surface energy and structural data, showing the importance of surface tensiometry contribution in the noninvasive and rapid evaluation of the presence of carnitine in semisolid formulations.



INTRODUCTION

The Integrated Analytical Approach (IAA) is an investigative methodology that integrates various pieces of information obtained from different analytical techniques within a specific analytical field to study complex systems. Over the past 20 years, IAA has been applied across a wide range of research fields. For example, in the health sector, the IAA has enabled the identification and quantification of androgenic substance residues in the plasma and urine of slaughtered animals through the integrated use of qualitative and quantitative analyses (such as high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry).[1] In the chemical-mineralogical field, IAA has been applied for the characterization of residues of organic glass components through the integrated use of gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR).[2] The literature defines the term'integrated analytical approach' as the integration of data from different analytical techniques within the same field of investigation (e.g., chemical).[3−6] The concept of an integrated analytical approach has also been extended to the integration of information in fields such as economics[7] and psychology.[8]

In the chemical-physical field, the IAA mainly focuses on the integration of various destructive analytical techniques, including spectroscopic, diffractometry, and qualitative-quantitative methods. More recently, surface tensiometry has been introduced into the IAA to investigate the physicochemical properties of thermal waters intended as complex systems containing elements of varying nature.[9] Additionally, IAA has been used in the analysis of Lourdes waters[10,11] as part of an international project with the primary objective of assessing chemical-surface correlations. IAA was recently defined as'an integrated approach to study the chemical composition (e.g., by spectroscopic methods), bulk structure (e.g., by rheological methods), and surface properties (e.g., by surface tensiometry

Received: May 1, 2024
Revised: August 6, 2024
Accepted: August 7, 2024

approach) of the same material sample and at the same time (IAA^s_t).[12] The IAA^s_t, which applies to the study of the "same sample at the same time" allows the simultaneous management of data from various analytical techniques used across diverse research areas, limiting data losses over time and including surface tensiometry as a noninvasive analytical approach. Surface tensiometry is applied to liquid and solid substrates, but it can also be used for semisolid materials, such as semisolid preparations for cutaneous applications that are considered complex, heterogeneous systems.[13] The physical and mechanical properties of semisolid formulations directly affect the interface between the skin and the formulation; therefore, it is important to study the rheology, consistency, surface tensiometry, and sensory properties of these preparations to evaluate this interaction.[14] The rheological investigation establishes the relationship between the effects induced by the application of forces external to the system, the molecular structure of the complex system under consideration, and the intermolecular interactions.[15] In general, rheology provides extensive information about the structure and consistency of the preparation. The rheological analyses are carried out for multiple reasons, including understanding the nature of the system under examination and checking the quality. The complexity of these systems leads to multiple interconnections and interactions between their various components, often making it difficult to characterize them and, consequently, to understand, describe, manage, and modify their nature.

Therefore, the main aim of this study is to characterize the behavior of the *structure-surface* correlations of lipogel, oil-in-water (W/O) cream, and polyethylene glycol gel (PEG-gel) in the application of IAA^s_t, using surface tensiometry by the contact angle method (CA). The goal of characterizing these structure-surface correlations is to introduce surface tensiometry into the technological characterization of pharmaceutical semisolid formulations, thereby developing a noninvasive and fast method for their quality assessment.

EXPERIMENTAL SECTION

The materials used for the preparation of the PEG gels, O/W creams, and lipogels were cetostearyl alcohol, beeswax, cholesterol, olive oil, liquid paraffin, PEG 400, PEG 4000, and white paraffin, all purchased from A.C.E.F. (Italy). Carnitine was obtained from Biokosmes (Italy), Cetomacrogol 1000 was purchased from Farmalabor (Italy), and Nipagina was purchased from Fagron (Italy).

Preparation of PEG Gels and Lipogels. The compositions of PEG-gels and lipogels are reported in Tables 1 and 2. The preparation

Table 1. PEG-Gel Composition

	% w/w	
materials	A	B
PEG 400	70	60
PEG 4000	30	40

of PEG gels and lipogels followed the same formulation steps. All components were melted at 70 °C, and once the mixture reached a clear and homogeneous consistency, gelation was carried out under constant stirring with a paddle stirrer equipped with a wire mesh rotor set at 60 rpm. The PEG-gels and lipogels were then transferred to a glass container and stored at room temperature away from light.

Preparation of O/W Creams. The compositions of the O/W creams are shown in Table 3. For the preparation of O/W cream, the aqueous phase and the oily phase were prepared separately. All

Table 2. Lipogel Composition

	% w/w		
materials	A	B	C
beeswax	10	8	6
cholesterol	3	3	3
cetostearyl alcohol	4	3	2
Milli-Q water	-	-	-
white paraffin	-	up to 100	-
olive oil	up to 100	-	up to 100

Table 3. O/W Cream Composition

phases	materials	% w/w
oily		
	liquid paraffin	21
	cetostearyl alcohol	5
	Cetomacrogol 1000	1.8
water	Nipagin	0.15
	Milli-Q water	up to 100

components of the oily phase were melted at 70 °C. The aqueous phase was prepared by boiling the water and solubilizing the nipagin. Emulsification was carried out by pouring the aqueous phase at 75 °C into the oily phase at 70 °C using a turbo emulsifier (Silverson, SL2, Italy) operated at 700 rpm for 2 min. Gelation was improved using a paddle stirrer at 40 rpm. The preparation was transferred to a glass container and stored at room temperature away from light.

