# WETTABILITY OF HYBRID CHITOSAN/PHOSPHOLIPID COATINGS

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*Abstract*

*Solution spreading and Langmuir-Blodgett/Schaefer techniques were used for preparation of chitosan/phospholipid (DPPC) films on air plasma treated polyethylene terephthalate (PET) plates. The surface wetting properties were determined based on the measurements of the advancing and receding contact angles of water, formamide and diiodomethane. The contact angle hysteresis model of Chibowski was applied to estimate the total surface free energy values. The coatings properties were found to be affected by molecular organisation and packing depending on the preparation technique. Phospholipid molecules modified the chitosan film surface by changing the kind and magnitude of interactions, which is revealed in the values of surface free energy. These findings may be helpful for the development of new generation polymer-supported biocompatible coatings with anti-thrombogenic and anti-bactericidal properties, thus expanding the spectrum of chitosan applications.*

## 1.  Introduction

Biocompatible polymers and coatings have been of great interest to researchers in recent years owing to their applications in many fields, e.g. in molecular recognition, membrane fusion, controlled drug delivery systems and prosthesis fabrication. Endovascular metallic stents are commonly covered with polyethylene terephthalate (PET) [1, 2]. This is also a widely accepted material for synthetic ligaments in knee injury (above-knee bypasses) [2, 3]. However, the disadvantage of PET is its low surface free energy and chemical inertness, leading to poor wettability and weak adhesion. Hence, PET ligaments can cause serious side-effects such as poor tendon–bone healing, bolt loosening and synovitis [4] or, during stent placement in the bloodstream, it can stimulate thrombosis and restenosis [2]. To overcome these limitations, the PET surface can be modified by plasma treatment to increase wettability, roughness and effective surface area [5], and/or it can be covered with bioactive coatings for enhanced biocompatibility of the surrounding tissues [1]. The biopolymer chitosan (Ch) has emerged as a suitable candidate for PET surface coating. Chitosan is a linear polysaccharide built of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units, which are distributed along the biopolymer chain. It is known for its non-toxic, non-immunogenic, biodegradable, wound healing and antimicrobial properties [6–8]. Despite displaying enhanced biocompatibility, chitosan itself can induce platelet activation and thrombus formation as a consequence of interactions between the positively charged chitosan and the negatively charged blood components [9, 10]. To improve the haemostatic properties of the chitosan film, hybrid chitosan/phospholipid coatings were created. Phospholipids, being major constituents of biological membranes, could provide a more favourable host tissue response. Simultaneously, by modifying the chitosan film surface charge they could make it less thrombogenic.

In this paper, novel mixed chitosan/phospholipid films on the air plasma activated PET surface (PET$_{air}$) were prepared by the chosen methods, i.e. solution spreading and/or Langmuir-Blodgett/Schaefer techniques [11–13]. The latter techniques provided tightly packed molecular films. In each stage of PET surface modifications, its properties were evaluated based on the advancing and receding contact angle measurements of probe liquids. Then the apparent surface free energy was determined using the contact angle hysteresis approach proposed by Chibowski [14]. Modification of the activated PET surface by the hybrid chitosan/phospholipid films can deliver a next generation of stents with biomimetic coverage towards antithrombogenicity, reduction of inflammation and acceleration of wound healing.

## 2.  Materials and Methods
### 2.1. Materials

Polyethylene terephthalate (PET) (Axpet Standard, Bayer) was a support for biological film fabrication. Plates with 60 mm x 50 mm x 3 mm dimensions were used. Chitosan (MW 100,000–300,000, deacetylation degree DD 82%) was purchased from Acrōs Organics (Belgium) while 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, 99%) was provided by Sigma-Aldrich (USA) and used without purification. Chloroform (p.a., POCH S.A., Poland) and ethanol (96%, p.a., POCH S.A., Poland) were applied as received for compound dissolution. Water purified by a Milli-Q Plus system with the resistivity of 18.2 MΩcm, pH 5.6, was a subphase for the Langmuir-Blodgett technique as well as along with formamide (99.5%, Acrōs Organics, Belgium) and diiodomethane (99%, Sigma-Aldrich, USA) were used for contact angle measurements.

