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**Study on the surface and rheological properties of
gel-type biopolymers formed on cold plasma
activated plates**

PhD Dissertation

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LUBLIN 2020

*To my parents,
the ones who always believe in me.*

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks to dr hab. Konrad Terpiłowski, for providing me the opportunity to join his research group and for his guidance and encouragement throughout my research work. I will never forget our trips into the Tatra mountains.

I would also like to extend my sincere thanks to my supervisor dr hab. Marta Tomczyńska-Mleko and to Prof. hab. Stanisław Mleko, for sharing their experience and valuable insights during my research.

A special mention of gratitude to my family, in particular to Fonsi, for always cheering me up, and my other half, Cristina, for her support and undying love.

Last but not least, I am very thankful to my wise friend, Prof. Dr. Fernando González-Caballero, for his unconditional support, motivation and strong encouragement. Thank you for opening the door to knowledge. You changed my life.

COMPENDIUM OF SCIENTIFIC PAPERS OF THE THESIS

1.- S. Pérez-Huertas, K. Terpiłowski, M. Tomczyńska-Mleko, M. Wesółowska-Trojanowska, M. Kawecka-Radomska, M. Nastaj, S. Mleko, Surface Properties of Whey Protein Gels, **Journal of the Chemical Society of Pakistan**, 41 (2019) 956-964.

IF₂₀₁₉= 0.393; IF₅=0,457; MNISW=20

The percentage share of Mr. Pérez-Huertas is estimated to be at 60%

Author's contribution to the paper:

Participation in the elaboration of the content of research that was to be included in the paper. Elaboration of literature review. Preparation of the preliminary and final version of the manuscript

2.- S. Pérez-Huertas, K. Terpiłowski, M. Tomczyńska-Mleko, S. Mleko, Ł. Szajneckid, Time-based changes in surface properties of poly (ethylene terephthalate) activated with air and argon-plasma treatments, **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, 558 (2018) 322-329.

IF₂₀₁₈= 3.131; IF₅=3.091; MNISW=70

The percentage share of Mr. Pérez-Huertas is estimated to be at 85%

Author's contribution to the paper:

Formulation of the research problem. Preparation of some given samples to study their wettability. Measurements of contact angles. Surface free energy calculations by the two approaches described in the text and later interpretation of the obtained results. Calculation of surface roughness parameters and interpretation of the results obtained. Surface composition analysis by XPS technique and the interpretation of the results. Interpretation of the results as well as the preparation of both, the preliminary and the final version of the manuscript.

3.- S. Pérez-Huertas, K. Terpiłowski, M. Tomczyńska-Mleko, K. Nishinari, S. Mleko, Surface and rheological properties of egg white albumin/gelatin dispersions gelled on cold plasma-activated glass, **Food Hydrocolloids**, 96 (2019) 224–230.

IF₂₀₁₉= 5.839; IF₅=6.103; MNISW=140

The percentage share of Mr. Pérez-Huertas is estimated to be at 80%

Author's contribution to the paper:

Formulation of the research problem. Preparation of the samples to carry out all experiments. Contact angle measurements. Surface free energy calculations by the approach described in the text and interpretation of the obtained results. Surface roughness parameters calculation and the interpretation of the results obtained. Dynamic oscillatory measurements and interpretation of the viscoelastic properties. Interpretation of the results as well as the preparation of the preliminary and the final version of the manuscript.

4.- S. Pérez-Huertas, K. Terpiłowski, M. Tomczyńska-Mleko, S. Mleko, Surface Modification of Albumin/Gelatin Films Gelled on Low Temperature Plasma-Treated Poly (Ethylene Terephthalate) Plates, **Plasma Processes and Polymers**, 17 (2019) 1900171.

IF₂₀₁₉=3.173; IF₅=3.075; MNISW=70

The percentage share of Mr. Pérez-Huertas is estimated to be at 85%

Author's contribution to the paper:

Formulation of the research problem. Performance of all measurements. Writing, editing and acting as a corresponding author.