Ointments Containing Carnitine. Formulations named Lipogel C and the Cream were prepared by incorporating 4% (w/w) of carnitine. To prepare carnitine-containing lipogel, the active was dissolved in a small amount of Milli-Q water at 50 °C. The aqueous solution was added to the preparation at the onset of gelation. The fully gelled lipogel was then transferred to an aluminum-wrapped glass container and stored at room temperature, away from light. To prepare the carnitine-containing cream, carnitine was added to the aqueous phase prior to the emulsification process. After gelation, the cream was transferred to an aluminum-wrapped glass container and stored at room temperature, away from light. The composition of each preparation is reported in Tables 4 and 5.

Table 4. Composition of Lipogel C with Carnitine

materials	% w/w
carnitine	4
beeswax	6
cholesterol	3
cetostearyl alcohol	2
Milli-Q water	10
olive oil	up to 100

Surface Tensiometry Analysis. The liquid tests used for the surface tensiometry analysis to evaluate the wettability of the formulations included benzyl alcohol, diiodomethane, ethylene glycol, glycerol, formamide, Fomblin HC/25 PFPE, and Milli-Q water.

Table 5. Composition of O/W Cream with Carnitine

phases	materials	% w/w
oily	liquid paraffin	21
	cetostearyl alcohol	5
	Cetomacrogol 1000	1.8
water	carnitine	4
	Nipagin	0.15
	Milli-Q water	up to 100

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

Table 6. Properties of the Test Liquids Used for Wettability Measurements

liquid	MW (g/mol)	viscosity (mPa·s) at 20 °C	density (g/cm³)	surface tension (mN/m)	DC (mN/m)	PC (mN/m)
benzyl alcohol	108.18	6.60	1.04	38.90	29.00	9.90
diiodomethane	267.84	2.80	3.30	50.00	50.80	0.00
ethylene glycol	62.07	21.00	1.11	48.00	29.30	19.00
glycerol	92.90	1410	1.23	62.70	21.20	41.50
formamide	45.04	3.75	1.13	59.00	39.40	19.60
Fomblin HC/25 PFPE	-	5560	1.90	18.10	18.00	0.10
Milli-Q water	18.02	1.00	1.00	72.80	21.80	51.00

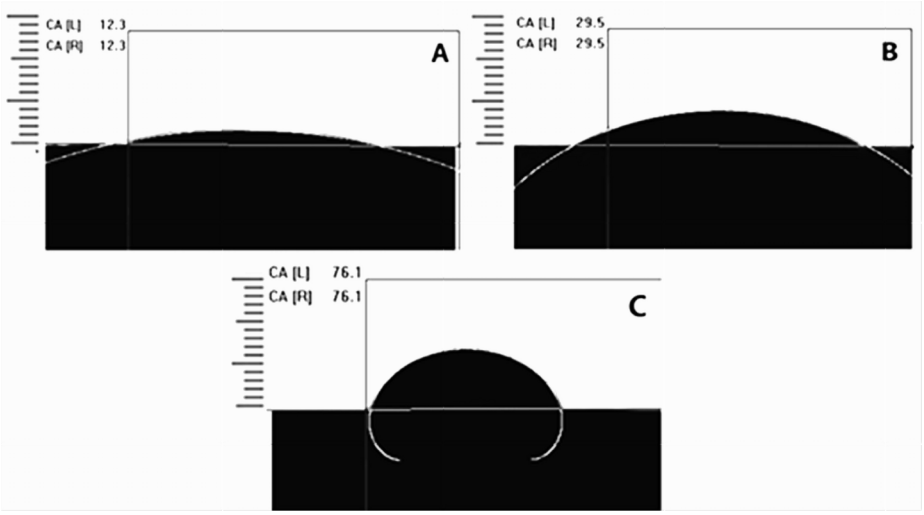


Figure 1. Water drop profiles on heterogeneous and smooth surfaces of (A) PEG-gel, (B) O/W creams, and (C) lipogel.

Benzyl alcohol, diiodomethane, ethylene glycol, glycerol, formamide, and Milli-Q water were purchased from Sigma-Aldrich (Germany), while Fomblin HC/25 PFPE was sourced from Solvay (Belgium). The properties of all the liquid tests are listed in Table 6.

Wettability analyses of semisolid preparations for skin application loaded with 4% (w/w) carnitine were conducted 7 days after preparation to ensure complete stabilization of the system. The analyses were performed at a controlled temperature of 20 °C ± 2 using a static optical goniometer (Drop Shape Analyzer DSA30, Kruss, Germany), equipped with a camera in line with a light source and operated with DSA4 software (Kruss, Germany). The sample was placed on a support and positioned between the light source and the camera, resulting in an image of the test liquid drop on the surface. The liquid test drop was produced by manually actuating the plunger of a syringe placed orthogonally above the sample and fixed on a support. Surface tensiometry measurements were taken every 2 frames/s for a total of 2500 frames, corresponding to a total duration of 200 s. The instrument is equipped with dedicated software (Drop Shape Analysis software, Kruss, Germany) that performs wettability measurements over time. For each test liquid, three measurements were conducted (N = 3). Generally, the contact angle (CA: deg) method was used to determine the Interfacial Free Energy (IFE: mJ/m^2) using the Young-Duprè equation, as well as the Surface Free Energy (SFE) of solid substrates and the Surface Tension (ST) of liquids. The ST of liquids is expressed in mN/m due to variations in the liquid drop surface. The SFE of solid substrates is expressed in mJ/m^2 because of the variations in the surface area (SA: mm^2) during the spreading of the liquid on their surface. The apparent CA can be measured macroscopically[16] using the Young−Laplace (Y-L) method, and it is related to the nature of the surface of the solid substrate.[16] Semisolid preparations for skin applications, such as ointments and O/W and W/O creams, are chemically heterogeneous but exhibit a smooth surface due to the presence of liquid components that reduce surface roughness. For this reason, the differences between actual and apparent CAs measured onto the surface of semisolid substrates by a static contact angle (SCA) are negligible. The Circle Fitting (CIR) method allows measuring the apparent CA[17] in nonhydrophobic and nonsuperhydrophobic situations (<180°), although the drop volume (μL) should be as low as possible (critical water drop volume 2 μL corresponding to CA error of 3°).[18] For example, in the case of a volume of water drop of 6.7 μL, the error that occurred in the CIR is −5.7°.[18] The method of CIR allows us to obtain an apparent CA value that is not influenced by the observed distortion. In general, the distortions (or flattening) in the measurements of the CAs carried out in static mode (SCA) occur at the point of contact between drops of water and solid matrices due to the force of gravity. In this work, the CIR fitting was used in the measurement of apparent drops CA over time applying the Kinetic Contact Angle (KCA) methodology[10] to evaluate its suitability for the wettability characterization of semisolid preparations. CAs can be determined in static (Static Contact Angle, SCA) and kinetic (Kinetic Contact Angle, KCA) modalities.[10] The SCA mode considers the measurement of the CA formed at the solid/liquid interface after 0.5 s from the contact between the test liquid and the substrate. The CA measure by SCA is determined mainly by the Young−Laplace method when it is not affected by distortions due to the surface roughness factor (r) and for values <180°. Differently from the dynamic contact angle (DCA) methodology, the KCA method allows us to determine only the spread ratio of the drop of a liquid left on the surface of a semisolid preparation over time. The spreading speed of a drop on a solid surface can be determined in different ways. For example, the spreading velocity of a liquid drop (V; m/s) can be calculated using eqs 1 and/or 2.[19,20]