## 2.2. Methods

### 2.2.1. *PET cleaning and activation*

The PET plates were cleaned in an ultrasonic methanol bath for 15 min, placed into an ultrasonic bath in Milli-Q water twice for 15 min, then rinsed thoroughly with Milli-Q water to approach the conductivity of pure water. After washing, all slides were dried under vacuum. For activation, the polymer surface was treated for 1 min with the low-temperature plasma Pico system (Diener Electronic, Germany) at the pressure of 0.2 mbar, a plasma gas flow rate of 22 sccm and operating power of 460 W.

### 2.2.2. *Chitosan film preparation*

Chitosan powder was dissolved in a 0.1% acetic acid solution to obtain a final concentration of 0.1 mg/ml. Then the solution was stirred in an ultrasonic bath for 20 min at room temperature. A chitosan film on the PET polymer was obtained by pouring 5 ml of acidic solution onto the non-activated and activated surface. In the latter case, the chitosan solution was poured onto the PET surface within 2 min of its activation. The samples were allowed to dry for 24 h in the air. In the next step, the dried films were immersed first in a 0.5% NaOH solution for acetic acid neutralisation and next in ethanol for NaOH removal. The biopolymer surface becomes resistant to dissolution due to deacidification [15]. In the last step, samples were thoroughly rinsed with Milli-Q water and dried in a vacuum oven at room temperature overnight.

### 2.2.3. *DPPC monolayer preparation*

The DPPC monolayer was prepared on the dried chitosan film ($PET_{air}$/Ch) in different ways:

(1)   solution spreading technique (S)

The ethanol solution of 5 ml volume with 7.3 μg of DPPC was spread on the $PET_{air}$/Ch surface using an automatic pipette; this covered the support with one statistical DPPC monolayer. The $PET_{air}$/Ch/$DPPC_S$ samples were dried under vacuum for 24 h after evaporating the solvent in air. In order to prepare the reference sample, 5 ml of ethanol without DPPC was poured onto the chitosan film ($PET_{air}$/Ch/$EtOH_S$).

(2)   Langmuir-Blodgett (LB) technique by vertical dipping (LB↓) or withdrawing (LB↑)

The floating DPPC monolayer was prepared at the air–water interface using the Langmuir-Blodgett KSV 2000 standard-trough (KSV Instruments Ltd., Finland). The Wilhelmy plate method was used for measuring the surface pressure. The water subphase was maintained at a constant temperature (20°C) due to water system circulation (thermostat Alpha RA 8, Lauda). A 1 mg/ml concentration of the solution was obtained by dissolution of phospholipid DPPC powder in chloroform. Then, by means of a Hamilton microsyringe, the droplets of solution (total volume of 80 μl) were placed on the subphase surface top. When the solvent evaporated (10 min), symmetrical compression at a compression rate of 10 mm/min was applied to the monolayers until the surface pressure of 35 mN/m was obtained. Before transfer started, a 20 min equilibration at that pressure took place. Next, still at 35 mN/m, deposition of the monolayers onto $PET_{air}$/Ch proceeded by vertically drawing the support previously dipped in the subphase (LB↑) or dipping it vertically while floating the monolayer at the air–water interface. The speed of transfer was 5 mm/min. The obtained samples were designated as follows: $PET_{air}$/Ch/$DPPC_{LB↑}$ and $PET_{air}$/Ch/$DPPC_{LB↓}$, respectively.

(3)   Langmuir-Schaefer (LS) technique

Based on the horizontal orientation of the support, the horizontal transfer of the condensed DPPC monolayer onto the $PET_{air}$/Ch resulted in deposition of the

phospholipid film on the chitosan with a surface pressure of 35 mN/m ($PET_{air}$/Ch/$DPPC_{LS}$).

The pressure of 117 mbar was used while drying all surfaces in a vacuum apparatus at room temperature for about 24 h.

### 2.2.4. *Contact angle measurements*

The sessile drop method using a GBX Contact Angle Meter (France) was applied for measuring the contact angles of the three probe liquids, namely water, formamide and diiodomethane. The stable conditions were ensured owing to the nitrogen constant flow in a closed thermostatic chamber. From the microsyringe, 6 µl droplets were injected to obtain the advancing contact angle, whereas the receding contact angle was determined after sucking 2 µl of the droplet back into the syringe. The contact angles were measured on both right and left side of 15–20 droplets of each liquid put on each surface.

