

WETTING PROPERTIES OF CHITOSAN-MODIFIED AND PLASMA-TREATED PEEK SURFACES

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Abstract

In this paper, the wettability of chitosan/phospholipid (1,2-dipalmitoyl-sn-glycero-3-phosphocholine – DPPC), chitosan/lipid (cholesterol – Chol) and chitosan/protein (cyclosporine A – CsA) films on air plasma activated polyetheretherketone (PEEK) plates was studied. The layers were prepared using the solution spreading technique and their surface wetting properties were determined based on the measurements of the advancing and receding contact angles of water, formamide and diiodomethane. Moreover, based on the contact angle hysteresis model of Chibowski, values of total surface free energy were estimated. Significant changes in PEEK polarity were observed after plasma activation and modifications with Ch/DPPC, Ch/Chol and Ch/CsA layers. These molecules modulate the chitosan film surface by changing the type and magnitude of interactions, which is revealed in the values of surface free energy. These results may be important for the development and implementation of highly biocompatible bone substitution polymers coated with chitosan film with anti-fungal and anti-bactericidal properties. Those systems based on chitosan may also carry and release biologically active substances which could be relevant in the new generation of drug delivery systems.

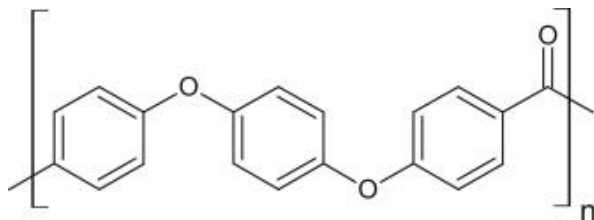
Keywords: *plasma activation, PEEK, chitosan films, contact angle, surface free energy*

Received: 28.03.2018

Accepted: 08.05.2018

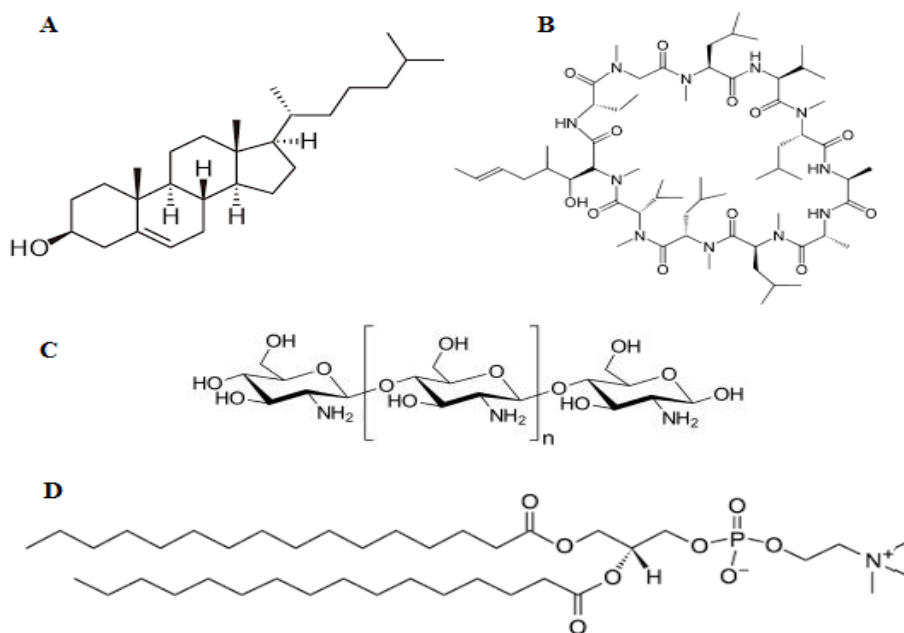
1. Introduction

The development of cheap, non-toxic and effective controlled drug delivery systems as well as highly biocompatible bone substitute materials is a major challenge for modern medicine and pharmacology. One of these materials is polyetheretherketone (PEEK), which is widely used in orthopaedics as a bone implant, and soon may dominate obsolete materials used for the production of implants, such as titanium or platinum alloys due to the low price, better mechanical properties and susceptibility to modifications shown by PEEK [1]. Scheme 1 shows the structure of PEEK.



