

WETTABILITY OF CHITOSAN-MODIFIED AND LIPID/POLYPEPTIDE-COATED PEEK SURFACES

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Abstract

In the present paper, cold plasma-activated and chitosan-coated polyetheretherketone (PEEK) was covered with thin films of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, cholesterol, cyclosporine A, and their mixtures using the Langmuir-Blodgett technique. The thermodynamic function, i.e., surface free energy, of those systems was determined based on the contact angle hysteresis (CAH) approach. This parameter seems to be essential in determination of cell adhesion to polymeric materials and molecular interactions with living tissues. The obtained results show that the wettability and surface free energy of PEEK can be changed depending on the composition of the coating.

Keywords: *plasma-activated PEEK, lipid-peptide films, surface free energy, cyclosporine drug delivery*

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1. Introduction

Polyetheretherketone (PEEK) (Fig. 1) is a synthetic, highly biocompatible polymer whose mechanical properties are very similar to those of human bones. For this reason, it has been used in regenerative medicine for over twenty years as a bone replacement material. In contrast to metal bone substitute materials, such as titanium or platinum alloys, PEEK does not cause allergies, and, in its volume, much less mechanical stresses arise. Moreover, it exhibits excellent sterilization resistance and radiolucency, which facilitate much better imaging using X-rays compared to metal alloys [1–3]. Obviously, economic aspects, such as low price and susceptibility to physicochemical surface modification, put PEEK above Pt, Ti, and its alloys. This fact will probably lead to the displacement of metal materials from orthopaedic implants within a dozen years. Unfortunately, a significant problem of the PEEK surface is a lack of osseointegration or insufficient integration with the bone tissue caused by the polymer hydrophobicity and low surface free energy [4,5].

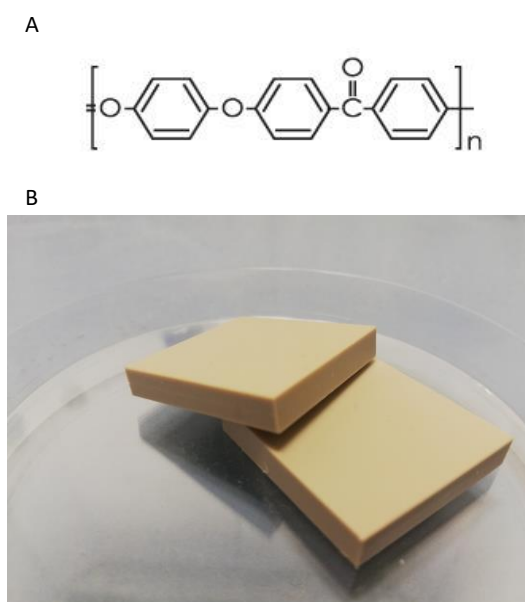


Figure 1. A) Structure of the PEEK monomer. B) PEEK plates used in the experiment

To overcome this imperfection and increase adhesion and growth rate of osteoblasts on the PEEK surface, physical and/or chemical modification is widely used, including cold plasma treatment, sandblasting, mineral coatings of titanium and hydroxyapatite, as well as organic polymeric coatings [6–10].

During the experiment described in the presented manuscript, the PEEK surfaces were activated using low-temperature and low-pressure air plasma. The increase in micro-roughness and creation of new functional surface groups [11] facilitate adhesion and bonding of various bioactive molecules, such as chitosan, to their surfaces. Chitosan (Ch) is a natural polysaccharide that has antibacterial and antifungal properties, good biocompatibility, and adsorption properties [12]. What should be taken into account is that the Ch biopolymer is an alternative to collagen in bone tissue engineering and,

according to literature, it has already been tested, with positive results as a polymer matrix for embedding bioactive molecules [13–16]. Also, the phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol (Chol) (Fig. 2A, B) are widely used for film formation on implant surfaces [17,18], which mimic the natural cell membrane [19].

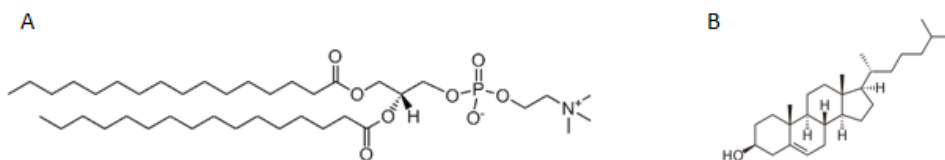


Figure 2. A) Structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).
B) Structure of cholesterol (Chol).