ABSTRACT

Recent progress in plasma science has led to the development of cold plasmas with temperature close to room conditions. Non-thermal plasma is considered as an ideal technology to treat heat-sensitive materials. Nevertheless, substantial limitations are found when working with biopolymers, especially gel-types ones. Direct application of cold plasma on investigated hydrogels is impossible, as it leads to sample destruction. A brand-new methodology, *indirect plasma treatment* is proposed, which allows to pre-treat gel-type biomaterials by the use of activated cold plasma supports. The methodology is analysed and evaluated by means of a set of studies, including contact angle measurements, surface free energy determination, topography studies, confocal microscopy observations, surface chemical composition analysis and rheological studies. This work first focuses on the changes induced by air- and argon-cold plasma treatments on the surface properties of poly (ethylene terephthalate) - PET plates, which are used as activated supports for the gels production. Moreover, the effect of time storage is evaluated as well. Plasma treatment leads to dramatic changes in the surface polarity (wettability) of the polymer, especially in the case of argon-plasma activation. The wettability enhancement is induced by the formation of polar groups on the polymer surface upon reaction with plasma generated reactive species, increasing not only the polar component (and finally the total surface free energy - SFE) of the PET plates, but also their roughness parameters. The appearance of these functional groups is confirmed by the changes in the surface chemistry of PET, given by the survey scan X-ray photoelectron spectrometer (XPS) spectra. PET surface chemical composition changes as a function of time, indicating that there are mechanisms contributing to the loss and further recovery of its hydrophobicity. In the second part of the work, thin layers of egg white albumin – EWA/ gelatin gels have been formed *in situ* on activated supports, previously treated with cold plasma, using oxygen, air and argon gases. After gelation, the gels are detached from treated supports and their pre-treated surfaces studied. Activated supports of different nature are used: PET and glass plates. The effect of increasing concentration of gelatin in the gel is also studied. The gels produced according to the indirect methodology exhibit an enhanced version in terms of mechanical properties. The effect of plasma pre-treatment to the gels is to provide a more hydrophilic character to their surfaces, which is manifested by the SFE increment. The SFE of the gels is shown to be a function of the gelatin concentration in the EWA protein gels and the type of plasma pre-treatment. The most effective pre-treatment is obtained by the oxygen plasma, which induces the higher

hydrophilicity on the samples. The surface roughness can also be tailored by the indirect plasma treatment. Protein-based gels surface becomes rougher with the plasma treatment of the supports, regardless of the gas used, or the support's nature. These studies also demonstrate that the stiffness of the gels can be tuned too, as manifested by the dynamic oscillatory measurements. The hydrogels produced on activated supports exhibit an unusual modification of their bulk properties, as all treated gels enhanced their rheological properties in terms of rigidity. Oxygen- plasma pre-treatment induced outstanding changes, not only in the surface but also in the rheological properties of hydrogels, regardless the nature of the support. These findings entail an original contribution with respect to the standard uses of the cold plasma treatment, which are based on direct alteration of only the surface properties. The results obtained for the gels gelled on both PET and glass plates show a strong similarity in the patterns followed by the SFE values, the roughness parameters and the complex rigidity modulus, corroborating that indirect methodology works accurately in activated supports of different nature (plastic and glass). This Dissertation provides scientific basis regarding the targeted use of indirect plasma treatment to adjust the above-mentioned properties of materials when they are unable to be exposed to the plasma working conditions. Further development in surface and rheological characterization of hydrogels when using this methodology, may be a promising approach for obtaining novel materials with technological applications.

Key words: cold plasma; egg white albumin; gelatine; rheology; surface properties