Scheme 1. Structure of polyetheretherketone (PEEK).

Low temperature air plasma can be used to activate the PEEK surface (PEEKair); this procedure is also a modern method for the disinfection of a wide range of materials used in medicine [2]. As previously reported, plasma activation of the surface of PEEK increases its micro-roughness and allows chitosan layers to be deposited more efficiently [3]. This kind of hybrid system may find application in a new generation of drug carriers due to the unique properties of the chitosan biopolymer. Chitosan (Ch) is a linear amino-polysaccharide comprised of (1–4)-linked D-glucosamine residues and randomly distributed N-acetyl-d-glucosamine units. After cellulose, chitosan is the second most abundant polymer on Earth. In nature, it is a semi-crystalline powder and builds the exoskeleton of insects and crustaceans, like shrimps and crabs. In the laboratory chitosan may be produced through the exhaustive deacetylation of chitin. Since the early 1990s, chitosan has been widely used in pharmaceutical areas mainly in slimming, wound healing and tissue modifications. Due to its unique cationic character, the chitosan biopolymer has drawn increasing attention within the pharmaceutical and biomedical industries, owing to its abundant availability, mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties such as biocompatibility, biodegradability, non-toxicity and low-immunogenicity [4-6]. In order to investigate the type and magnitude of interactions between PEEKair/Ch, living cells and possibly carried drugs, monolayers of cholesterol (Chol), phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and immunosuppressive cyclosporine A (CsA) were deposited using the spreading technique. Chol and DPPC are the main components of biological membranes, while CsA is widely used in medicine as a drug to prevent the rejection of organs following transplantation. The structures of all substances studied are shown in Scheme 2. The surface properties were evaluated based on the advancing and receding contact angles of probe liquids. Afterwards, the apparent surface free energy was determined using the contact angle hysteresis approach proposed by Chibowski [7]. Air plasma-modified PEEK surfaces coated with chitosan film may be a new generation of bone substitute materials which reduce inflammation, accelerate convalescence and contain therapeutic substances that could be released in a controlled manner.



Scheme 2. Structures of: A) Cholesterol (Chol), B) Cyclosporine A (CsA), C) Chitosan (Ch) and D) 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).

2. Materials and Methods

2.1 Materials

Polyetheretherketone (PEEK) (TECAPEEK natural, Proflex) plates with dimensions of 30mm x 20mm x 5mm were used as the base matrix for monolayer deposition. Chitosan (MW 100,000–300,000 and deacetylation degree DD 82%) was purchased from Acrōs Organics (Belgium) while 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, 99%), cholesterol (Chol, 99%) and cyclosporine A (CsA, 98.5%) were provided by Sigma-Aldrich (USA). Methanol (p.a.) and ethanol (96%, p.a.), both from Avantor Performance Materials Poland S.A., were used as received for compound dissolution. Formamide (99.5%, Acrōs Organics, Belgium) and diiodomethane (99%, Sigma-Aldrich, USA), purified by a Milli-QPlus system water with the resistivity of 18.2M Ω cm, pH=5.6, were used as probe liquids for contact angle measurements.

2.2 Methods

2.2.1 Purifying the surface of polyetheretherketone (PEEK) plates

PEEK tiles were placed in a beaker with a solution of 2 mL of neutral extran in 200 mL of pure MilliQ water and then placed in an ultrasonic bath for 15 minutes. After removing the excess extran solution, the plates were covered with methanol in the same beaker and placed in an ultrasonic bath for 15 minutes. After this step, methanol was poured out and the polymers were covered with pure water and again placed in an ultrasonic bath for 15 minutes. This last step was repeated 2-3 times until the conductivity value of the water above the plates corresponded to the specific conductivity of pure water from the MilliQ system, i.e. about 4.5 μ S/cm. Then, dry plates were placed in a vacuum oven for 24 hours at room temperature. After this time,

the plates were transferred to a desiccator, where they were stored for further modification.

