

SURFACE CHARACTERISTICS OF DPPC MONOLAYERS DEPOSITED FROM TITANIUM DIOXIDE–CHITOSAN–HYALURONIC ACID SUBPHASES ON A GLASS SUPPORT

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Abstract

The Langmuir-Blodgett technique was used to transfer the monolayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) from the subphase containing chitosan (Ch) and/or titanium(IV) oxide (TiO₂) and/or hyaluronic acid (HA) on glass plates after a low-temperature air plasma treatment. The surface free energy and its components were determined on the basis of advancing and receding contact angle measurements of water (W), formamide (F), and diiodomethane (DM). To estimate the total surface free energy (γ_s^{tot}), the contact angle hysteresis (CAH) model was used, and the obtained data were compared with the results calculated from the Lifshitz-van der Waals/acid–base (LWAB) approach. It was found that the subphase components modify the surface of the phospholipid monolayer by changing the type and magnitude of interactions. They were reflected as significant differences in the obtained values of total surface free energy and its components. These findings can help in the development of composite materials, thereby expanding the spectrum of applications for chitosan, TiO₂, and HA, as well as understanding the interactions between biomaterial and cell.

Keywords: *topography, surface free energy, titanium dioxide, chitosan, hyaluronic acid, DPPC*

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1. Introduction

Chitosan is a natural polysaccharide that is often chosen for research because of its relative reactivity and, thus, remarkable simplicity of modification. The interactions of chitosan with the environment are due to the amino groups present in its structure, which are protonated in acidic solutions, as well as its primary and secondary hydroxyl groups, which are responsible for the adsorptive capacity of the compound.

Studies show that despite the differences in the properties of chitosan [degree of deacetylation (*DD*), molecular weight (*MW*)] caused by its origin and the receiving methods, chitosan obtained both from chitin by chemical methods and that isolated from fungi has very good biocompatible properties [1]. However, various biological applications require different properties that change as an effect of the *DD* and *MW* values [2]. For example, studies carried out on the influence of the *DD* value of the biopolymer on protein stability using the molecular dynamics simulation technique have shown that high values of this parameter prevent the conformational changes. Moreover, the polymers with high *DD* values were better adsorbed and more resistant to the temperature effect [3]. On the other hand, commercial chitosan with a 95% deacetylation degree and an average molecular weight of 600,000 g/mol exhibited high biocompatibility with mouse cell lines in *in vivo* studies. Cells grown on nanocomposite chitosan adhered and proliferated and, in addition showed well-developed typical spindle morphologies and formed tight intercellular connections with neighbouring cells [4]. The high biocompatibility of commercial chitosan (*DD*=92%) in the animal model was also observed when, after implantation of porous polymer scaffolds in mice, no signs of infection were noted. [5]. Furthermore, chitosan (*DD* of about 75%, extracted from mycelium *Aspergillus Niger*) implanted into animal biomodels as a tissue transplant was completely absorbed, leaving no traces of grafted material or signs of inflammation or embrace of foreign bodies, also indicating high biological compatibility [6]. In addition, the growing interest in chitosan results from its low production cost and the ability to form chemical bonds with various inorganic compounds, such as titanium dioxide.

TiO₂ is a multifunctional material quite often found in medical, pharmaceutical, and cosmetic applications. Literature data indicate that nanoparticles can successfully be used in tissue engineering and orthopaedics, fulfilling the function of promoters to protein adhesion, thus contributing to the enhancement of cell proliferation [7,8].

Both chitosan and TiO₂ have antibacterial, antifungal, and non-toxic properties. In addition, these substances are biocompatible and completely biodegradable in the human body. Immune active chitosan also performs protective functions by retarding product aging and water loss, while titanium dioxide improves chitosan thermal stability and oxidation resistance [8]. Due to the unique physicochemical and antibacterial nature, both compounds can be components of skin substitutes. On top of it, chitosan shows structural and qualitative similarity to the proteoglycan backbone, resulting in minimal immune response [9]. Therefore, in order to enhance biocompatibility, a highly biocompatible hyaluronic acid (HA), which is a component of the dermis, was introduced into the system.