$$V = dH/dt \tag{1}$$

$$V = \sqrt{2 \cdot g \cdot H} \tag{2}$$

Where H is the drop height, dH is the variation of the drop height over time, dt is the time, and g is the gravity constant. To improve the precision and accuracy of the analysis of the wettability of heterogeneous semisolid preparations, the spreading velocity of the

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

drop (V) on the smooth surfaces of ointments and creams was determined as a function of the variation in surface area (SA: mm^2) over time (t). In this context, the spreading drop speed over time was defined as the Horizontal Drop Speed (HDS) to distinguish the horizontal movement of the drop on solid or semisolid surfaces from the Vertical Drop Contact Angle (VDCA). Infact, VDCA led to the evaluation of the vertical diffusion rate of a drop of liquid through a liquid film of liquid perfluoropolyether (PFPEf) used as a "solid substrate" within the Solid-like Methodology (SLM).[21,22]

The HDS equation is as follows (eq 3):

$$V = \mathrm{d}SA/\mathrm{d}t \tag{3}$$

where V is the Horizontal Drop Speed (HDS), dSA is the variation of the surface area (mm^2), and dt (ms or s) is the time.

Rheology. The rheological characterization of the formulations was performed 7 days after their preparation. A rheometer (Anton Paar, MCR 92, Italy) equipped with plate-cone geometry was used for the analysis. Measurements were carried out at a temperature of 25.0 ± 0.1 °C kept constant by a Peltier system.[23] The shear rate was set from 0 to 50 s^{-1} to observe the thixotropic behavior of the preparations. The analysis was carried out for 5 min to obtain outward and return curves. Each measurement was performed in triplicate.

RESULTS AND DISCUSSION

Figure 1 contains reported examples of the heterogeneous and smooth surface nature of the ointments and cream.

The CAs measured by SCA using the Y-L method showed different water drop profiles on heterogeneous and smooth surfaces of PEG-gel (A) (CA = 12.3°), O/W creams (B) (CA = 29.5°), and lipogel (C) (CA = 76.1°) demonstrating the measurability of test liquids CAs on ointments and cream surfaces.

PEG-Gel. Figure 2 shows the comparison between the HDS values of benzyl alcohol for the two PEG-gel formulations:

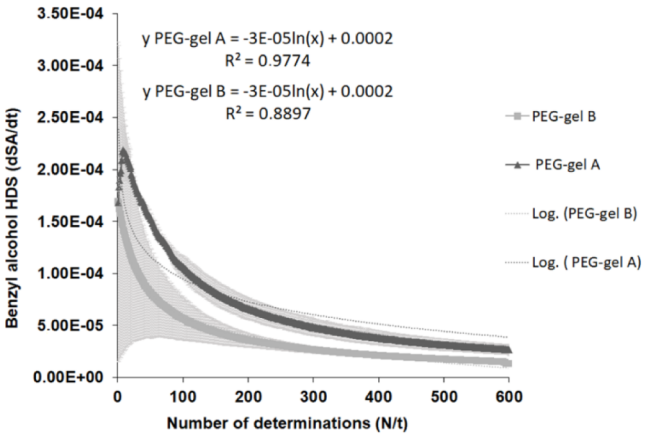


Figure 2. Comparison of the HDS measured over time of benzyl alcohol drops deposited on PEG-gel at 60% and 70% (w/w) of PEG 400 respectively (N = 3 for each preparation). Values are expressed as mean ± SD.

PEG-gel A (containing 70% (w/w) PEG 400) and PEG-gel B (containing 60% (w/w) PEG 400).

Figure 2 shows that the HDS values of benzyl alcohol determined over time were significantly higher in the case of PEG-gel A (drop volume = 0.29 μL) than in PEG-gel formulation B (drop volume = 0.30 μL). It is possible to hypothesize that this result could be due to the liquid−liquid interactions (l/l) established on the surface of the formulation with the higher content of the liquid PEG 400 excipient. These l/l interactions could alter the drop spreading rate because they can interfere with the formation of a liquid−solid interface (l/s) between benzyl alcohol and the excipient PEG 4000. Figure 2 demonstrates that the surface tensiometry parameter HDS effectively discriminates between the two PEG-gel formulations using benzyl alcohol as the test liquid, indicating its suitability for semi-solid formulations in general.