2.2.2 Low temperature plasma treatment of PEEK surface

Cleaned samples were placed in the chamber of the Pico plasma system (Diener Electronic, Germany). The polymer surface was exposed to low pressure (0.2 mbar) and low temperature air plasma (460W) for 1 minute, with a continuous air flow of 22 sccm (*standard cubic centimetres per minute*). After equalising the pressure in the chamber of the device with atmospheric pressure, the plates were transferred onto Petri dishes, activated upwards. Immediately after activation with plasma, solutions of the tested compounds were used.

2.2.3 Solutions preparation

Solutions of DPPC, Chol and CsA at a concentration of 1 mg/mL were obtained by weighing the appropriate amounts of DPPC, Chol and CsA, and then dissolving them in 1 mL of ethanol. The solutions were prepared immediately before use. The exception was the chitosan (Ch) solution, which was prepared in a 100 mL flask by dissolving 10 mg of chitosan in 0.1% acetic acid. The 0.1 mg/mL solution prepared in this way was reusable when stored in a refrigerator.

2.2.4 Monolayers formation on PEEK matrix using the spreading technique

PEEK plates were placed on a platform equipped with a spirit level. Then, 0.5 mL of the chitosan solution was applied to the polymer surface using an automatic pipette. After the evaporation of water and drying, the samples were moved to a vacuum oven. The ethanol solutions of biologically active substances with a volume of 0.5 mL were poured onto the modified PEEK surface. The number of substance molecules deposited on the PEEK surfaces was chosen based on their π -A isotherms at a surface pressure of 20 mN/m, thereby forming a self-assembling monolayer. After the evaporation of ethanol, the samples were placed in a vacuum oven until the remaining solvent was completely removed. The samples were then further analysed within 24 hours of the monolayer preparation. In the case of PEEK surfaces activated with air plasma, solutions of biologically active substances were applied within 2 minutes from the end of the plasma process, which ensured the complete wettability of the carrier.

2.2.5 Advancing and receding contact angle measurements of water, formamide and diiodomethane

Prepared samples were placed in the chamber of the contact angle measuring system (DGD ADR model with GBX S.A.R.L, WinDrop++ software). During the measurements, the chamber was filled with nitrogen pumped from a cylinder. The droplets were dropped using a micro-syringe, successively MilliQ water, diiodomethane and formamide. Here, 6 μ L of each liquid drop was released and the advancing contact angle was measured. 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, with 5-8 single droplets of each probe liquid were placed on each sample, taking readings of the contact angle on both the left and right sides of the droplet. This provided 10-16 advancing and 10-16 receding contact angles of one liquid with a standard deviation of 1-5°. The averaged contact angles were used for surface free energy calculations.

2.2.6 Surface free energy determination using hysteresis of advancing and receding contact angles (CAH approach)

In this model, estimation of the total free surface energy (γ_s) were based on the advancing (Θ_a) and receding (Θ_r) contact angles of three liquids of known surface tensions (γ_L) [7].

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

In the next parts of this paper, the total surface energy (γ_s^{tot}) was presented. It represents the arithmetic mean of surface free energy values (γ_s) calculated separately from the contact angle hysteresis of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}).

3. Results and discussion

3.1 Contact angles and hysteresis

The wettability of the untreated and plasma treated PEEK surface and that coated with chitosan (after plasma treatment) or/and the DPPC, Chol and CsA films was examined using advancing (Θ_a) and receding (Θ_r) contact angle measurements of three liquids: pure water (MilliQ), formamide and diiodomethane. The contact angles of polar liquids on the unmodified PEEK surface were $\Theta_a = 87.5^\circ/\Theta_r = 69.5^\circ$ and $\Theta_a = 70^\circ/\Theta_r = 54.8^\circ$ for water and formamide, respectively, while for non-polar diiodomethane these values were $\Theta_a = 23.7^\circ/\Theta_r = 16.5^\circ$ (Fig.1.A). These results are in agreement with those published previously [3].