Hyaluronic acid is a high molecular weight polysaccharide, which consists of linear macromolecular chains of a repeating disaccharide unit, glucuronic acid and *N*-acetyl-D-glucosamine. It contains charged carboxylate units and hydrophobic patches; both units open possibilities for interactions [10,11]. During the preparation of materials whose potential applications are associated with contact with living cells, determination of the impact of the biomaterial surface on the cellular response is necessary. The cell

membrane, consists of the amphiphilic phospholipids, is the first barrier that maintains a stable metabolic intracellular environment and is responsible for regulating and selecting substances in and out of cells. To test the wide spectrum of cell properties in the presence of other substances the techniques based on the creation of a model biological membrane are used. Langmuir-Blodgett layers belong to the group of thin materials deposited on a solid support that can mimic the biological cell membrane. They are obtained by transferring the Langmuir monolayer, spread over the aqueous subphase surface, to a solid surface by means of the Langmuir-Blodgett method. This technique is commonly used due to the possibility of controlling the thickness of the deposited layers [12]. In addition, it allows for obtaining materials that can be subjected to further analysis, allowing for determination of the type and strength of interactions of the phospholipid with the subphase components, as well as those with liquids of a different nature (hydrophilic–hydrophobic character and surface free energy) to obtain the expected response from the biological environment in the presence of a biomaterial.

This article presents studies on the impact of chitosan (Ch), hyaluronic acid (HA), and titanium dioxide (TiO₂) on a model biological membrane prepared by use of the phospholipid DPPC. Specifying the factors that allow for understanding the interactions between the cell and the material can provide the basis for proper control of the surface properties of the biomaterial. The analysis of chemical composition, wettability, and surface free energy should be helpful in achieving this goal. In addition, knowledge of the type and strength of interactions of the Ch/HA/TiO₂ subphase components with the cell membrane can allow for evaluating the expected cell behaviour in the presence of the material and, finally, for skilfully creating a new generation of skin substitutes.

The aim of this article was the determination of the total value of the surface free energy and its components for glass plate surfaces, activated by air plasma, in the presence of the DPPS monolayer or without the DPPC monolayer transferred from the different subphases. In order to precisely estimate the energy changes induced by the individual components of the subphase and/or DPPC, the cleaned glass plates before and after plasma modification were also interpreted.

2. Materials and Methods

2.1. Materials

For preparation of the subphase, chitosan with a molecular weight of 100,000–300,000 and deacetylation degree (DD) of 82% from Acrōs Organics (ACRS34905), a commercial 1% solution of high-molecular hyaluronic acid, and titanium dioxide P-25 from Evonik (additionally purified according to the procedure described by Preočanin and Kallay [13]) were used. These ingredients were dissolved/dispersed in an aqueous solution of acetic acid (99.9% purity) from Avantor Performance Materials. For the biological membrane model, the phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), with a purity >99%, from Sigma was used. It was dissolved in chloroform (98.5% purity) purchased from Avantor Performance Materials. As a solid support for the deposition of the DPPC monolayers, optical glass plates (36 mm × 24 mm × 1 mm, As Polonia, Poland) were used after degreasing using ethanol (96%) from Avantor Performance Materials. As test liquids for contact angle measurements, water purified by a Milli-Q Plus 185 system from Millipore (USA) (18.2 MΩ cm conductivity), formamide, and diiodomethane (analytical grade, Sigma) were used.

2.2. Preparation of Solutions and Mixtures

Chitosan (Ch), titanium dioxide (TiO₂), and hyaluronic acid (HA) were combined with acetic acid (AA) in various scheme (various combinations of used ingredients) in

order to get as much information as possible about the effect of each component. Thus, solutions and mixtures were prepared as follows: AA, AA/Ch, AA/TiO₂, AA/HA, AA/Ch/TiO₂, AA/Ch/HA, AA/HA/TiO₂, and AA/Ch/HA/TiO₂, wherein each sample contained 1.2 mg/mL TiO₂, 0.1 mg/mL chitosan, and 0.5 mL/L hyaluronic acid, which were dissolved in 0.1% acetic acid.

2.3. Preparation of the Plates

Purified and degreased glass plates were treated with active gas (plasma). In the study, low-temperature plasma (pressure, 0.2 mbar; plasma gas flow rate, 22 sccm; working power, 460 W) of air for 1 min in the Pico system (Diener Electronic, Germany) was used.