PEG-gel A (70% PEG400) showed pseudoplastic behavior with more reproducible thixotropy compared to PEG-gel B (60% PEG400). Consequently, further structure-surface correlations were performed on PEG-gel A.

Figure 3 reports the structure-surface correlations between surface tensiometry HDS and the viscosity parameters referred

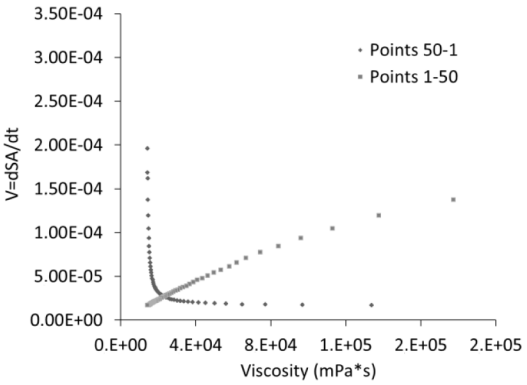


Figure 3. Correlation models between HDS (dSA/dt) and viscosity (mPa·s) for shear rates 50−1 (1/s) and 1−50 (1/s) of PEG-gel A.

to as PEG-gel A (drop volume= 0.30 μL) at 1−50 and 50−1 (1/s), respectively.

The nonlinear regression for 1−50 (1/s) shear rate fitted with a power parameter equation $f = y_0 + a{\cdot}x^b$ showing R = 0.9931, $p < 0.0001$ for coefficient y_0, p = 0.0231 for coefficient a and $p < 0.0001$ for coefficient b. The normality test resulted in p = 0.4090.

For the 50−1 (1/s) shear rate, the nonlinear regression fitted with a rational parameter I equation $f = (a + b{\cdot}x)/(1+c{\cdot}x)$ demonstrating R = 0.9912, p = 0.0004 for coefficient a, $p <$ 0.0001 for coefficients b and c with a normality test of p = 0.0001.

In contrast, after the logarithmic linearization of the nonlinear regression for shear rates from 1 to 50 (1/s) shear rate, Pearson's correlation coefficient was r_{xy} = 0.8961.

The data in Figure 3 demonstrate the correlations between the rheological data (viscosity) and surface tensiometry (HDS) in terms of the increase (1−50) and decrease (50−1) of the shear rate applied to PEG gel A. In particular, low viscosity values indicate a breakdown of the system, with modifications of the volume elements due to their orientation in the flow direction. The destructuration of the system reflects greater destruction of the surface and a consequent increase in HDS values.

Lipogel. The lipogel consists mainly of two components, beeswax and olive oil, each with distinct properties. Beeswax, the solid component, is characterized by high dispersive components (DC = 28.6 mJ/m^2) and low polar components (2.5 mJ/m^2). Olive oil, the liquid component, and is characterized by high dispersive components (DC = 14.2 mN/m) and low polar (PC = 7.2 mN/m) components. The base lipogel maintains the same balance between DC and PC of its components showing high dispersive (DC = 42.93 ± 5.30

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

mJ/m^2) and low polar (PC = 3.43 ± 1.29 mJ/m^2) components. The SFE properties of a lipogel affect its wettability with a liquid test having a specific ST. The variations of the lipogel wettability depend on variations in the concentration of its components. Figure 4 shows the

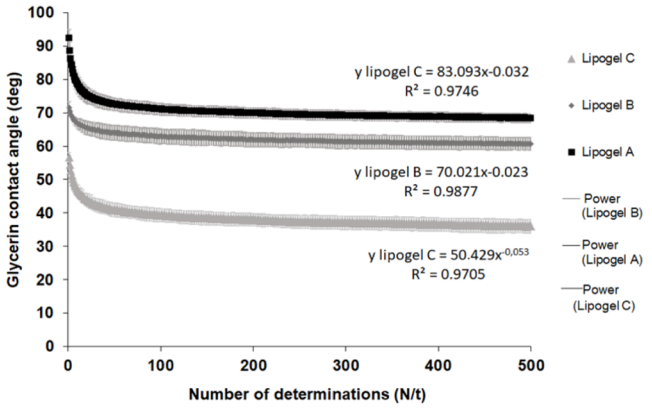


Figure 4. Comparison between contact angles measured in Lipogels A (10% w/w beeswax), B (8% w/w beeswax), and C (6% w/w beeswax) containing increasing concentrations of beeswax (N = 3 for each preparation). Values are expressed as mean ± SD.

comparison between the CAs obtained using glycerol as a polar liquid test with the three lipogel formulations containing different amounts of beeswax, droplets of which were left on the surface of Lipogel A (10% w/w beeswax), Lipogel B (8% w/w beeswax), and Lipogel C (6% w/w beeswax). The volume of glycerol droplets at the interface with Lipogels A, B, and C were, respectively, 0.78 ± 0.02 μL, 0.73 ± 0.08 μL, and 0.75 ± 0.04 μL (<2 μL).

Figure 4 demonstrates the high discriminating capacity of the glycerol in relation to the lipogel formulations with respect to the differences in percentages of beewax and olive oil. In particular, CAs of glycerol are higher due to the high concentrations of beeswax characterized by a low polar component (PC = 2.5 mJ/m^2) and appears to agree with the typical SFE, DC, and PC of the lipogel. The increase in beeswax concentration improves the consistency of the formulation, followed by a progressive decrease in the system polarity. Consequently, glycerol CAs measured over time tend to increase significantly due to their high surface tension (ST = 62.7 mN/m) and its polar component (PC = 41.5 mN/m).