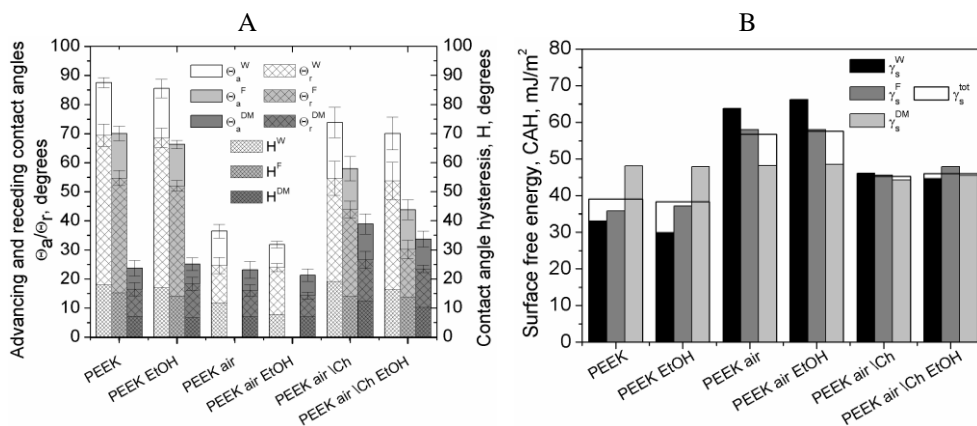


Figure.1. A –Advancing and receding contact angles of water (W), formamide (F) and diiodomethane (DM) and their hysteresis (H); B – Surface free energy γ_s estimated from the contact angle hysteresis of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}) and their arithmetic mean (γ_s^{tot}) for PEEK basic modified surfaces.

Activation of the polymer surface with air plasma results in a significant change in polar properties which reveals a reduction in the average values of contact angles of water and formamide by $\Theta_a = 51.1^\circ/\Theta_r = 44.8^\circ$ for water; in the case of formamide, the strength of its interactions with the surface is so large that the contact angles of its droplets cannot be measured ($0^\circ/0^\circ$), while those of diiodomethane remain mostly

unchanged. Water and formamide are polar liquids and interact by both hydrogen bonds and dispersion forces; however, diiodomethane, which can be considered a non-polar liquid, mostly interacts by dispersion forces. Low values of contact angles of water and total spreading of formamide droplets after PEEK plasma activation show that PEEK plasma treatment significantly changes its surface polarity. On the other hand, it is known that the PEEK plasma treatment raises its roughness on the microscale and the adhesion of chitosan, hence its film is deposited more efficiently [3]. The surface obtained by coating the activated PEEK surface with chitosan film shows very interesting wetting properties. The initial advancing and receding contact angles measured on the modified and chitosan layer covered PEEK surface are $\Theta_a = 73.8^\circ/\Theta_r = 54.7^\circ$, $\Theta_a = 57.9^\circ/\Theta_r = 43.9^\circ$ and $\Theta_a = 38.9^\circ/\Theta_r = 26.6^\circ$ for water, formamide and diiodomethane, respectively (Fig.1.A). These findings suggest that the chitosan film is relatively hydrophobic and does not allow the probe liquids to make contact with the activated PEEK surface. Similar results and trends of changes were observed in other experiments where polyethylene terephthalate (PET) plates were used instead of PEEK [8]. Moreover, the influence of ethanol on the PEEK surfaces modified with chitosan film has been studied. The results show that there are no significant changes in sample wettability after contact with ethanol (Fig.1.A); therefore, solutions of DPPC, Chol and CsA were prepared using ethanol as a solvent. PEEK modifications with the layers of chitosan, DPPC, Chol and CsA will be discussed in the next part of this work.

The formation of DPPC, Chol and CsA films on the unmodified PEEK surface caused an increase in its polarity. This was revealed in the reduction of water and formamide advancing contact angles of water and formamide on the PEEK\DPPC, PEEK\Chol and PEEK\CsA surfaces, by 25° , 20.8° , 19° and 27.6° , 17.3° , 20.1° , respectively, as compared to the reference system ($\Theta_a = 85.5^\circ$ water; $\Theta_a = 64.7^\circ$ formamide) (Fig.2.A). These findings suggest that the molecules deposited onto the PEEK surface rotate the hydrophilic parts towards the gas phase.