ABSTRAKT

Ostatnie postępy w nauce o plazmie doprowadziły do rozwoju technik badawczych opartych na zimnej plazmie, której temperatura zbliżona jest do warunków panujących w pomieszczeniu. Plazma niskotemperaturowa jest uważana za idealną technologię do obróbki materiałów wrażliwych na ciepło. Niemniej jednak, znaczne ograniczenia użycia niskotemperaturowej plazmy dotyczą pracy z biopolimerami, zwłaszcza tymi występującymi w postaci żelu. Bezpośrednie stosowanie zimnej plazmy na badanych hydrożelach jest niemożliwe, ponieważ prowadzi do zniszczenia próbki. Zupełnie nowa metodologia, zwana technologią *pośredniego działania plazmy*, pozwala na wstępną obróbkę biomateriałów typu żelowego poprzez wykorzystanie aktywowanych zimną plazmą podłoży do przenoszenia zmian powierzchniowych na żelowe biopolimery. Efektywność techniki oceniana jest poprzez pomiar szeregu właściwości: kąta zwilżania, obliczania swobodnej energii powierzchniowej, badań topograficznych, mikroskopii konfokalnej, analizy składu chemicznego powierzchni i badań reologicznych. Praca ta skupia się przede wszystkim na zmianach wywołanych działaniem plazmy uzyskanej z powietrza i argonu na właściwości powierzchniowe Poli (tereftalanu etylenu) PET oraz otrzymanych na jego powierzchni żeli. Ponadto ocenia się również wpływ czasu na efekt działania plazmy na płytki podkładowe. Obróbka plazmowa prowadzi do dramatycznych zmian polarności powierzchni polimeru, szczególnie gdy plazmę uzyskano z argonu. Wzrost zwilżalności wywołany jest przez tworzenie się grup polarnych na powierzchni polimeru po kontakcie z gazem aktywującym, zwiększa się znacznie składowa polarna swobodnej energii powierzchniowej, a co za tym idzie jej całkowita wartość, wzrastają także wartości parametrów opisujących chropowatość powierzchni. Pojawienie się tych grup funkcyjnych potwierdzą zmiany w chemii powierzchni PET, a zbadane one zostały techniką spektroskopii fotoelektronów w zakresie promieniowania X (XPS). Skład chemiczny powierzchni PET zmienia się w funkcji czasu, co wskazuje, że istnieją mechanizmy przyczyniające się do utraty i odzyskania jego hydrofobowości. W drugiej części pracy cienkie warstwy albuminy białka jaja - żele EWA/żelatyna powstały *in situ* na podłożach uprzednio poddanych działaniu zimnej plazmy. Jako gazy, z których uzyskano plazmę, zastosowano tlen, powietrze oraz argon. Po żelowaniu żele odłącza się od potraktowanych plazmą podłoży i bada ich właściwości powierzchniowe. Jako materiał podłoża stosowane były płytki PET oraz szkło. Badany jest również wpływ wzrostu stężenia żelatyny w żelu. Żele produkowane zgodnie z pośrednią metodologią wykazują ulepszoną właściwość pod względem właściwości

mechanicznych. Efektem wstępnej obróbki plazmowej żeli jest nadanie ich powierzchni bardziej hydrofilowego charakteru, co przejawia się przyrostem SFE (ang. surface free energy). Wykazano, że SFE żeli jest funkcją stężenia żelatyny w żelach białkowych EWA i rodzaju obróbki wstępnej w plazmie. Najskuteczniejszą obróbkę wstępną uzyskuje się stosując plazmę tlenową, która indukuje większą hydrofilowość próbek. Obróbka płytek podkładowych ma także wpływ na chropowatość powierzchni żeli. Powierzchnia ta staje się bardziej szorstka dzięki obróbce plazmowej podłoża, niezależnie od zastosowanego gazu lub rodzaju podłoża. Badania pokazują również, że plazma ma wpływ także na sztywność żeli, co przejawiają się w dynamicznych pomiarach oscylacyjnych. Obróbka wstępna plazmą tlenową wywołała największe zmiany, nie tylko na powierzchni, ale także we właściwościach reologicznych hydrożeli, niezależnie od rodzaju podłoża. Przeprowadzone badania są oryginalnym wkładem w standardowe zastosowania obróbki zimną plazmą, które są oparte na bezpośredniej zmianie tylko właściwości powierzchni. Wyniki uzyskane dla żeli żelowanych zarówno na płytkach PET, jak i szklanych wykazują silne podobieństwo wartości SFE, parametrów szorstkości i złożonego modułu sztywności potwierdzając, że metodyka pośrednia działa w przypadku aktywowanych podłoży, takich jak tworzywa sztuczne i szkło. Niniejsza rozprawa stanowi podstawę naukową dotyczącą pośredniego działania plazmą w celu dostosowania zmiany ich właściwości, gdy nie mogą być one narażone na warunki pracy plazmy. Dalszy rozwój charakterystyki powierzchniowej i reologicznej hydrożeli przy zastosowaniu tej metodologii może być obiecującym podejściem do pozyskiwania nowych materiałów o zastosowaniach technologicznych.