Syms et al. demonstrated the relationship between the surface tension (ST: mN/m) of a liquid, CA, torque (μN·m) due to its ST (surface tension torque T_γ), Laplace pressure (T_p), and the torque resulting from the action of an external device (T_f) on a general liquid drop (e.g., weight).[24] On this basis, this work identifies torque as a possible rheological correlation parameter for solid substrates, hypothesizing a link with the CAs depending on the surface free energy (SFE: mJ/m^2) of a semisolid preparation for cutaneous application such as lipogel. Figure 5 shows the correlations between the average data of the CAs of glycerol (N = 802) and torque (1/s = 1−50).

The Pearson's correlation coefficients calculated for average CAs of 1, 2, and 3 measurement series and torque values showed respectively r_{xy} = 0.99, r_{xy} = 0.98, and r_{xy} = 0.97.

This confirms the close relationship between the surface tensiometry properties of lipogel and its physical characteristics. The increase of glycerol CAs is associated with higher

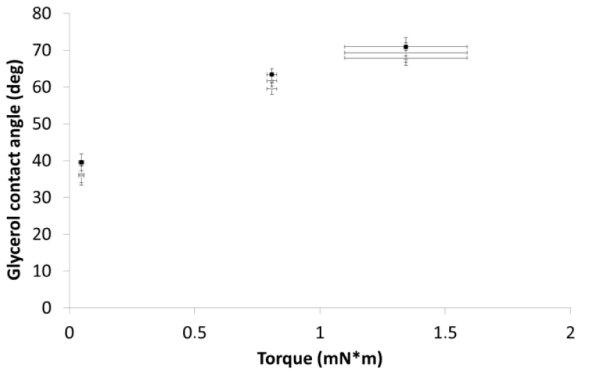


Figure 5. Comparison between the average CA of glycerol in 6, 8 and 10% beeswax of the lipogel and the average torque performed on the CAs (N = 3) over time.

torque values because of the deformation of the lipogel surface after the application of force F.

Further structure-surface investigations have been performed on Lipogel C because it showed pseudoplastic behavior with more regular respect to Lipogels A and B.

Figure 6 compares the spread behavior of water drops on Lipogel C (6% w/w beeswax) containing 4% w/w carnitine with the same formulation without the active.

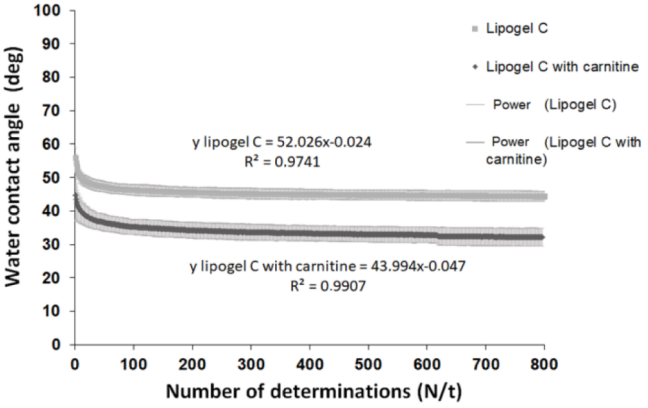


Figure 6. Lipogel C water wettability profiles with and without 4% w/w carnitine (N = 3 for each preparation). Values are expressed as mean ± SD.

Figure 6 shows that water is a test liquid capable of significantly discriminating Lipogel C with and without carnitine presenting drop volumes of 0.7 ± 0.12 and 0.47 ± 0.019 μL, respectively. The decrease in the water contact angle values over time is due to an increase in the polarity of the system after the addition of the carnitine, which is localized on the surface. In particular, the presence of 4% (w/w) carnitine on the surface of Lipogel C increases the polar interactions at the s/l interface and demonstrates the migration over time of the carnitine solutions toward the surface of the formulation. In addition, the presence of carnitine solution seems to interfere with the spreading of the water drop because it reduces the formation of the s/l interface between the beeswax and the liquid test.

In Figure 7, the comparison between surface tensiometry HDS (dSA/dt) and in Figure 8 rheological shear stress (Pa), HDS, and viscosity (mPa·s) are reported.

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

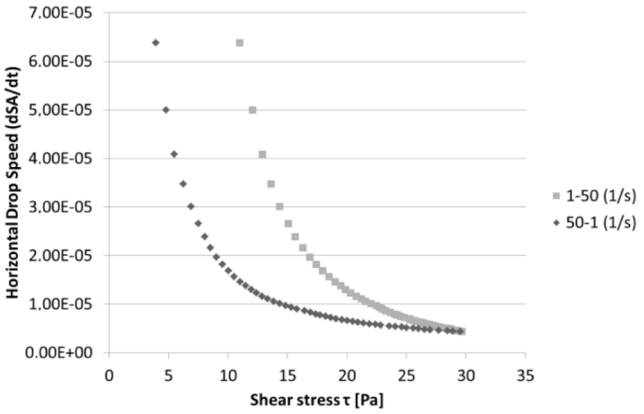


Figure 7. Comparison between HDS (d*SA*/d*t*) and shear stress (Pa) at 1−50 and 50−1 shear rates (1/s).

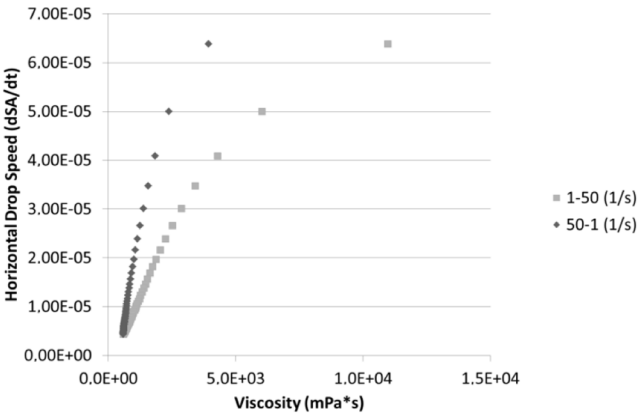


Figure 8. Comparison between HDS (d*SA*/d*t*) and viscosity (mPa·s) at 1−50 and 50−1 shear rates (1/s) of lipogel.