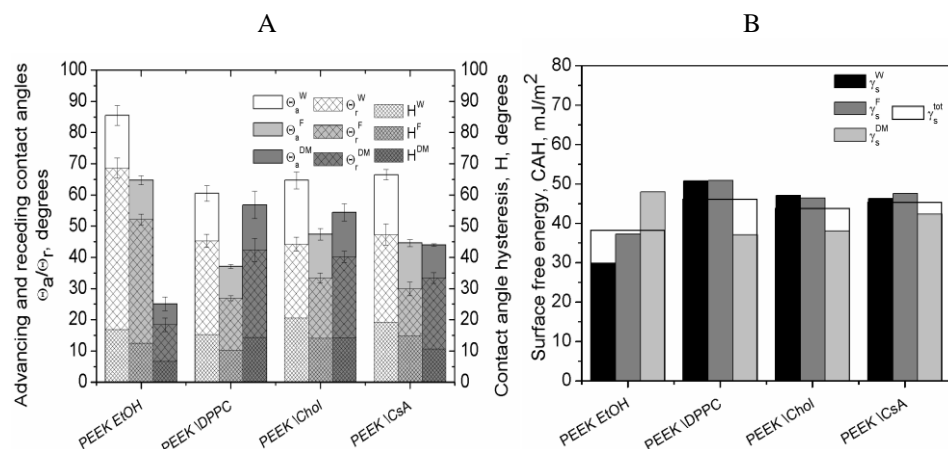


Figure 2. A – Advancing and receding contact angles of water (W), formamide (F) and diiodomethane (DM) and their hysteresis (H); B – Surface free energy γ_s estimated from the contact angle hysteresis of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}) and their arithmetic mean (γ_s^{tot}) for PEEK modified surfaces.

As is already known, diiodomethane interacts with other materials, mostly via Lifshitz-van der Waals interactions, which mainly include dispersion forces of London [8]. In this system, a significant decrease in dispersion interactions is observed after the deposition of DPPC, Chol or CsA layers due to an increase of diiodomethane advancing contact angles by 24° ; 21.8° and 15° for DPPC, Chol and CsA, respectively, compared to PEEK EtOH system ($\Theta_a = 18.4^\circ$) (Fig.2.A).

Air plasma process treatment of the PEEK surface allows it to be coated with chitosan film. The PEEK activation with plasma causes high surface oxidation and its polarity increases; therefore, the interactions between chitosan molecules and the polymer surface become stronger. The measured contact angles of water and formamide are $\Theta_a = 66.5^\circ$ and $\Theta_a = 38.8^\circ$, respectively, whereas on the reference system (PEEKair) those liquids form drops of advancing contact angles of $\Theta_a = 31.8^\circ$ (water) or they totally spread (Θ_a of formamide is assumed 0°) (Fig.3.A). Also, the dispersion forces after chitosan deposition weaken. The average advancing contact angle of diiodomethane measured on the PEEKair\Ch surface is $\Theta_a = 37.9^\circ$, and that examined on PEEKair EtOH is $\Theta_a = 21.3^\circ$. Other substances deposited on the activated PEEK surface also modify its polarity. On the DPPC, Chol and CsA layers, the advancing contact angles of water account for $\Theta_a = 20.1^\circ$; $\Theta_a = 40.5^\circ$ and $\Theta_a = 30.8^\circ$, respectively. The lowest values obtained for DPPC may be explained by a specific arrangement of the hydrophilic DPPC heads towards to the gas phase.