2.4. Transfer of the Subphase to the Solid Support and Deposition of the DPPC Monolayer

The DPPC films formed by applying the appropriate amount of 1 mg/mL DPPC chloroform solution on the subphase were deposited on glass plates using the Langmuir-Blodgett (LB) method, taking advantage of the Langmuir-Blodgett trough (KSV 2000). First, the bath was filled with subphase. The glass plate was immersed in a vertical position and then the DPPC layer was applied. The monolayer was compressed at a constant speed of 10 mm/min until reaching a pressure of 35 mN/m because this corresponds to the pressure of the cell membranes at which the transfer of the monolayer took place. The transfer of the DPPC monolayer from the subphase to the glass plate occurred at a rate of 5 mm/min. The temperature during the measurements was constantly controlled by means of a built-in thermostat and amounted to 20±1°C. The reference samples were plates transferred from the subphase in the absence of the monolayer. The plate was immersed in the subphase solution for the appropriate time (about 45 minutes) and transferred at zero surface pressure. Two attempts were made to ascent a plate from each type of subphase (one with the DPPC monolayer and the second without the DPPC monolayer). Detailed information about the course of the study are described by us in [14].

2.5. Contact Angle Measurements

The contact angle measurement was carried out using a GBX apparatus (France) equipped with a camera for taking photos of liquid drops. Three test liquids were used for the tests: water, formamide, and diiodomethane. As a result of the wettability analysis, between two and eight drops were recorded, depending on the liquid used and the test system. On the other hand, readings were made on the left and right side of the drop, which gave 4 to 16 results, with a relatively small mean standard deviation in the range of 0.7–4.1 degrees for respective samples. The exact course of the analysis has been described in our previous article [14].

2.6. Surface Free Energy Estimation

Surface energy is a very important and helpful parameter in surface characteristics in the aspect of intermolecular interactions of substances in contact. When making measurements of advancing contact angles of three liquids differing in chemical nature (but defined composition and value of surface tension), using the appropriate theoretical equations, it is possible to define components of free surface energy and, ultimately, total free surface energy. One of the models that uses these data to calculate the total free surface energy and its components is the Lifshitz-van der Waals/acid–base (LWAB) approach [15,16]. This model suggests the division of total free surface energy into two

components: apolar Lifshitz–van der Waals (γ_S^{LW}), related to long-range interactions (dispersion, orientation, and induction), and polar (Lewis) acid–base (γ_S^{AB}) (1) [17–19].

$$\gamma_S = \gamma_S^{LW} + \gamma_S^{AB} \quad (1)$$

In turn, the acid–base component consists of electron-donor (γ_S^-) and electron-acceptor (γ_S^+) parameters (2):

$$\gamma_S = \gamma_S^{LW} + \sqrt{2(\gamma_S^- \gamma_S^+)} \quad (2)$$

Knowing the additional data, such as values of receding contact angles, it becomes possible to calculate the contact angle hysteresis (CAH) and, thus, the total surface free energy from different theoretical models proposed by Chibowski [20–24]. In the CAH approach, the calculation of the total surface free energy of the solid (γ_S) (3) takes into account the surface tension of the test liquid (γ_L) and the contact angle hysteresis, which is the difference between the advancing (θ_a) and receding (θ_r) contact angle.

$$\gamma_S^{tot} = \frac{\gamma_L(1+\cos\theta_a)^2}{2+\cos\theta_r+\cos\theta_a} \quad (3)$$

The mentioned models were used to verify changes in the apparent surface free energy and its components obtained for the glass plates with or without the DPPC monolayer transferred from the various subphases.

3. Results and Discussion

The surfaces of biomaterials in aqueous solutions interact with the molecules of the solvent (water). The proximity of polar groups strictly determines the behaviour of the surface in contact with the biological environment. The fundamental for biological applications, because the most important, are the interactions between water molecules and acid-base and/or apolar groups present on the surface. The strength of the sample liquid interactions of different kinds can be determined based on the values of surface free energy and its components using theoretical models.

3.1. Analysis of the Glass Plate Surface Before and After Plasma Treatment

3.1.1. Surface Free Energy Calculations Using the LWAB Approach

The use of plasma treatment was aimed at introducing additional functional groups to the support surface, which allowed for effective attachment of the transferred compounds. The quantities characterizing the glass plate surface, as well as the plate designed (or planned) for the air plasma modification, were determined using the LWAB model, and the obtained results are presented in Table 1.