The nonlinear regression for shear rates 1−50 (1/s) (Figure 7) fitted with an exponential decay single parameter equation $f = a \cdot \exp(-b \cdot x)$ and demonstrated $R = 0.9890$, $p < 0.0001$ for coefficients a and b, and the normality test resulted in $p = 0.0001$.

For the shear rate of 50−1 (1/s), the nonlinear regression also fit an exponential decay single parameter equation, demonstrating $R = 0.9679$ and $p < 0.0001$ for coefficients a and b, with a normality test result of $p < 0.0001$.

After the logarithmic linearization of the nonlinear regression (d*SA*/d*t*) for 1−50 (1/s) and 50−1 (1/s) shear rates, the Pearson's correlation coefficients were respectively $r_{xy} = -0.9922$ and $r_{xy} = -0.9530$.

For the nonlinear regression shown in Figure 8, for the shear rate of 1−50 (1/s), the data fitted with a Power 2 parameter equation of the form $f = a \cdot x^b$ with $R = 0.9707$ and $p < 0.0001$ for coefficients a and b, with a normality test result of $p = 0.0007$.

Regarding the shear rate of 50−1 (1/s), the nonlinear regression demonstrated $R = 0.9441$ and $p = 0.0062$, with $p < 0.0001$ for coefficients a and b, and a normality test result of $p = 0.0131$.

The logarithmic linearization of the nonlinear regression (mPa·s) for shear rates of 1−50 (1/s) and 50−1 (1/s) showed Pearson's correlation coefficients of $r_{xy} = 0.9781$ and $r_{xy} = 0.9983$, respectively.

Figures 7 and 8 illustrate the comparison between HDS and shear stress (Pa), and between HDS and viscosity (mPa·s) for shear rates of 1−50 (1/s) and 50−1 (1/s), respectively.

Figure 9 reports the comparison between surface tensiometry HDS (dSA/dt) and viscosity (mPa·s) of Lipogel C with the same formulation containing 4% (w/w) carnitine.

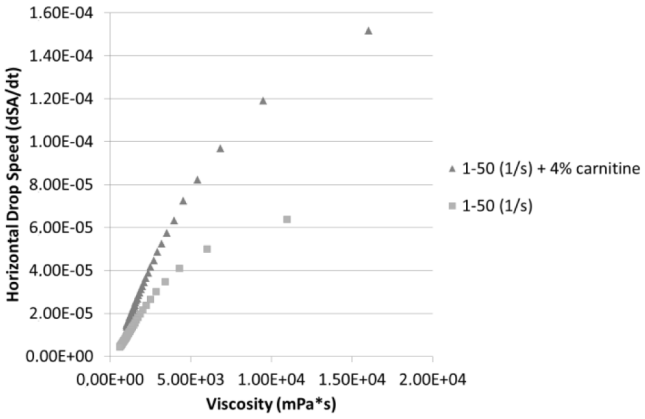


Figure 9. Comparison between HDS and mPa·s of the lipogel base charged with 4% carnitine.

For lipogels charged with 4% carnitine, the nonlinear regression shown in Figure 9 (1−50 1/s) fitted with a Power 2 parameter equation $f = a \cdot x^b$ with $R = 0.9798$ and $p < 0.0001$ for coefficients a and b, with the normality test result of $p = 0.0281$. The logarithmic linearization of the nonlinear regression (mPa·s) for the shear rate of 1−50 (1/s) showed a Pearson's correlation coefficient of $r_{xy} = 0.9796$.

Figure 9 demonstrates the higher discriminating capacity of the rheological surface tensiometry approach to evaluate the effect of the presence of carnitine on the surface and structure properties of lipogels. Figures 7−9 confirm the link between the destructuring surface of lipogels and HDS that expresses the CA behaviors of test liquid as demonstrated in the case of PEG-gel (see Figure 3). In particular, the effects of carnitine added to the lipogel resulted in higher shear stress values, which confirmed an increase in the consistency of the formulation and, consequently, a pseudoplastic trend with thixotropy. This work shows the great discrimination capacity of the surface tensiometry technique applied by KCA because the highest number of determinations of CAs data over the time ($N = 800$) corresponding at τ ($N = 100$) represented by shear rates of 1−50 and 50−1 (1/s). The differences in the data obtained with the different formulations highlight the possibility of integrating the two approaches to increase their discrimination capacity.

The results obtained from the combined rheological-surface tensiometry approach highlight the relationship between the rheological parameter, the torque (mN·m), and the glycerol (deg). Since ST (mN/m) of glycerol affects its CA, these results demonstrated the link between torque and glycerol ST, expressed, respectively, in mN·m and mN/m, within the structure-surface correlations related to Lipogel C, containing 6% (w/w) of beeswax.