Słowa kluczowe: zimna plazma; albumina białka jaja; żelatyna; reologia; właściwości powierzchniowe

I. BACKGROUND

In this section, an overview of the most important features of plasma technology is given, with special focus on the non-thermal/cold plasma treatments. A comprehensive literature review is conducted, covering several aspects of the usefulness of this technology applied to polymers, mainly concerning PET. This section also deals with the basic concept of hydrogels, their general properties, and applications. A brief introduction about the fundamentals of surface thermodynamics is also given.

Plastic is one of the world's most-used materials, roughly 150 million tons (about 20 kg per person) of synthetic polymers are produced worldwide annually. In recent years, a remarkable growth in the use of polymer and biopolymers films for engineering purposes has been observed. These films can be found in extensive applications in packaging [1-11], automotive and air craft industry [12], electrical and electronic equipment [13] or in medical fields ranging from biocompatible implants and coatings to tissue engineering scaffolds, drug delivery system and a wide variety of hospital equipment [14,15]. Nevertheless, many of these applications require the films to have specific properties, including high strength, hardness, resilience, resistance to corrosion, lack of conductivity (thermal and electrical), transparency or proper adhesion; in other words, they need to be functional. To satisfy industrial needs, there is an emerging technique, *cold-plasma treatment*, which is able to provide the material with engineered properties.

Plasma is commonly defined as “a fourth state of matter, distinct from solid or liquid or gas and is present in stars and fusion reactors [16]. It was first described by the American chemist Irving Langmuir in the 1920s [17,18]. Some estimates suggest that up to 99% of the entire visible universe is in the plasma state [19], but here on Earth, with a few spectacular exceptions, such as lightning and the high auroras (during a solar eclipse, plasma can be observed as a bright circle of light around the sun), plasmas can seldomly be found naturally. Recently the use of plasma has become very frequent in everyday life, as in the case of plasma TV sets that can easily be found in everyone's house. Therefore, it is inevitable that many researches and development teams take this chance to broaden the application abilities of something so unique and intriguing as the plasma is [20]. The idea to use plasma to modify materials has been at the forefront, specifically, the so-called ‘cold plasma’. When plasma comes into contact with the material, an extra energy is transferred from the plasma to allow

for subsequent reactions to take place on the material surface. This ‘extra energy’ acts on the surface of the substrate and can modify a wide range of its characteristics, such as: surface energy, roughness, hydrophilicity, surface charge, biocompatibility or reactivity; additionally, this treatment is able to modify the nature of polymer surfaces, and the possibility of provoking some changes of their bulk properties becomes an open question. The use of plasma to improve the wettability of different materials has become increasingly popular. Printing, painting, or adhesive bonding are some of the technologies which could reap the benefit of these treatments. The success of this technique is based on its ability to change the surface properties of a material by physical or chemical modification of its most external layers ($\leq 1 \mu\text{m}$), without, in principle, modifying its bulk characteristics. In this way, reengineering of surface chemistry can completely open new fields of application to conventional polymers. It should be also underlined that this technology has substantial limitations. The effect of plasma exposure can eventually erode away the materials in the exhaust chamber, hence it may damage or destroy the treated materials. In particular, the material must be capable of enduring the operating conditions reached in the chamber. This limitation concerns the gel-type biopolymer materials, which are noticeably damaged during the activation. Therefore, there is still an outstanding need to overcome this problematic issue. The development of new biomaterials to substitute petroleum-based plastics has become an important challenge nowadays. Hence, protein-based biomaterials are a new generation of bioplastics and many researchers are working on their further development. However, in general, wide industrial uses have not been found yet for these materials, since the development is still at the experimental stage. Meeting some improved properties, these materials still show inferior performances in terms of functional and structural properties [21]. Adjusting the biomaterial surface and bulk properties, allows obtaining ‘smart’ biomaterials with new features and enhancements, opening a whole new world of industrial applications. Some of them can have a crucial role as drug delivery systems, tissue scaffolds or active packaging.