Table 1. Surface free energy and its component values of a glass plate before and after plasma treatment (LWAB model)

Support	Surface free energy and its components (mJ/m ²)				
	γ_S^{LW}	γ_S^-	γ_S^+	γ_S^{AB}	γ_S^{tot}
Glass plate	37.8 ± 1.9	40.3 ± 6.7	0.4 ± 0.1	8.3 ± 1.3	46.1 ± 3.2
Glass plate/Air plasma	40.2 ± 1.0	52.6 ± 0.8	1.4 ± 0.2	17.0 ± 1.1	57.2 ± 0.1

As can be seen, the modification of glass caused an increase in the total surface free energy by 11.1 mJ/m² compared to unmodified plates. This is due to the introduction of additional functional groups on the glass surface, derived from plasma (N₂, N₂⁺, O₂, NO, and OH), which allow for creating strong bonds with the contacting substances. The changes in the total surface free energy values obtained from the LWAB model are similar to those obtained using the CAH model (**Table 2**), but they are slightly lower.

3.1.2. Surface Energy Calculations Using the CAH Approach

Higher values of the total surface free energy in the case of the CAH model compared to the that from the LWAB approach are a consequence of using both contact angles, advancing and receding, in the calculations in the first case.

Table 2. Surface free energy values of a glass plate before and after plasma treatment (CAH model)

Support	Surface free energy (mJ/m ²)			
	water	formamide	diiodomethane	total
Glass plate	61.9 ± 4.3	51.1 ± 2.0	43.7 ± 0.9	52.2 ± 2.4
Glass plate/Air plasma	71.9 ± 0.3	57.9 ± 0.0	44.5 ± 0.6	59.4 ± 0.3

3.2. Plate Surface and Lipid Film Analysis Using the CAH Approach

The following chapter presents the surface characteristics based on the contact angle hysteresis (CAH) model. **Fig. 1** shows the values of the surface free energy calculated from the contact angle hysteresis separately for water (γ_s^W), formamide (γ_s^F), and diiodomethane (γ_s^{DM}). In contrast, the total surface free energy (γ_s^{tot}) is the arithmetic average of the results obtained for all liquids. Depending on the chemical nature of the liquid used for the measurement, differences in the calculated values of γ_s are obtained. Its changes reflect the various forces occurring between the liquid and the surface. The values represented by γ_s^W and γ_s^F suggest both kinds of interactions by hydrogen bonding and dispersion forces, whereas γ_s^{DM} indicates the existence of only dispersion forces. γ_s^{tot} contains information about averaged interactions coming from the surface and is useful for comparison when considering the total surface free energy evaluated from another model.

Despite the similar chemical nature of water and formamide molecules, it should be taken into account that they have different values of surface tension and its components. Water is characterized by identical values of electron-acceptor and electron-donor parameters, while higher values of electron-donor and lower values of electron-acceptor parameters are ascribed to formamide [25]. As a result, the strength of interactions may be slightly different [26].

The values of surface free energy obtained for glass plates contacted with the subphase solutions (Fig. 1) suggest that the strongest interactions occur in the case of water taking into account the values obtained for formamide and diiodomethane. They oscillate from value 63.9 mJ/m² for AA/Ch/HA system, up to 71.6 mJ/m² for AA/HA/TiO₂. The weakest interactions appeared in the case of diiodomethane, here we obtained lower values than in the case of water. The differences in the value between

individual systems are included in the range from 42.6 mJ/m² for AA to 49.6 mJ/m² for the AA/TiO₂ system. On the other hand, the surface free energy values obtained using formamide took intermediate values between those received using water and diiodomethane and changed from 55.8 mJ/m² for AA/Ch to 58.0 mJ/m² for AA/HA/TiO₂. The γ_s^F values are also the closest to the total surface free energy for all systems.