They are applied for preparing implant coatings by means of numerous techniques, such as Langmuir-Blodgett/Schaefer, solution spreading, self-assembly, or liposome adhesion techniques [20–23]. The obtained films with lipids should strengthen the intimate interface between the cells and the artificial material [24]. Fig. 3 represents the schematics of possible obtained structures of DPPC-CsA and Chol-CsA thin films deposited on the plasma-activated PEEK support with and without chitosan coating. The addition of the immunosuppressive drug cyclosporine A (CsA) to the one-component DPPC or Chol films can result in an interesting system serving as a carrier for therapeutic substances. When the biomaterial comes into contact with living tissue, the optimal hydrophilic–hydrophobic character of its surface and, hence, the appropriate surface free energy values, play a major role in the process of cell adhesion to artificial materials and osseointegration on their surface [25]. For this reason, in the present experiment, the obtained PEEK surfaces subjected to physicochemical modifications have been tested for wettability and surface free energy changes estimated on the basis of contact angle hysteresis (CAH) model.

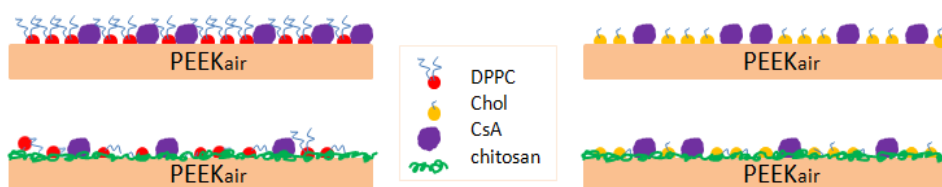


Figure 3. Schematic representation of the created structures of the DPPC-CsA and Chol-CsA thin films deposited on the PEEK air surfaces with and without chitosan coating.

2. Materials and Methods

2.1 Materials

PEEK plates (20 mm × 30 mm × 5 mm) were cut from commercial TECAPEEK natural, Proflex. The chitosan [MW of 100,000–300,000 and deacetylation degree (DD) of 82%] was purchased from Acrōs Organics (Belgium). The monolayer compounds DPPC and Chol were purchased from Sigma-Aldrich (USA), while CsA was from Alfa Aesar (USA). All had purities above 99%. Solvents for the Langmuir-Blodgett (LB) procedure, methanol and chloroform, were purchased from Avantor Performance

Materials Poland S.A. For wettability testing, three model liquids were applied, including water from a Milli-Q system (with the resistivity of 18.2 M Ω cm and pH=5.6), formamide (99.5% Acr \ddot{o} s Organics, Belgium), and diiodomethane (99%, Sigma-Aldrich, USA).

2.2 Methods

The chitosan solution was prepared by dissolving the proper amount of chitosan in 0.1% acetic acid to obtain the final concentration of 0.1 mg/mL. Such prepared solution was stored in the fridge for further usage. Before any PEEK modification, the polymer tiles were cleaned with the procedure already described, which can be found in our previous paper [26]. Purified and dry plates were activated with the low pressure (0.2 mbar) and low temperature air plasma (460 W) for 1 minute, with a continuous air flow of 22 sccm (*standard cubic centimetres per minute*) in the chamber of the Pico plasma system (Diener Electronic, Germany). Special scaffold was applied to provide activation of both sides of the plate. After equalizing the pressure in the chamber of the device with atmospheric pressure, the plates were modified with chitosan by means of the dip-solution technique. First, the plates were transferred to beaker with 50 mL of the chitosan solution for 5 minutes with the pliers. Then, the plates with chitosan layer were washed with MilliQ water three times to remove the acetic acid ions and after that transferred to vacuum oven for 24 hours at room temperature.

In the next steps of the experiment using the Langmuir-Blodgett (LB) technique the plasma activated and chitosan coated PEEK supports were modified with the thin films of phospholipid–1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), sterol–cholesterol (Chol) and polypeptide–cyclosporine A (CsA). The Langmuir-Blodgett trough (LB 2000, KSV, Finland) coupled with automatic dip-arm was applied to cover the modified PEEK surfaces. Accurate amounts of DPPC, Chol, CsA were dissolved in chloroform-methanol solution (4:1 v/v). Mixtures of CsA-DPPC and CsA-Chol were prepared by mixing appropriate volumes of one-component solutions. All systems were used just after preparation and precise solution volumes were spread using a micro-syringe (Hamilton) onto water subphase filling the clean Langmuir trough. After 10 minutes of solvent evaporation the following procedure was done by the LB technique. Moving barriers compressed substances spread onto air/water interface to obtain given value of surface pressure, high enough to coat the solid support with the packed monolayer. The surface pressure of transfer has been chosen basing on the π -A isotherms curves (surface pressure versus area per molecule) as well as referring to the other authors [27]. The obtained surfaces were transferred onto Petri dishes and dried in vacuum oven at room temperature for 24 hours. Such prepared surfaces were ready for wettability measurements.