This dissertation is mainly focused on featuring and developing a novel methodology, where the foremost goal is to obtain novel gel-type biopolymers. As it is impossible to improve the properties of a such material by direct plasma, the indirect method has been mainly focussed. Gel biopolymers were produced on surfaces activated previously by cold plasma. Polyethylene terephthalate (PET) and glass have been used as activated support materials, so the study on the influence of cold plasma on the polyethylene terephthalate (PET) was also performed. In the next sections, all these issues have been justified and developed.

I.1 Plasma Physics and Chemistry

Plasma, a state of matter naturally occurring in our universe, is also used in everyday applications such as chip etching and torches, serving not only as propulsion but also as sustained energy production via controlled fusion [22].

Plasmas were first identified in a discharge tube (or Crookes tube) and described by Sir William Crookes in 1879 (he described plasmas as “radiant matter”) [23]. The nature of the Crookes tube “cathode ray” matter was subsequently identified by the British physicist Sir J.J. Thomson in 1897 [24] and dubbed “plasma” by Irving Langmuir in 1928 [25], perhaps because it reminded him of a blood plasma [26]. To top it all, Langmuir wrote: “*Except near the electrodes, where there are sheaths containing very few electrons, the ionized gas contains ions and electrons in about equal numbers so that the resultant space charge is very small. We shall use the name plasma to describe this region containing balanced charges of ions and electrons*” [25].

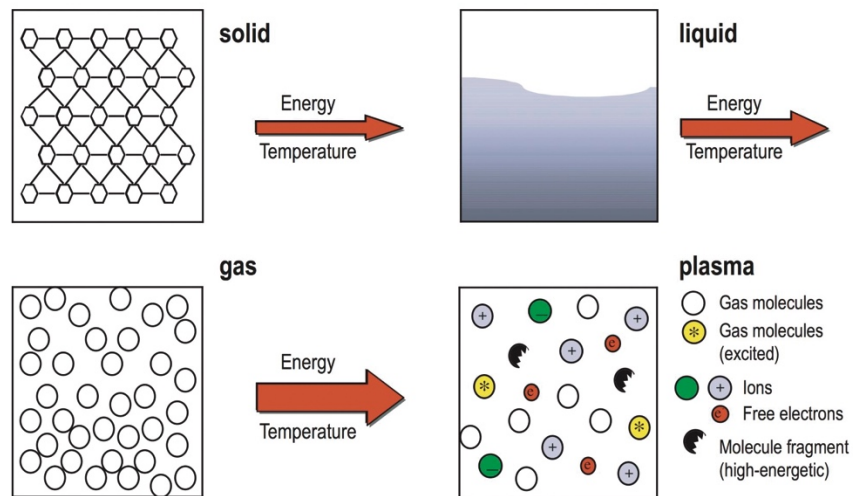


Figure 1¹. Illustration of the state change occurring as a result of temperature increase of the material

In Fig.1, it can clearly be seen how an array of particles can be arranged in different configurations, depending on the temperature conditions. The effect of heating makes the material going from solid to gaseous state. When sufficient energy is supplied to a gas, it is ionized and goes into the energy-rich plasma state, the fourth state of matter. So, in essence,

¹ <https://www.plasmatreat.com/copyrights.html>

plasma can be considered as an “exotic state” consisting of a mixture of particles with different charges and masses. In contrast to the three ordinary states, the transition to plasma is not a transition phase per se, thermodynamically speaking, but the ionization increases ‘gradually’ when more energy is coupled to the system. In turn, sufficient ionization affects the electrical properties of the system to the point of becoming plasma. In addition, the properties found in plasma are vastly different from those seen in regular gas. For instance, a plasma is electrically conductive, its motion can be influenced by electric and magnetic fields. Therefore, it is called ‘ionized gas plasma’ if it meets the requirements of being quasi-neutral and its properties are always dominated by electric and/or magnetic forces. This state of matter is associated with a number of characteristic properties, which are either connected to the high particle temperatures or are caused by the existence of free charge carriers, causing, consequently, a high electrical conductivity and giving rise to collective effects as “wave phenomena”.