### 2.2.5. *Surface free energy determination from the contact angle hysteresis (CAH) model*

From the CAH model [14], the total surface energy $\gamma_S$ was determined based on the advancing $\Theta_a$ and receding $\Theta_r$ contact angle measurements for three liquids of known surface tension $\gamma_L$.

$$\gamma_S = \frac{\gamma_L(1+cos\Theta_a)^2}{(2+cos\Theta_r+cos\Theta_a)}$$

The obtained values of $\gamma_S$ are apparent because they depend on the kind of liquid. In this paper $\gamma_S^{tot}$ is expressed as the arithmetic mean of $\gamma_S^W$, $\gamma_S^F$, and $\gamma_S^{DM}$, which are estimated from the contact angle hysteresis of water (W), formamide (F) and diiodomethane (DM), respectively.

## 3. Results and Discussion

### 3.1. Contact angle and its hysteresis

The wetting properties of the PET surface, untreated or plasma treated and covered with chitosan or chitosan/DPPC films, were determined based on the measurements of advancing and receding contact angles of three liquids, and the surface free energy calculation.

The initial advancing and receding ($\Theta_a$/$\Theta_r$) contact angles measured on the untreated PET were 75.6°/66.6°; 61.7°/50.5° and 26.4°/20.0° for water, formamide and diiodomethane, respectively (Fig. 1). The values of contact angle hysteresis (*H*), defined as the difference between the advancing and receding contact angles, are also plotted. The Ch film support was polyethylene terephthalate (PET). However, its low surface free energy is responsible for poor wettability and Ch adhesion. The deposition of a chitosan layer on the non-activated PET (PET/Ch) surface had practically no effect on the contact angles of liquids in comparison to those measured on the bare sheet, while the contact angle hysteresis of polar liquids increased. This can be explained by heterogeneity of the coverage, making the PET surface readily accessible to the test liquids. Hence, the overall wettability of PET/Ch is mainly determined by contribution of the PET surface properties.

Air plasma treatment of PET induces drastic changes in the surface wettability. The contact angles of water and formamide are significantly reduced by about 55°, while those of DM increase by 7° compared to those measured on the unmodified PET (Fig. 1). Water and formamide as polar liquids are capable of interacting by both hydrogen
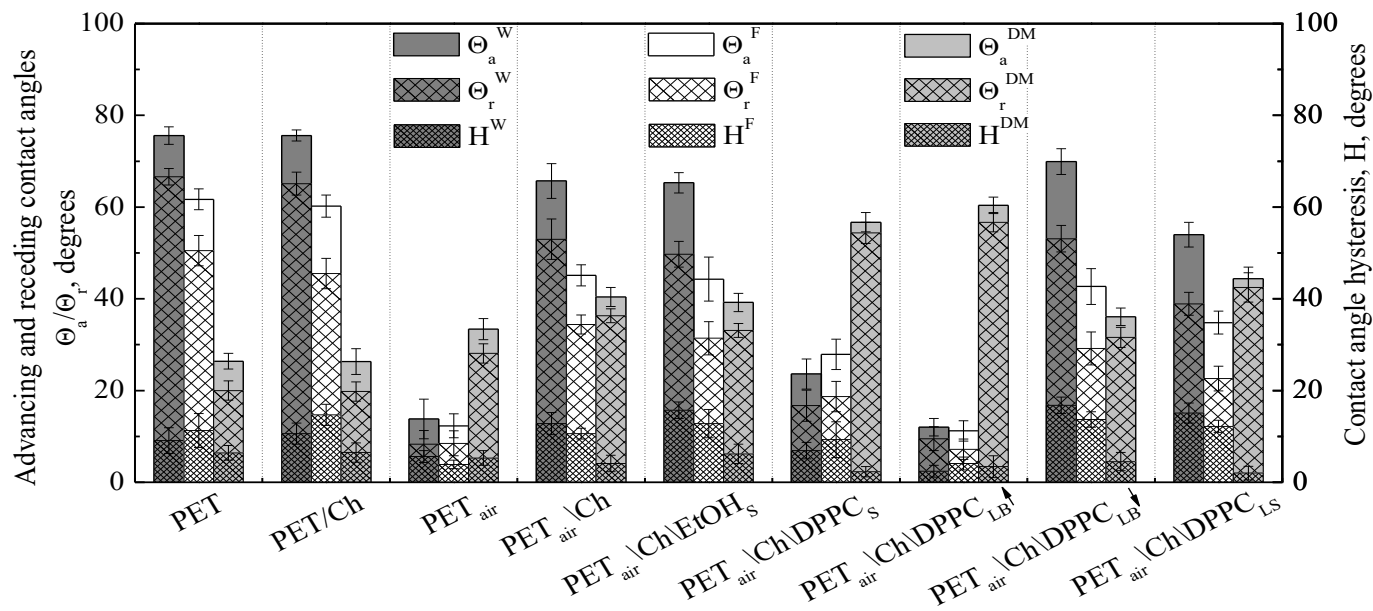
**Figure 1.** Advancing ($\Theta_a$) and receding ($\Theta_r$) contact angles of water (W), formamide (F) and diiodomethane (DM) and their hystereses *H*.