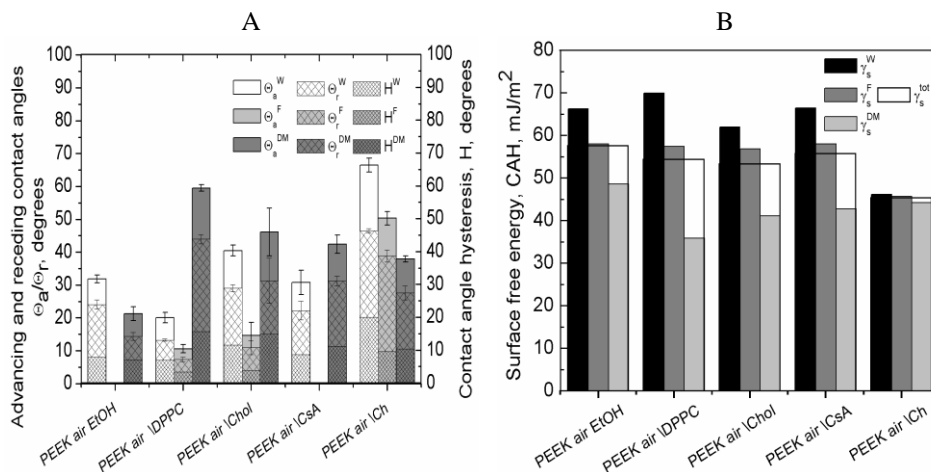


Figure 3. A – Advancing and receding contact angles of water (W), formamide (F) and diiodomethane (DM) and their hysteresis (H); B – Surface free energy γ_s estimated from the contact angle hysteresis of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}) and their arithmetic mean (γ_s^{tot}) for PEEKair-modified surfaces.

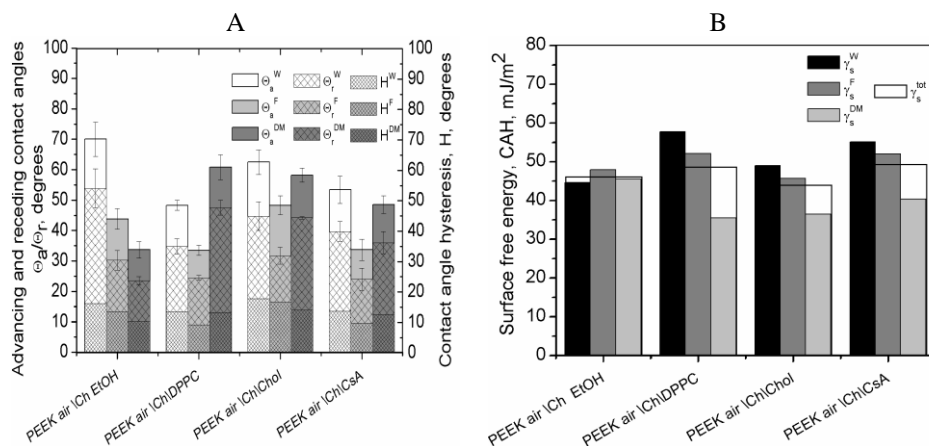


Figure 4. A – Advancing and receding contact angles of water (W), formamide (F) and diiodomethane (DM) and their hysteresis (H); B – Surface free energy γ_s estimated from the contact angle hysteresis of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}) and their arithmetic mean (γ_s^{tot}) for PEEKair\chitosan-modified surfaces.

Since the chitosan molecules possess a positive charge, they strongly interact with cell membranes; therefore, it may be used in biomedical applications such as drug carriers. Deposition of the bioactive layers of DPPC, Chol, or CsA onto the PEEKair\Ch surface results in changes in its polar properties. The highest hydrophobicity reduction compared to PEEKair\Ch EtOH system ($\Theta_a = 70^\circ$ water contact angle) is observed on DPPC ($\Theta_a = 48.3^\circ$) and on CsA ($\Theta_a = 53.4^\circ$) while the Chol layer reveals a water advancing contact angle equal to $\Theta_a = 62.5^\circ$. Significant changes in non-polar interactions are observed in these systems, where the average advancing contact angle of diiodomethane is $\Theta_a = 33.7^\circ$ for the reference system and increases to $\Theta_a = 60.8^\circ$ for DPPC, $\Theta_a = 58.2^\circ$ for Chol and $\Theta_a = 48.5^\circ$ for CsA after the layer deposition. All surfaces obtained on the activated PEEK (Fig.4.A) coated with chitosan film show greater hydrophilicity compared to those obtained on unmodified PEEK (Fig.2.A). The hydrophilicity property is desirable in the case of contacting materials with living cells and body fluids. Moreover, the fusion of highly biocompatible polymers such as PEEK with chitosan, which is bactericidal and fungicidal, may result in new generation drug delivery systems.