The samples were placed in the chamber of contact angle measuring system (DGD ADR model with GBX S.A.R.L, WinDrop++ software). During the measurements the droplets were dropped using a micro-syringe, successively MilliQ water, diiodomethane and formamide. 6 μ L of liquid drop was released and the advancing contact angle was measured according to the procedure describing in our previous papers [11,20,21]. Subsequently, a volume of 2 μ L was withdrawn from the drop to measure the receding contact angle. Those measurements were completed for every system twice, where on each sample 5–8 single droplets of each probe liquid were placed taking readings the contact angle both on left and right side of the droplet. It provided 10–16 advancing and also 10–16 receding contact angles for each liquid tested. As a final point, the averaged contact angles were used for surface free energy calculation.

2.2.1 Surface free energy determination using the advancing and receding contact angle hysteresis (CAH approach)

In this model estimation of the total surface free energy (γ_s) bases on the advancing (θ_a) and receding (θ_r) contact angles of three liquids of known surface tensions (γ_L) [28].

$$\gamma_s = \frac{\gamma_L(1 + \cos\theta_a)^2}{(2 + \cos\theta_r + \cos\theta_a)}$$

In the following parts of this paper the total surface free energy (γ_s^{total}) values were presented. They represent the arithmetic mean of surface free energy values (γ_s) calculated separately from the contact angle hysteresis of water (γ_s^{water}), formamide ($\gamma_s^{formamide}$) and diiodomethane ($\gamma_s^{diiodomethane}$).

3. Results and Discussion

The main goal of the experiment was to estimate the impact of the lipid-peptide coatings on polarity and surface free energy of plasma activated and chitosan coated PEEK surfaces. Cold plasma treatment is well known method of not only adhesion and hydrophilicity increase but also of the sterilization process [29]. Taking into account the antibacterial, curative and mucoadhesive properties of chitosan this kind of surfaces can be valuable for creation of new generation coatings with the possibility of transferring and releasing therapeutic agents.

3.1 Contact Angles and Surface Free Energy

3.1.1 Chitosan Dip-Coated on the Activated PEEK Surface

The wettability of unmodified PEEK surfaces and after cold plasma activation was already described in our previous paper [26] as well by the other authors [30,31]. The surface free energy calculated from the contact angle hysteresis of water (γ_s^{water}) for plasma activated PEEK changed from 33.1 mJ/m² to 58.0 mJ/m² while that from the contact angle hysteresis of formamide ($\gamma_s^{formamide}$) rose from 39.0 mJ/m² to 56.7 mJ/m² compared to the unmodified PEEK surface (Fig. 4B). The observed phenomenon results from introducing new, highly polar surface functional groups rich in oxygen and/or nitrogen during air plasma treatment [11,32]. However, the contact angles of diiodomethane measured on PEEK and PEEKair as well as determined from them surface free energy values were not affected by cold plasma activation and did not change significantly (Fig. 4A, A'). This effect can be explained taking into account non-polar character of diiodomethane test liquid, which interacts with the surface mainly by dispersive forces. This phenomenon suggests that plasma treatment of the PEEK polymer does not affect dispersive interactions.

The significant change in wettability of the PEEKair surface coated with chitosan and in consequence change of its surface free energy confirms presence of chitosan coating. Advancing contact angles of all test liquids measured on the PEEKair/Ch surface raised markedly compared to the PEEKair surface: θ_a^{water} from 36.4° to 57.5°, $\theta_a^{formamide}$ from 0° to 36.2° and $\theta_a^{diiodomethane}$ from 23.1° to 52.1°. As an effect surface free energy-calculated from the contact angle hysteresis of each liquid decreased, γ_s^{water} from 63.8 mJ/m² to 52.4 mJ/m², $\gamma_s^{formamide}$ from 58.0 mJ/m² to 51.4 mJ/m² and $\gamma_s^{diiodomethane}$ from 48.2 mJ/m² to 39.0 mJ/m². In general, γ_s^{total} decreased from 56.6 mJ/m² to 47.6 mJ/m² after chitosan embedding to the activated PEEK surface. Slightly different effect of the activated PEEK surface coating with chitosan was observed in our