bonds and dispersion forces while apolar diiodomethane interacts mainly by dispersion forces. Formamide forms droplets of slightly lower contact angles than those of water ($\Theta_a^W/\Theta_r^W = 13.8°/8.3°$ and $\Theta_a^F/\Theta_r^F = 12.3°/8.5°$, respectively). These findings show that the plasma surface activation results in the increased surface polarity.

It seems interesting to show here the changes of the water (W) contact angles as a function of time of the activated PET storage in the air, taking a moment of plasma treatment end as a starting point (Fig. 2).
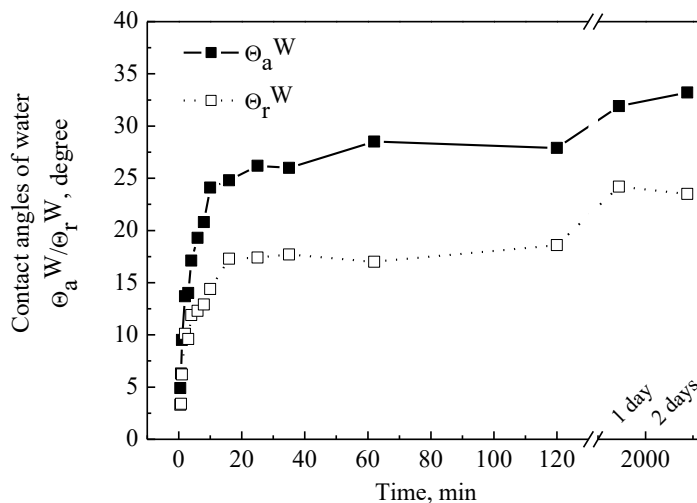


**Figure 2.** Advancing ($\Theta_a$) and receding ($\Theta_r$) contact angles of water (W) as a function of exposure time of the PET plates in air after plasma treatment.

These results demonstrate that the activated PET surface is very hydrophilic. The advancing contact angles measured within the first 30 seconds of activation were only 5°, whereas after 2 min, they reached about 14°. The contact angle increased with storage time. The greatest changes occurred within the first 20–30 min and further exposure to the air caused only slight changes in the hydrophilic character of the surface. To ensure complete spreading, the aqueous chitosan solution was poured onto the activated PET plates within the first 2 min of plasma treatment. This time offset was imposed by technical settings. The corresponding values of contact angles of all liquids on PET$_{air}$ are shown in Fig. 1.

The chitosan coating increased the measured contact angles of all liquids compared to those on PET$_{air}$. Higher contact angle hystereses of polar liquids were also observed. These results indicate that molecules of polar liquids can permeate the chitosan structure during the receding contact angle measurement, thus strengthening the hysteresis. Moreover, the chitosan surface wettability did not alter after the PET$_{air}$/Ch treatment with pure ethanol (PET$_{air}$/Ch/EtOH$_S$ in Fig. 1). In contrast, the presence of DPPC in the ethanol solution, after solvent evaporation and drying (PET$_{air}$/Ch/DPPC$_S$), caused drastic reduction of water and formamide contact angles and an increase of the diiodomethane contact angles. The observed growth in the surface polarity may be a consequence of orientation of the DPPC molecules with their polar heads towards the air. Moreover, the

DPPC film deposited by the LB↑ technique (PET$_{air}$/Ch/DPPC$_{LB↑}$) led to further enhancement of the surface polarity, probably due to more ordered organisation and/or condensation of the monolayer. Interestingly, the contact angles of diiodomethane were higher than those on PET$_{air}$ and PET$_{air}$/Ch, suggesting reduced dispersion interactions due to screening of the hydrocarbon tails by polar heads.