Creams O/W. Figures 10 and 11 report, respectively, the comparison between the discriminating capabilities of rheological and surface tensiometry for two cream formulations (formulation E) with and without carnitine by using benzyl alcohol as a liquid test. The volume of benzyl alcohol

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

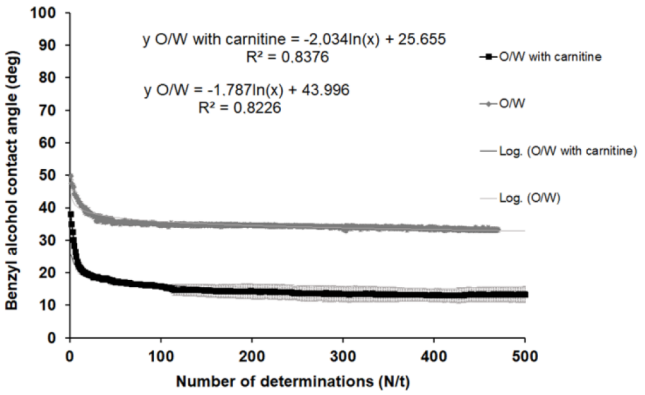


Figure 10. Comparison between surface tensiometry data of the O/W cream base and with 4% w/w carnitine represented by benzyl alcohol CA (deg).

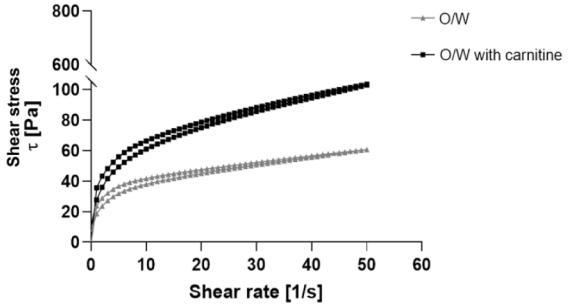


Figure 11. Comparison between rheological data of the O/W cream base and with 4% w/w carnitine represented by shear stress (Pa).

drops deposited on the surfaces of the creams with and without carnitine were 0.22 ± 0.03 μL and 0.063 ± 0.0035 (<2 μL), respectively.

Figure 10 shows that the presence of carnitine led to a significant decrease in the CAs of benzyl alcohol measured by KCA over time. Furthermore, Figure 11 shows the flow curves for the O/W cream and demonstrates that the cream containing carnitine exhibits a pseudoplastic pattern with thixotropy. Furthermore, by comparing the average flow curves of the two formulations, it was observed that the cream containing carnitine required a greater shear effort before it began to flow. The comparison between the rheological data of the O/W cream with and without carnitine showed differences due to the energetic surface variations and the high discrimination capacity of the two techniques, which were statistically significant, allowing integration of the respective data within the IAA_t^S.

CONCLUSIONS

The application of the CA method to evaluate semisolid preparations introduces a new type of interface that can be indicated with the symbol ss/l (semisolid/liquid interface). This work demonstrated the suitability of the KCA methodology for studying wettability in semisolid preparations using CIR. The small droplet volumes (<2 μm) of benzyl alcohol, water, and glycerol allowed for the assessments of the influence of carnitine on structure-surface correlations for lipogels and PEG-gels. The HDS parameter demonstrated a close correlation between the surface and the structural data. The high degrees of structure-surface correlations validated the surface tensiometry approach applied to semisolid preparations within the new definition of the Integrated Analytical Approach, and the contribution of surface tensiometry was found to be fundamental for the integrated structure-surface study of the PEG-gel, O/W cream, and lipogel formulations. Due to the speed and noninvasiveness of the surface tensiometry technique, the contact angle (CA) method applied under kinetic conditions (KCA) using CIR methodology has shown high discriminating capacity toward "as such" preparation samples.

From a practical viewpoint, this work opens the possibility of assessing drug release kinetics by evaluating the correlation between surface tensiometry and rheological data concerning the composition of different formulations.

Finally, this work paves the way for investigating correlations between structure, surface characteristics, and the amount of drug released *in vitro* or permeated under *ex vivo* conditions using IAA_t^S.

AUTHOR INFORMATION

Corresponding Author

Davide Rossi − *Department of Pharmaceutical and Pharmacological Sciences, Padua University, Padua 35121, Italy*; orcid.org/0000-0002-2649-2889; Email: davide.rossi@unipd.it

Authors

Giulia Lazzari − *Department of Pharmaceutical and Pharmacological Sciences, Padua University, Padua 35121, Italy*

Erica Franceschinis − *Department of Pharmaceutical and Pharmacological Sciences, Padua University, Padua 35121, Italy*

Elisa Vettorato − *Department of Pharmaceutical and Pharmacological Sciences, Padua University, Padua 35121, Italy*

Nicola Realdon − *Department of Pharmaceutical and Pharmacological Sciences, Padua University, Padua 35121, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.langmuir.4c01613

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Draisci, R.; Montesissa, C.; Santamaria, B.; D'Ambrosio, C.; Ferretti, G.; Merlanti, R.; Ferranti, C.; De Liguoro, M.; Cartoni, C.; Pistarino, E.; et al. Integrated Analytical Approach in Veal Calves Administered the Anabolic Androgenic Steroids Boldenone and Boldione: Urine and Plasma Kinetic Profile and Changes in Plasma Protein Expression. *Proteomics* **2007**, *7*, 3184−3193.

(2) Ribechini, E.; Modugno, F.; Baraldi, C.; Baraldi, P.; Colombini, M. P. An Integrated Analytical Approach for Characterizing an Organic Residue from an Archaeological Glass Bottle Recovered in Pompeii (Naples, Italy). *Talanta* **2008**, *74*, 555−561.

(3) Fermo, P.; Mearini, A.; Bonomi, R.; Arrighetti, E.; Comite, V. An integrated Analytical Approach for the Characterization of Repainted Wooden Statues Dated to the Fifteenth Century. *Microchem. J.* **2020**, *157*, 105072.

(4) Caggiani, M. C.; Coccato, A.; Mazzoleni, P.; D'Alessio, A.; Russo, A.; Barone, G. Integrated Analytical Approach to Unveil the Secrets of the Recently Discovered "Sphinx Room": A New Piece of Domus Aurea Puzzle. *Herit. Sci.* **2020**, *8* (1), 124.