previous studies [26] using the spreading technique of chitosan solution onto the PEEKair surfaces. In that case the advancing contact angles of water, formamide and diiodomethane were measured as: $\theta_a^{water}=66.5^\circ$, $\theta_a^{formamide}=50.3^\circ$ and $\theta_a^{diiodomethane}=37.9^\circ$. In consequence the values of surface free energy estimated from contact angles of three liquids were $\gamma_s^{water}=46.1$ mJ/m², $\gamma_s^{formamide}=45.6$ mJ/m² and $\gamma_s^{diiodomethane}=44.2$ mJ/m². Finally, the values of total surface free energy were slightly lower ($\gamma_s^{total}=45.3$ mJ/m²) than those determined for the chitosan dip-coated PEEK (47.6 mJ/m²) [26]. Based on these results, it can be concluded that depending on the coating technique the chitosan molecules can be organized in different way. The PEEKair/Ch surface obtained by dipping technique was more polar than that obtained by spreading procedure. Moreover the former one revealed weaker interactions with non-polar diiodomethane. This can suggest that this surface has definitely more polar groups towards the gas phase compared to the chitosan spreading-coated PEEK surface.

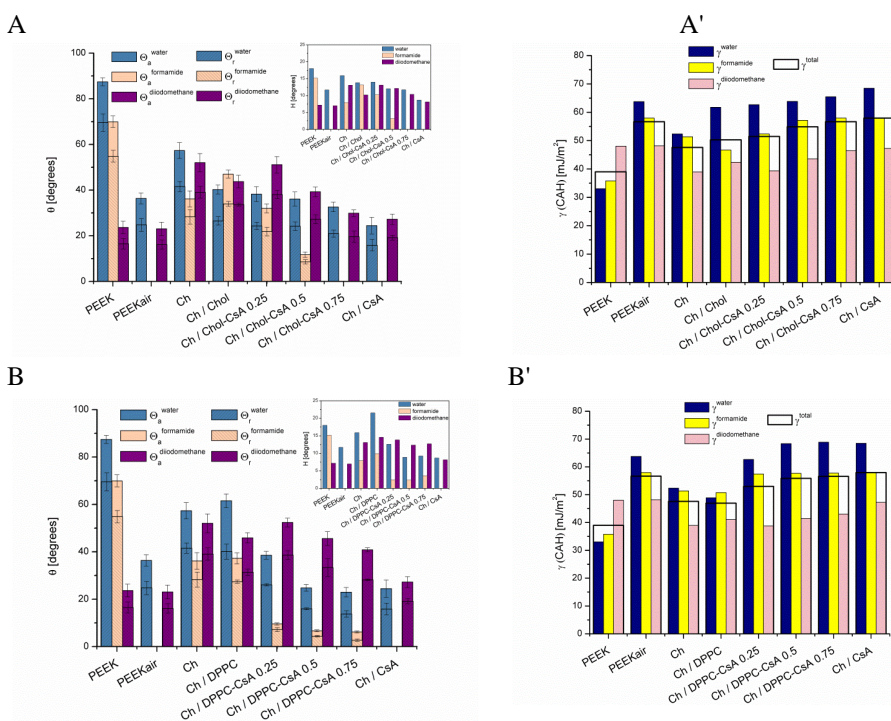


Figure 4. A, B) Advancing and receding contact angles of water, formamide and diiodomethane and their hysteresis (H) (inset A, B); A', B') Surface free energy (γ_s) estimated from the contact angle hysteresis of water, formamide and diiodomethane and their arithmetic mean (γ_s^{total}) for the modified PEEK surfaces.

It has been already confirmed by other authors basing on the FTIR, SEM and thermal analyses that cold plasma activation of the PEEK surface improves adhesion of the chitosan molecules and allows creation of strong physical bonding, however simultaneous formation of chemical bonds are not excluded. The activated PEEK surface itself seems to have no potential medical application, due to the return of the activated polymer surface to the state before the modification in time. The production of an additional cover with chitosan provides a sterile surface with the higher surface free

energy than that of unmodified PEEK and other useful properties resulting from the presence of chitosan [12,29].