When vertical dipping (LB↓) was used for DPPC transfer (in the case of PET$_{air}$/Ch/DPPC$_{LB↓}$), the contact angles of all liquids were similar to those measured on PET$_{air}$/Ch. However, when the LS technique was applied (in the case of PET$_{air}$/Ch/DPPC$_{LS}$), their values were between those on PET$_{air}$/Ch/DPPC$_{LB↓}$ and PET$_{air}$/Ch/DPPC$_{LB↑}$, but closer to the former ones. Hence, one may conclude that in both cases, DPPC deposition is not sufficiently effective and/or it does not considerably influence the surface wetting properties.

On PET$_{air}$/Ch/DPPC$_{LB↑}$, the contact angle hysteresis of all test liquids was very small, within 2.4–4.1° (Fig. 1). As a low hysteresis was obtained in this case, it is believed that the developed mixed film constituted a well-prepared and stable surface [16]. Larger contact angle hysteresis indicates a better penetration of the liquid into the film structure during the receding contact angle measurement due to the lower condensation and/or ordering of molecules in the layer.

### 3.2. Surface free energy from contact angle hysteresis (CAH)

It should be taken into account that the contact angle is only a rough indicator of the surface character. To obtain more detailed characteristics, the surface free energy should be determined. In this paper, the contact angle hysteresis (CAH) model proposed by Chibowski was applied. Fig. 3 presents the values of the total surface free energy calculated separately from the contact angle hysteresis (CAH) of water ($\gamma_S^W$), formamide ($\gamma_S^F$) and diiodomethane ($\gamma_S^{DM}$), as well as their arithmetic mean ($\gamma_S^{tot}$). Depending on the type of liquid used for contact angle measurements (two polar and one apolar), different values of $\gamma_S$ were obtained. Therefore, the values of $\gamma_S$ should be treated as apparent. Nevertheless, their changes can provide important information on the intermolecular interactions coming from the surface after contact with the bulk liquid. $\gamma_S^W$ and $\gamma_S^F$ reflect both hydrogen bonding and dispersion forces, while $\gamma_S^{DM}$ indicates practically only dispersion forces. $\gamma_S^{tot}$ bears information on the averaged interactions originating from the surface and it is useful for comparison when the total surface free energy evaluated from a different model is considered [17].

The trend of $\gamma_S^W$ and $\gamma_S^F$ changes versus the kind of surface is the same but a little higher values of $\gamma_S^W$ are observed. As mentioned above $\gamma_S^W$ and $\gamma_S^F$ as determined from the contact angles of polar liquids indicate the interactions mainly by hydrogen bonding. Since water and formamide differ in the surface tension and its contributions ($\gamma_W = 72.8$ mJ/m$^2$, $\gamma_W^- = \gamma_W^+ = 25.5$ mJ/m$^2$, $\gamma_W^{LW} = 21.8$ mJ/m$^2$; $\gamma_F = 58.0$ mJ/m$^2$, $\gamma_F^- = 39.6$ mJ/m$^2$, $\gamma_F^+ = 2.28$ mJ/m$^2$, $\gamma_F^{LW} = 39.0$ mJ/m$^2$) [18], i.e. formamide exhibits higher $\gamma_L^-$ but lower $\gamma_L^+$ values than those of water, the strength of interactions can alter. Stronger interactions are obtained using water (except for PET$_{air}$/Ch/DPPC$_{LB↓}$). In all cases, the increase/decrease of $\gamma_S^W$ and $\gamma_S^F$ is accompanied by the decrease/increase of $\gamma_S^{DM}$, which is a consequence of the increased/decreased surface polarity, respectively. Diiodomethane is a liquid for which one can assume that $\gamma_{DM} = \gamma_{DM}^{LW} = 50.8$ mJ/m$^2$ [18]. This means that DM interacts solely by the Lifshitz-van der Waals interactions, which mainly include dispersion forces of London. As can be seen in Fig. 3, $\gamma_S^{DM}$ is higher than $\gamma_S^W$ and $\gamma_S^F$ for PET and PET/Ch. Calculating the difference between $\gamma_S^W$ or $\gamma_S^F$ and $\gamma_S^{DM}$, negative values are obtained, which, despite having no physical meaning, indicate that water and formamide interact with PET and PET/Ch mainly by the dispersive forces