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX

(5) Neff, D.; Reguer, S.; Bellot-Gurlet, L.; Dillmann, P.; Bertholon, R. Structural Characterisation of Corrosion Products on Archaeological Iron. An Integrated Analytical Approach to Establish Corrosion Forms. *J. Raman Spectrosc.* **2004**, *35*, 739−745.
(6) Randazzo, L.; Ricca, M.; Ruffolo, S.; Aquino, M.; Petriaggi, B. D.; Enei, F.; Russa, M. F. L. An Integrated Analytical Approach to Define the Compositional and Textural Features of Mortars Used in the Underwater Archaeological Site of Castrum Novum (Santa Marinella, Rome, Italy). *Minerals* **2019**, *9* (5), 268.
(7) Alaouchiche, Y.; Ouazene, Y.; Yalaoui, F. Economic and Energetic Performance Evaluation of Unreliable Production Lines: An Integrated Analytical Approach. *IEEE Access* **2020**, *8*, 185330−185345.
(8) Curran, P. J.; Hussong, A. M. Integrative Data Analysis: The Simultaneous Analysis of Multiple Data Sets. *Psychol. Methods* **2009**, *14*, 81−100.
(9) Rossi, D.; Dobrzynski, D.; Moro, I.; Zancato, M.; Realdon, N. The Importance of an Integrated Analytic Approach to the Study of Physicochemical Characteristics of Natural Thermal Waters used for Pelotherapy Aims: Perspectives for Reusing Cooled Thermal Waters for Treatments Related to Thermalism Applications *Geothermal Water Management* CRC Press 2017365389.
(10) Rossi, D.; Rossi, S.; Dobrzyński, D.; Dolmella, A.; Realdon, N. Assessment of spring waters from Lourdes (France) by contact angle method. *Surf. Interfaces* **2020**, *19*, 100471.
(11) Rossi, D.; Dobrzyński, D.; Moro, I.; Zotto, M. D.; Moschin, E.; Realdon, N. Contact angle keying method for an integrated analytical approach to characterising waters in the Lourdes area (Pyrenees, France). *Int. J. Water* **2020**, *14* (1), 29−68.
(12) Rossi, D.; Realdon, N. Surface Tensiometry Approach to Characterize Cosmetic Products in the Beauty Sector. *Surf. Sci. Adhes. Cosmet.* **2021**, 309−352.
(13) Demortier, G. PIXE Analysis of High Z Complex Matrices. *Nucl. Inst. Methods, Phys. Res. B* **1999**, *150*, 520−531.
(14) Calixto, L. S.; Maia Campos, P. M. B. G. Physical−Mechanical Characterization of Cosmetic Formulations and Correlation Between Instrumental Measurements and Sensorial Properties. *Int. J. Cosmetic Sci.* **2017**, *39*, 527−534.
(15) Malkin, A. Y.; Isayev, A. *Rheology - Concept, Methods, And Applications* Chemical Technology Publishing 2017 https://www.elsevier.com/books/rheology/malkin/978-1-927885-21-5.
(16) Marmur, A.; Della Volpe, C.; Siboni, S.; Amirfazli, A.; Drelich, J. W. Contact Angles and Wettability: Towards Common and Accurate Terminology. *Surf. Innovations* **2017**, *5*, 3−8.
(17) Xu, Z.; Lu, F. F. A Static Contact Angle Algorithm and its Application to Hydrophobicity Measurement in Silicone Rubber Corona Aging Test. *IEEE Trans. Dielectr. Electr. Insul.* **2013**, *20*, 1820−1831.
(18) Extrand, C. W.; Moon, S. I. Contact Angles of Liquid Drops on Super Hydrophobic Surfaces: Understanding the Role of Flattening of Drops by Gravity. *Langmuir* **2010**, *26* (22), 17090−17099.
(19) Wu, Z.; Hao, J.; Lu, J.; Xu, L.; Hu, G.; Floryan, J. M. Small Droplet Bouncing on a Deep pool. *Phys. Fluids* **2020**, *32*, 012107.
(20) Range, K.; Feuillebois, F. Influence of Surface Roughness on Liquid Drop Impact. *J. Colloid Interface Sci.* **1998**, *203*, 16.
(21) Rossi, D.; Rossi, S.; Realdon, N. Determination of Surface Tension Components of Aqueous Solutions Using Fomblin HC/25® Perfluoropolyether Liquid Film as a Solid Substrate *Advances In Contact Angle, Wettability And Adhesion, Int. Ed.* Wiley Online Library 2019145177 .
(22) Rossi, D.; Dall'acqua, S.; Rossi, S.; Zancato, M.; Pittia, P.; Franceschinis, E.; Realdon, N.; Bettero, A. Method Development for Measuring Contact Angles of Perfluoropolyether Liquid on Fomblin HC/25®PFPE Film *Advances In Contact Angle, Wettability And Adhesion, Int. Ed.* Wiley Online Library 2018 8197
(23) Park, E.-K.; Song, K.-W. Rheological Evaluation of Petroleum Jelly as a Base Material in Ointment and Cream Formulations: Steady Shear Flow Behaviour. *Arch. Of Pharmacol. Res.* **2010**, *33*, 141−150.
(24) Syms, R. R. A.; Yeatman, E. M.; Bright, V. M.; Whitesides, G. M. Surface Tension Powered Self Assembly of Microstructures: The State of the Art. *J. Microelectromech. Syst. Int. Ed.* **2003**, *12*, 387−417.

https://doi.org/10.1021/acs.langmuir.4c01613
Langmuir XXXX, XXX, XXX−XXX