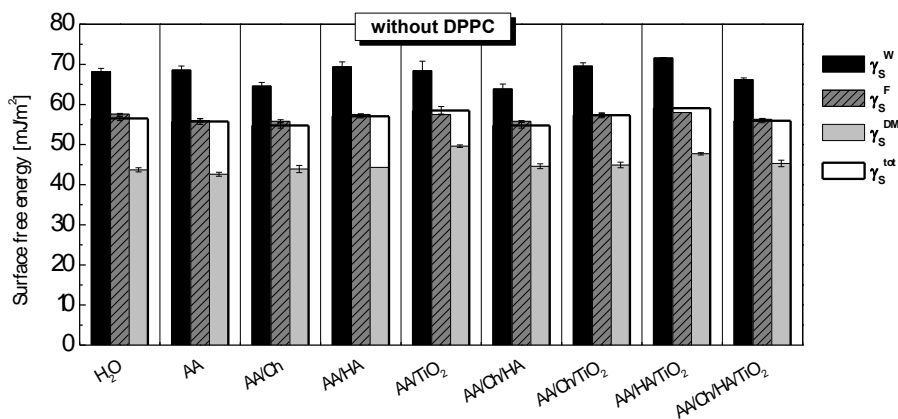


Figure 1. Surface free energy (γ_s) of water (W), formamide (F), and diiodomethane (DM) and arithmetic average of these values (tot) obtained for the glass plates pull out from the various subphases without DPPC

The following systems are worth mentioning: AA/TiO₂, which had the highest values of γ_s^{DM} , and AA/HA/TiO₂, which had the highest γ_s^W and γ_s^F values and one of the highest γ_s^{DM} values. As an effect in these two cases, the highest values of γ_s^{tot} were recorded. This may suggest the interactions with the subphase components, i.e. TiO₂ and/or HA.

Deposition of the DPPC phospholipid monolayer from the subphase on the glass plate reduced its surface free energy (Fig. 2) compared to the plates without the DPPC monolayers (Fig. 1) as well as the commercial activated plates (Table 2). This is due to the specific orientation of the phospholipid molecules with polar heads towards the support and the hydrocarbon chains towards the air. In addition, biological modification causes quite different trend of γ_s values changes, both qualitatively and quantitatively, for all tested liquids in each system (Fig. 2), compared to those plotted in the case of systems without a monolayer (Fig. 1).

The highest changes in γ_s were recorded using formamide for measurements. In this case, the smallest values, from 12.1 mJ/m² for AA/Ch/TiO₂ and AA/Ch/HA/TiO₂ systems up to 27.3 mJ/m² for the H₂O subphase, were obtained, in comparison to glass plates without the DPPC monolayer. The smallest changes occurred for the AA/TiO₂ and AA/HA/TiO₂ systems, where there was a reduction of only 4.6 mJ/m² and 0.4 mJ/m², respectively. In these two cases, the highest values of γ_s^F and one of the highest γ_s^W values were obtained, and, consequently, the highest values of γ_s^{tot} were also found.

When analysing systems with the DPPC monolayer, it can be seen that in the case of systems containing chitosan, i.e. AA/Ch, AA/Ch/HA, AA/Ch/TiO₂, and AA/Ch/HA/TiO₂, some of the highest values of γ_s^W were obtained, which are 61.9

mJ/m², 57.4 mJ/m², 60.0 mJ/m², and 61.4 mJ/m², respectively. Only the AA/HA/TiO₂ system showed a higher (the highest) value of 68.8 mJ/m². In addition, in systems containing only polysaccharides (Ch and HA), a slight difference between the values of γ_s^F and γ_s^{DM} were noted, whereas systems with an inorganic component (TiO₂) showed significant deviations. As an effect the difference between these values decreases when an additional component in the form of Ch and/or HA was introduced into the AA/TiO₂ system. Among them the effect of chitosan is greater because for AA/TiO₂ $\gamma_s^F - \gamma_s^{DM}$ equals 13.1 mJ/m², 11.1 mJ/m² for AA/HA/TiO₂, and only 5.9 mJ/m² for AA/Ch/HA/TiO₂.

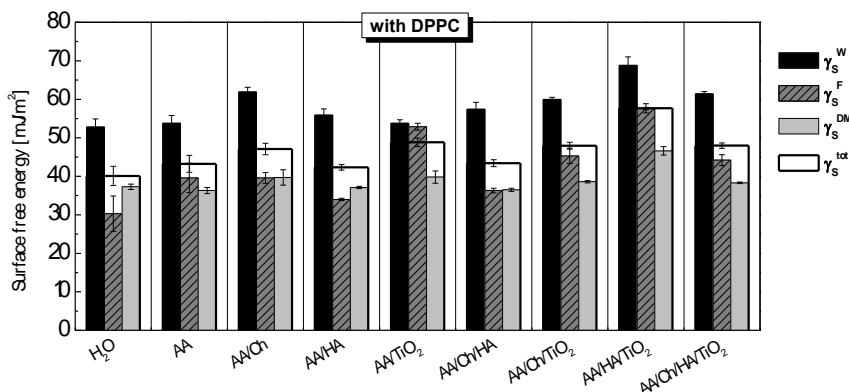


Figure 2. Surface free energy (γ_s) of water (W), formamide (F), and diiodomethane (DM) and arithmetic average of these values (tot) obtained for the glass plates with the DPPC monolayer transferred from the various subphases