which are weaker than those of diiodomethane. For $PET_{air}$, uncovered and covered with films, both the dispersive forces and H-bonds can be considered, because $\gamma_S^W$ and $\gamma_S^F$ are higher than $\gamma_S^{DM}$.
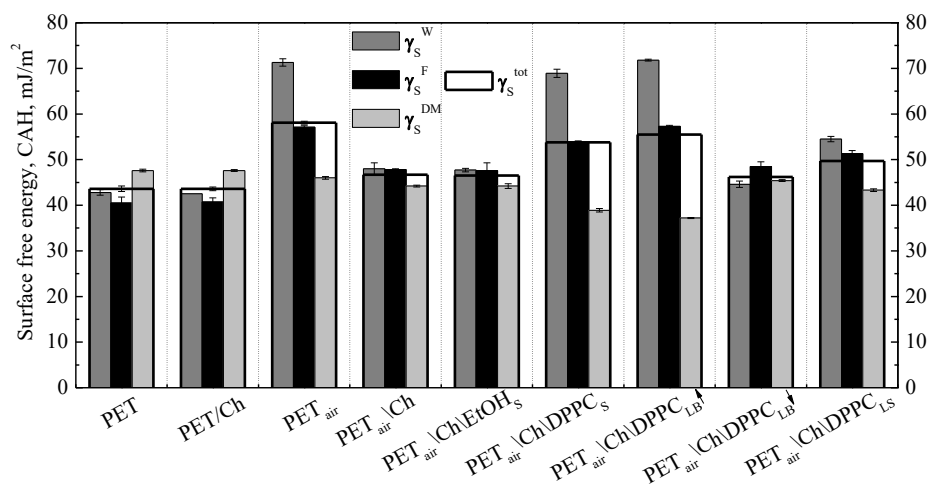


**Figure 3.** Surface free energy $\gamma_S$ estimated from the contact angle hysteresis of water ($\gamma_S^W$), formamide ($\gamma_S^F$) and diiodomethane ($\gamma_S^{DM}$) and their arithmetic mean ($\gamma_S^{tot}$).

PET surface activation by plasma significantly enhances its polarity, evidenced by the increased $\gamma_S^{tot}$ value, particularly $\gamma_S^W$. The PET surface, untreated and treated by air plasma, was subject to X-ray photoelectron spectroscopy (XPS) analysis conducted by Yang et al. [5] and Pandiyaraj [19]. This showed a significant decrease in C–C/C–H bonds when treated by plasma and an increase of new oxygen- and nitrogen-containing polar groups –OH, C–O, O=C–O, C=O and N–CO–N, on the polymer surface. The modification of PET results in functional group incorporation. Thus, improvement of surface polarity and, consequently, the chitosan solution spreading to form its homogeneous film is due to the ability of functional group interactions to act through bonds of electron donors and/or electron acceptors (mainly by hydrogen bonding). Considering our previous paper describing the investigation of chitosan adhesion onto activated polyetheretherketone ($PEEK_{air}$) [20], it follows that acid-base interactions between PET functional groups, as well as hydroxyls and amines (–OH or –$NH_2$/–$NH_3^+$) groups of chitosan are mainly responsible for the adhesion of chitosan film to the $PET_{air}$ surface. However, electrostatic interactions cannot be excluded. Surface roughness is additionally increased by treating the PET surface with plasma, thus facilitating chitosan film $PET_{air}$/Ch formation.

The presence of Ch decreased $\gamma_S^{tot}$ for $PET_{air}$ so that it was only slightly higher than that for PET or PET/Ch, but otherwise Ch makes $PET_{air}$/Ch able to interact not only by dispersive forces but also by hydrogen bonding (both $\gamma_S^W$ and $\gamma_S^F > \gamma_S^{DM}$). In the present study, the degree of deacetylation (DD) of chitosan is about 82%. Both acetylated and deacetylated units of chitosan have proton donor/acceptor groups participating in hydrogen bonding [21]. Both the above and surface roughness seem to be crucial for effective DPPC attachment. As follows from our results, the surface free energy of

$PET_{air}$/Ch/DPPC is dependent on the method of DPPC film preparation on $PET_{air}$/Ch. The $PET_{air}$/Ch/DPPC$_S$ and $PET_{air}$/Ch/DPPC$_{LB\uparrow}$ surfaces prepared by the DPPC solution spreading and the vertical withdrawal by means of Langmuir-Blodgett techniques, respectively, are characterised by the highest $\gamma_S^{tot}$ values, particularly $\gamma_S^W$. These findings suggest a significant contribution of hydrogen bonds that can originate from the polar heads of DPPC, and possibly polar Ch groups. Simultaneously, the reduced $\gamma_S^{DM}$ evidences that the hydrocarbon parts are mainly hidden or shielded by the heads. Interestingly, the $\gamma_S^F$ value is close to $\gamma_S^{tot}$, which can be considered as a peculiarity of formamide allowing for precise characterisation of general energetic properties of the surface using only one liquid.