3.1.2 One- and Two-Component Monolayers Deposited on the Plasma-Activated and Chitosan Dip-Coated PEEK Surfaces

Deposition of one component monolayers of Chol, DPPC and CsA on the chitosan coated PEEKair surface resulted in changes of its wettability, especially significant changes were observed in the Ch/CsA system. Presence of the Chol molecules on chitosan coating led to decrease of advancing contact angles of water and diiodomethane, while in the case of formamide surprisingly increase of its advancing contact angle was observed (**Fig. 4A**). In consequence the total γ_s^{total} of this surface was estimated as $\gamma_s^{total}=50.3$ mJ/m² which is slightly (2.7 mJ/m²) higher comparing to the PEEKair/Ch coating (**Fig. 4A, A'**). The impact of DPPC thin film on behaviour of test liquids was smaller than that of Chol. Little increase of hydrophobic character of the surface was reflected in increase of the advancing contact angle of water θ_a^{water} from 57.4° to 61.6°, while contact angles of formamide remained within the limits of the standard deviation. Advancing contact angles of non-polar diiodomethane dropped from 52.1° to 46.0°. Overall the total surface free energy for this system was estimated as $\gamma_s^{total}=46.9$ mJ/m², which was very close to that of chitosan coating ($\gamma_s^{total}=47.6$ mJ/m²) (**Fig. 4B, B'**). The greatest influence on the change in the contact angles of all measuring liquids was observed when the PEEKair/Ch surface was modified with cyclosporine A film. The advancing contact angles of test liquids rapidly dropped from 57.4° to 24.5° (water), from 36.2° to 0.0° (formamide) and from 52.1° to 27.3° (diiodomethane). Mean value of total surface free energy increased significantly from 47.6 mJ/m² to 58.0 mJ/m² compared to that of PEEKair/Ch surface. Cyclosporine A is a cyclic polypeptide capable of adopting the different conformations dependent on surrounding molecules [34]. In described systems the similar conformational changes not only CsA molecules but also other components of layer (Chol and Ch) can take place which are sensitive to packing and the composition of the monolayer. As a result of these changes, the molecules of Ch can interact through Lifshitz-van der Waals forces or by H-bonds creation with various functional groups of the used substances. Cyclosporine A owns for example –OH; –C=O; >NH functional groups which in this case may determine its specific orientation on the chitosan surface. Taking into account the low values of the contact angles of the polar measuring liquids, it can be concluded that CsA probably interacts with the chitosan molecules on the PEEK surface and directs with its hydrophilic parts towards the gas phase.

This hypothesis seems to be also applicable to the two-component mixtures of Chol-CsA and DPPC-CsA, because the small addition of cyclosporine to Chol or DPPC determines the behaviour of the entire film. In each case the hydrophilicity of the surface increases, the stronger the higher amount of the cyclosporin is present in the layer. Fig. 4A and Fig. 4B present the changes in wettability of the mixed Chol-CsA and DPPC-CsA monolayers. For the Chol-CsA system the advancing contact angles of water are not strongly affected by addition of CsA and reach similar level on all mixed films, i.e. θ_a^{water} equals about 35°. However, in the case of formamide great decrease of contact angles from $\theta_a^{formamide}=34.0^\circ$ (PEEKair/Ch/Chol) to 21.8° at $X_{CsA}=0.25$ and 8.5° at $X_{CsA}=0.5$ was observed, and finally $\theta_a^{formamide}=0.0^\circ$ at $X_{CsA}=0.75$ (total spreading). Similar effect can be seen for the DPPC-CsA mixed film but the values of advancing contact angles of water significantly decreased at $X_{CsA}=0.25$ and $X_{CsA}=0.5$ from 61.6° (PEEKair/Ch/DPPC) to 38.6° and 24.8°, respectively.

Further increase of the CsA amount did not affect the surface wettability by water. In the case of formamide the trend was similar to that of Chol-CsA mixed film. The small amount of CsA added to the DPPC film resulted in great decrease of the formamide advancing contact angles from 37.4° to 9.6° comparing to those on the PEEKair/Ch/DPPC coating. However, further addition of CsA did not influence the advancing contact angles of formamide significantly (**Fig. 4B**). The trend of changes of γ_s^{total} in the case of Chol-CsA and DPPC-CsA is the same: for pure Chol $\gamma_s^{total}=54.9$ mJ/m² and for mixed Chol-CsA layers: $\gamma_s^{total}=50.3$ mJ/m² at $X_{CsA}=0.25$, $\gamma_s^{total}=51.5$ mJ/m² at $X_{CsA}=0.5$, $\gamma_s^{total}=54.9$ mJ/m² at $X_{CsA}=0.75$, for pure DPPC $\gamma_s^{total}=46.9$ mJ/m² and for mixed DPPC-CsA layers: $\gamma_s^{total}=53.0$ mJ/m² at $X_{CsA}=0.25$, $\gamma_s^{total}=55.9$ mJ/m² at $X_{CsA}=0.5$, $\gamma_s^{total}=56.6$ mJ/m² at $X_{CsA}=0.75$ (**Fig. 4A', B', Table 1**). The dependencies can suggest that the CsA presence determines behaviour of the film deposited on the chitosan coating and such modifications show noticeable impact of a given substance that can affect the hydrophobic-hydrophilic nature of the entire film.