PEEK plasma activation results in a significant decrease in the water contact angle hysteresis value from $H^W = 18.0^\circ$ (PEEK) to $H^W = 11.7^\circ$ (PEEKair) (Fig.1.A). This result confirms the large hydrophilisation of the PEEK surface. Deposition of the biologically active DPPC, Chol and CsA monolayers causes an increase in H^W for Chol and CsA by 3.6° and 2.3° but a decrease by 1.7° for the DPPC monolayer compared to the PEEK reference system (Fig.2.A). Very similar changes and trends are observed after the deposition of those molecules onto the activated PEEK surface (Fig. 3.A). The presence of the chitosan layer causes a significant increase in the H^W and H^{DM} values from $H^W = 11.7^\circ$ and $H^{DM} = 7^\circ$ measured for PEEKair to $H^W = 19.1^\circ$ and $H^{DM} = 12.4^\circ$ measured for the PEEK air\Ch layer (Fig.3.A). Changes in the contact angle hysteresis can prove an increase or decrease in the permeability of the films to the measuring liquids; usually, high hysteresis values indicate film permeability and an increase in the interaction

between the measuring liquid and the polymer matrix. In the next part, the surface free energy estimated from the hysteresis of contact angles will be discussed.

3.2 Surface free energy from contact angle hysteresis (CAH)

Values of average advancing and receding contact angles describe the polar character of the surface in a general way. For more accurate analysis, surface free energy should be determined. In this study, to estimate this parameter, the contact angle hysteresis (CAH) model proposed by Chibowski was applied. It should be taken into account that the values of γ_s are apparent because they depend on the type of measuring liquid. However, changes in their values can provide useful information on intermolecular interactions on the solid surface. Total surface free energies of differently modified surfaces which are presented in this paper were calculated separately from the CAH of water (γ_s^W), formamide (γ_s^F) and diiodomethane (γ_s^{DM}), as well as their arithmetic mean γ_s^{tot} . This system of probe liquids was selected due to their physical properties, water and formamide which are both polar substances and reflect both hydrogen bonding and dispersion forces, differing in the surface tension and its contributions ($\gamma_w = 72.8 \text{ mJ/m}^2$, $\gamma_w^+ = \gamma_w^- = 25.5 \text{ mJ/m}^2$, $\gamma_w^{LW} = 21.8 \text{ mJ/m}^2$, $\gamma_F = 58.0 \text{ mJ/m}^2$, $\gamma_F^- = 39.6 \text{ mJ/m}^2$, $\gamma_F^+ = 2.28 \text{ mJ/m}^2$, $\gamma_F^{LW} = 39.0 \text{ mJ/m}^2$), while diiodomethane, as mentioned before, indicates only dispersion forces $\gamma_{DM} = \gamma_{DM}^{LW} = 50.8 \text{ mJ/m}^2$ [9].

Air plasma activation of the PEEK surface resulted in a large increase in polarity, which was observed as an increase in the γ_s^W and γ_s^F parameters from 33.1 mJ/m^2 and 35.8 mJ/m^2 estimated for PEEK to 63.8 mJ/m^2 and 58 mJ/m^2 estimated for PEEK air (Fig.1.B). However, the values of γ_s^{DM} remain unchanged. These changes have already been studied by Terpiłowski et al. [10] and Wiącek et al. [3]; based on X-ray photoelectron spectroscopy (XPS), they proved that low temperature plasma treatment of PEEK surface causes in decrease of C-C and C-H bonds and a significant increase in polar groups, which include oxygen and nitrogen molecules (-OH, C-O, O=C-O, C=O and N-CO-N). Also, as mentioned before, this kind of modification increases the surface roughness. Moreover, the chitosan molecules have proton donor/acceptor groups [11] which may participate in hydrogen bonding and bonding with other activated or new groups which contain oxygen or nitrogen. This information points to the relationship between plasma polymer activation and increased chitosan adhesion. Coating the activated PEEK surface with the chitosan film leads to a reduction of γ_s^{tot} , but this value is still higher than that for untreated PEEK surfaces.