Literature data indicate that TiO₂ [27,28] with DPPC interacts mainly with electrostatic forces. Therefore, it can be suggested that the described behaviour may be a consequence of electrostatic repulsion between the positively charged TiO₂ particles and the negatively charged choline group of the DPPC molecule and the reduction of these forces due to the addition of Ch [29] and HA [10,11], which interact with phospholipid molecules not only electrostatically, but also by hydrophobic forces and hydrogen bonds. The reduction of repulsive forces between TiO₂ and DPPC is probably a consequence of a decrease in the phospholipid's access to the oxide due to the formation of the Ch/TiO₂ composite (chitosan particles surround particles of titanium dioxide), whose possible structure has been previously described by us [30–32].

However, some literature suggests that other effects exist, such as hydrogen bonds between TiO₂ and DPPC, or direct coordination of phosphate or oxygen of carbonyl lipids with available sites of titanium dioxide [33,34]. On the other hand, the CAH approach shows averaged values and, therefore, gives us only a general consideration of the nature of the interactions occurring between the liquid and the sample. Therefore, it is important to compare the calculated values using other theoretical models.

3.3. Plate Surface and Lipid Film Analysis Using the LWAB Approach

Surface free energy is an important thermodynamic function characterizing the surface for studying interactions between substances that are in contact. It gives the opportunity to answer the question regarding the relative magnitude of the polar γ_s^{AB} (being the geometric mean of the electron-donor γ_s^- and electron-acceptor γ_s^+ parameters) and non-polar Lifshitz–van der Waals γ_s^{LW} components. The electron-donor and electron-acceptor parameters are particularly important because their changes may indicate interactions between hydrogen bridges and/or π -electrons.

By analysing the values of surface free energy and its components obtained for the glass plates pulled out from the subphase without the DPPC monolayer (Fig. 3), it can be observed that the AA/TiO₂ and AA/HA/TiO₂ systems have the largest share of non-polar components. Simultaneously, the lowest values of γ_s^+ were recorded in these two cases. The general trend of changes in γ_s^{tot} is analogous to that obtained from the CAH model (Fig. 1), but the values calculated from the LWAB approach are slightly lower. On the other hand, the changes in the γ_s^{LW} parameter (Fig. 3) are comparable to the changes that were plotted in the case of γ_s^F (Fig. 1).

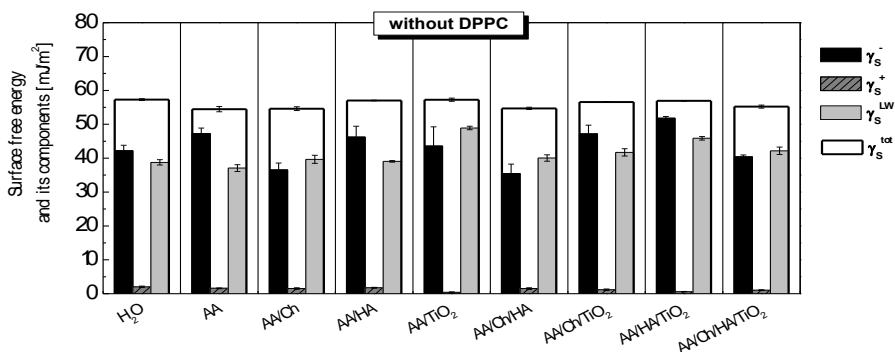


Figure 3. Surface free energy and its components obtained for the activated glass plates pulled out from the subphases without DPPC