Table 1. Total surface free energy [mJ/m²] of the PEEKair surfaces with and without chitosan film (from our previous studies [33]) coated with one- and two-component monolayers of Chol, DPPC, CsA, Chol-CsA and DPPC-CsA

| | Chol | DPPC | CsA | Chol-CsA | | | DPPC-CsA | | |
|--------------|------|------|------|-----------|------|------|----------|------|------|
| | | | | X_{CsA} | | | | | |
| | | | | 0.25 | 0.5 | 0.75 | 0.25 | 0.5 | 0.75 |
| PEEKair [33] | 45.9 | 45.5 | 54.6 | 48.4 | 52.6 | 54.2 | 46.4 | 51.3 | 55.1 |
| PEEKair/Ch | 46.9 | 50.3 | 58.0 | 51.5 | 54.9 | 56.7 | 53.0 | 55.9 | 56.6 |

The presence of chitosan coating on activated PEEK significantly affects the organization and formation of lipid-polypeptide monolayers on its surface. In our other paper [33] we described wettability process of Chol, DPPC and CsA films deposited on the activated PEEK surface by the LB technique. Those surfaces revealed total surface free energy $\gamma_s^{total}=45.9$ mJ/m²; 45.5 mJ/m² and 54.6 mJ/m² for DPPC, Chol and CsA respectively [33]. In present experiment, the same systems were investigated, but were deposited on the PEEKair/Ch surface. The presence of chitosan coating caused in each case increase of surface polarity what reflected in rise of the surface free energy values to 46.9 mJ/m²; 50.3 mJ/m² and 58.0 mJ/m² for DPPC, Chol and CsA films respectively (**Table 1**). The same trend of surface free energy values changes was observed in case of Chol-CsA and DPPC-CsA mixed films deposited on PEEKair substrate with and without chitosan. The total surface free energy increased as the amount of CsA molecules was higher in the monolayer, however, estimated values of γ_s^{total} were higher for those layers deposited on PEEKair with chitosan coating (**Table 1**). This observation proves the significant impact of chitosan on behaviour, organisation and orientation of different molecules on activated PEEK surfaces. Based on these findings, the possible molecular arrangement of the films was proposed and schematically presented in Fig. 3.

4. Conclusions

Interesting results were observed after deposition of one-component films on the activated and chitosan-coated PEEK surfaces as well two-component systems of DPPC-CsA and Chol-CsA with different CsA content ($X_{CsA}=0.25, 0.5, \text{ or } 0.75$). The PEEKair/Ch/DPPC system was found to be the most hydrophobic, and it revealed a

minimum value of surface free energy compared to the other systems. On the other hand, the most polar was the PEEKair/Ch system, with a CsA film characterized by the highest surface free energy values. What is important is the presence of the chitosan coating, which caused a noticeable growth of surface free energy of each thin film, single and also mixed, deposited on the PEEKair/Ch surface compared to the PEEKair surface. Moreover, by applying various techniques, it was possible to transfer molecules of different packing on the activated PEEK support, leading to the formation of a multifunctional bio-coating. It should be noted that CsA has a great impact on the behaviour of DPPC and CsA molecules. We believe that probable biomolecule organization on the PEEKair/Ch surface is reflected by the changes of its surface free energy evaluated from the CAH approach. The obtained significant changes in surface free energy of the PEEK surfaces are noteworthy and show that even very small amounts of used molecules can affect the surface properties of PEEKair or PEEKair/Ch. The development of polymer surfaces coated with substances with specific properties and controlling their hydrophobic–hydrophilic character is important in the aspect of numerous chitosan applications. Additionally, the drawn conclusions seem to be very helpful in future planning and the development of multifunctional coatings of polymers with a defined value of surface free energy, which is crucial in the case of biomedical applications. Received surfaces are intended to have potential application in regenerative medicine to develop biomimetic strategies for artificial bone grafts in tissue engineering, as well to enhance the polymer osseointegration process and simultaneous drug delivery systems.

5. References

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