The presence of the DPPC, Chol and CsA monolayers on the unmodified PEEK surface results in increased values of γ_s^{tot} for all substances from 38.3 mJ/m^2 for PEEK EtOH to similar values 46.1 mJ/m^2 ; 43.8 mJ/m^2 and 45.3 mJ/m^2 for DPPC, Chol and CsA, respectively (Fig.2.B). However, the same layers deposited on the activated PEEK surface show opposite changes to the properties, and γ_s^{tot} is reduced after molecule deposition (Fig.3B). These results may suggest that the molecules deposited on the activated carrier strongly interact with its surface and the formed film is dispersed so that the probe liquids have greater access to the polymer surface. This suggestion may be confirmed by the fact that chitosan, which strongly adsorbs to activated PEEK surfaces, shows γ_s^{tot} values of 45.3 mJ/m^2 compared to those of PEEK air, estimated as 57.6 mJ/m^2 . Coating the PEEKair\Ch surface with a DPPC, Chol or CsA monolayer always results in an increase in γ_s^W and a decrease in γ_s^{DM} (Fig.4.B). The highest values of γ_s^W were observed for the PEEKair\Ch\DPPC system. Since chitosan chains are flexible and are able to form tangles and loops, the penetration of DPPC molecules into porous chitosan film is possible. Hydrophobic backbones of chitosan may interact with the hydrocarbon DPPC chains by the Lifshitz-van der Waals forces. This may result in

exposure of the polar phospholipid heads towards the gas phase and hydrophilisation of the surface. The cholesterol layer caused significant changes in the γ_s^{DM} values, which decreased from 45.5 mJ/m² (PEEKair\Ch) to 36.5 mJ/m². As reported by Ohvo-Rekilä et al. [12], the cholesterol –OH group interacts with the chitosan –NH₂ group, creating H-bonds. Also, cholesterol hydrocarbon chains can interact with chitosan film, similar to DPPC, on the basis of dispersion forces. The CsA film deposited onto the PEEKair\Ch substrate showed the highest values of $\gamma_s^{tot} = 49.2$ mJ/m² compared to the rest of the layers deposited onto the PEEKair\Ch surface (Fig.4.B). It is worth mentioning that cyclosporine A is a cyclic peptide consisting of eleven amino acid residues. The structure of CsA (Scheme 1) exhibits many functional groups and few hydrophobic and hydrophilic areas. Its C=O and >NH groups can participate in the formation of hydrogen bonds, and the π electrons of double bonds can also participate in acid-base (polar) interactions, while –CH hydrocarbon fragments, which mainly determine the hydrophobicity of the peptide, interact through Lifshitz-van der Waals forces. Those results may be helpful for better understanding the type of interactions of chitosan films with biological membranes of living cells since DPPC and cholesterol are their main components, as well as the interactions of hybrid films with their surroundings.

4. Conclusions

Air plasma activation of PEEK increases the polarity and adhesion of chitosan to the polymer surface. This is the consequence of two facts: the surface roughness increases and new polar groups are introduced to the polymer surface with nitrogen and oxygen. It is possible that the plasma treatment of PEEK is the best way to increase chitosan biopolymer adhesion. PEEK modifications have a significant influence on the manner of molecular organisation and orientation of deposited monolayers (DPPC, Chol, CsA), which was observed as changes to the values of contact angles and surface free energies estimated by the CAH approach. The results obtained from depositing the biologically active substances on the PEEKair\chitosan biopolymer show interesting wetting properties and changes to free surface energy, which may be relevant in studies of chitosan–cell membrane interactions as well as drug delivery systems based on antibacterial chitosan films.

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