Using the LB↑ method, after $PET_{air}$/Ch submersion into the water subphase (pH = 5.6) for 30–60 min, the DPPC monolayer is formed. When the pH value is lower than the biopolymer p$Ka$, i.e. 6.1–6.5 [22, 23], the charge of chitosan is positive but the –NH$_3^+$ groups are close to deprotonation. The charge of the phosphate group –OPO$_3^-$ in the zwitterionic DPPC is negative and that of the ammonium group –N$^+$(CH$_3$)$_3$ is positive, thus maintaining uncharged monolayer at neutral pH [24]. Water was able to diffuse into the polymer matrix; however, no swelling in pure ethanol was observed [25]. In the process of DPPC transfer, it is likely that the phospholipid molecules are attached to the amine/ammonium and/or hydroxyl groups of chitosan with their polar heads by means of hydrogen bonding. Due to the flexibility of chitosan chains and the capability of forming loops and tangles, penetration of DPPC molecules into the empty space and adaptation within the porous scaffold of chitosan is possible as a result of the Lifshitz-van der Waals interactions between the hydrophobic backbone of chitosan and hydrocarbon chains of DPPC. This leads to the outside exposure of the polar phosphocholine groups. One can claim that inclusion of DPPC into chitosan leads to stable hybrid $PET_{air}$/Ch/DPPC$_{LB\uparrow}$ system formation. Its structure is believed to be more compact than that of $PET_{air}$/Ch. As for the spreading method, the DPPC film formation takes place by the self-organisation of molecules dissolved in 96% ethanol. This is probable, as in the case of LB↑, water can participate in creating interactions between chitosan and DPPC, which cause phospholipid attachment and molecule-specific orientation. The latter is retained even when water and/or ethanol traces are removed from the samples under vacuum. However, a slightly lower value of the surface free energy of $PET_{air}$/Ch/DPPC$_S$ can be attributed to the loosely packed structure, hence the probe liquid molecules have access to hydrocarbon parts of the film molecules, particularly when the receding contact angle is measured (higher contact angle hysteresis).

If the LB↓ or LS technique is applied to the DPPC monolayer deposition, the energetic properties of the obtained surfaces $PET_{air}$/Ch/DPPC$_{LB\downarrow}$ and $PET_{air}$/Ch/DPPC$_{LS}$, respectively, are not satisfactory. The transfer mechanism should impose formation of the polar surface with phosphocholine heads localised at the film–air interface which would improve its polarity. However, the contact angles and surface free energy are quite similar to $PET_{air}$/Ch (Figs. 1 and 3). Therefore, one can conclude that both methods are much less effective for the production of mixed coatings of hydrophilic character, which is desirable in surface biocompatibility. Chitosan, positively charged in contact with blood, can interact with negatively charged cell membranes and/or proteins, inducing platelet activation and thrombus formation [9, 10]. Development of hybrid chitosan/DPPC films can modify the density of positive charge in biopolymers to produce a less thrombogenic chitosan film of potential application in vascular haemostatic devices or prostheses made of or covered with PET.

## 4. Conclusions

Wetting properties and surface free energy from the Chibowski model were determined for nonactivated and air plasma activated PET covered with chitosan and/or phospholipid films. The kind and strength of interactions were strongly dependent on the surface chemistry and type of probe liquid. PET surface activation with plasma significantly improved its hydrophilic properties, which facilitated chitosan adhesion to form a homogeneous film of biopolymer. The surface free energy of the activated PET considerably decreased with the deposition of the chitosan layer and then increased when the Ch/DPPC film was present. The molecular organisation and packing were determined by way of the DPPC monolayer deposition on chitosan simultaneously affecting surface wettability. The most enhanced polar properties were found for the $PET_{air}/Ch/DPPC_{LB\uparrow}$ monolayer deposited by the LB↑ technique. Such hybrid systems seem to be promising in terms of biocompatibility of novel chitosan-based haemostatic devices. Studies using other techniques are currently being conducted.

## 5. Acknowledgements

## 6. References

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