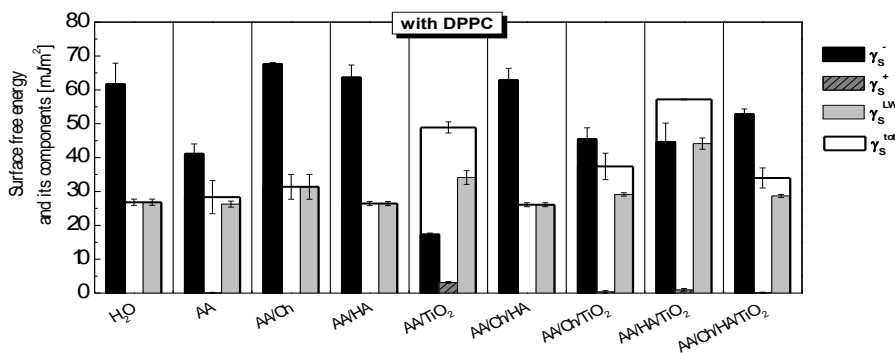


Figure 4. Surface free energy and its components obtained for the activated glass plates pulled out from the subphases with the floating DPPC monolayer

Transfer of the DPPC monolayer from the H₂O, AA/Ch, AA/HA, and AA/Ch/HA subphases completely eliminated the electron-acceptor interactions, but in these cases the highest values of electron-donor interactions were obtained (Fig. 4). This may be related to the access of polar liquid molecules to the basic DPPC groups capable of forming hydrogen bonds.

On the other hand, the combination of polymers (i.e., the AA/Ch/HA system) caused a slight decrease in the γ_s^- and γ_s^{LW} values and, consequently, γ_s^{tot} , which may suggest a slight decrease in the strength of interactions of the obtained surfaces with the probe liquids as a result of the interactions between both polysaccharides and the DPPC monolayer (Fig. 4).

In addition, the presence of the DPPC monolayer caused a drastic increase in the γ_s^- parameter for systems containing chitosan and hyaluronic acid, with the exception of AA/Ch/TiO₂ and AA/HA/TiO₂. The presence of oxide in the systems caused a decrease in the value of γ_s^- , but also the appearance of the γ_s^+ parameter.

The obtained values of the γ_s^- parameter in both AA/Ch/TiO₂ and AA/HA/TiO₂ systems are on similar levels. It may suggest that these polymers interact with the polar heads of DPPC mainly through the amine groups present in the polysaccharides. Both acetylated and deacetylated chitosan units and deacetylated units of hyaluronic acid have the donor/acceptor groups involved in the hydrogen bonding [10,11,35]. The reduction in γ_s^{LW} in the case of the AA/Ch/TiO₂ system compared to AA/HA/TiO₂ may indicate that the main contribution to this surface free energy component is derived from the interactions of TiO₂ with DPPC, which decrease in the presence of chitosan due to the orientation of the polymer on the surface of TiO₂ particles so that access to them is limited. Consequently, Ch also interacts with the DPPC, but to a lesser extent (some groups interact with TiO₂), which may be suggested by the decrease in the value of γ_s^- obtained for the AA/Ch/TiO₂ system in comparison with AA/Ch. In contrast, γ_s^{LW} in these cases are at the same level. All the above relationships can confirm the formation of a composite between TiO₂ and Ch.

In the case of the AA/TiO₂ subphase, the DPPC monolayer has the lowest electron-donor (γ_s^-) interactions, but the highest electron-acceptor (γ_s^+) interactions. As a result, one of the highest total surface energy (γ_s^{tot}) values was obtained (Fig. 4), which correlates with γ_s^{tot} determined from the Chibowski model (Fig. 2), but these values were higher. As mentioned earlier, this is a consequence of using the advancing and receding contact angles in the energy calculation, whereas the LWAB model only takes into account the advancing contact angles.

4. Conclusions

The total surface free energy and its components for the DPPC monolayers deposited on the activated glass plates from the subphases containing Ch, HA, and/or TiO₂ were determined based on two theoretical models available in the literature. The values obtained from the LWAB model using the advancing contact angles were compared with those from the contact angle hysteresis (CAH) model. The type and strength of interactions between Ch, HA, TiO₂, and DPPC seem to be crucial for efficient DPPC attachment and control of system wetting properties.

The subphase type determines the properties of the DPPC film. Differences are visible in the changes of surface free energy and its components. Variation of these quantities is dependent on the film permeability to the used test liquids, which is related to the characteristic orientation of the particles and molecules and/or their packing.

There have been obtained the systems with functional physicochemical properties which can be used in the controlled release of biological-active substances as well as in the production of antibacterial dressing or artificial skin. The study of wettability of the HA-based systems can be helpful in better understanding the mechanisms of water and different substances (polar or apolar) diffusion through membranes in aging and cellular transport processes.

5. Acknowledgment

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