A plasma consists of positively charged ions, negative electrons, neutral molecules, UV light and excited molecules, which can possess a huge amount of internal energy [27]. In the plasma treatment process, any or all of these reactive species before mentioned, may interact with a surface. By selecting the treatment parameters such as pressure, power, process time, gas flow, composition and other factors, the effects of plasma may be adjusted as desired. Plasma can be artificially generated in any neutral gas by providing enough energy for its ionization. In typical laboratory or technical applications, however, the ionization is mostly achieved by strong (direct or alternating current) electrical fields. Plasma phenomena research can lead to a source of unlimited, non-polluting energy.

1.2 Low temperature plasma

For the purpose of this Thesis, it is crucial to distinguish between thermal and non-thermal plasmas from the very beginning. This classification is based on the relative temperatures of electrons, ions and neutrals. In thermal plasma, all species are characterised by thermodynamic equilibrium, $T_{\text{gas}} \approx T_{\text{ions,neutrals}} \approx T_{\text{electrons}}$ [28,29]. The temperature of the thermal plasma may reach the values of several thousands of Kelvins ($10^4 < T \text{ (K)} < 10^8$) [30]. By contrast, in the case of non-thermal plasmas, the highly energetic electrons and the heavy particles (ions and neutrals) never achieve local thermodynamic equilibrium, while the electrons are at higher temperature (more than 10,000K) the heavy particles are at much lower temperature (nearby room temperature), $T_{\text{electrons}} \gg \gg T_{\text{gas}} \approx T_{\text{ions,neutrals}}$. However, the density of

the electrons in the plasma is very low compared to the density of the uncharged atoms, giving as a result, the before-mentioned 'cold' gas. The term 'cold' makes reference to plasma with temperatures oscillating between 40 to 70 °C [31]. This is certainly important when plasma is used to treat heat-sensitive biomaterials. Consequently, there are two main aspects in the low temperature/cold plasma treatments. First, cold-plasma is a source of high-temperature electrons at ambient conditions (room temperature and pressure), which means that costly devices are avoided, making cold plasma technology affordable. Secondly, when interacting with an open or controlled environment, many reactive species are produced. These plasmas have a high density of activated species, the energetic electrons collide with the background atoms and molecules causing thus enhanced level of dissociation, excitation and ionization. Then, a series of reactions takes place, hence, producing new chemical bonds, several reactive free radicals, excited state atoms and chemically active compounds, creating, as a result, its reactivity. Those reactive species can be used for many chemical reactions in different fields of science. The efficiency of plasmas is based on the ability of these excited species to induce chemical and physical modifications on the surface [32]. The surface modification of solid substrates by plasma exposure is mostly used for etching, cross-linking, surface activation (radical formation) and as pre-deposition process. Cold atmospheric plasma represents a great opportunity when referring to materials activation, as, *a priori*, the plasma minimizes the risk of damaging the heat-sensitive materials during the treatment. In the laboratory, cold plasmas are generally produced by the provision of electrical energy to the gas in a vacuum.

Many applications are being benefited from this treatment, a) food decontamination [33–43]; b) biomedicine [44–49]; c) medical treatments [50-52]; d) modification of the polymeric materials properties [53]; e) nanotechnologies [54] or surface engineering [55] as a case in point.

Some of the most significant benefits of cold plasma treatment for surface modification are:

- To modify surfaces by attachment or adsorption of functional groups to tailor surface properties for specific applications [56].
- To restructure polymer surfaces through crosslinking [57].
- To deposit polymer layers by plasma polymerization [58].
- To graft functional polymers or end groups onto plasma-activated surfaces [59].

- To prepare surfaces for subsequent processing, e.g.: film deposition or adsorption of molecules [60].
- To improve surface coverage and the spreading of coatings as well as enhancing adhesion between two surfaces [61].
- To modify wettability to render a surface hydrophilic or hydrophobic with the appropriate processed gas(es) [62].
- To change surface properties without affecting the bulk material [63].
- To remove organic contaminants by chemical reaction (O₂ or air plasma) or physical ablation (argon plasma) [64].

The generation of plasma at environmental temperature is interesting both technically and commercially: it can be implemented at ambient conditions, but it also reduces cost, increases treatment speed and enables industrial applicability. For those reasons, researchers are continuously looking for new applications that cold plasma science is offering.

I.3 Poly(ethylene terephthalate) (PET)

PET was first synthesized by the esterification of either terephthalic acid or dimethyl terephthalate with ethylene glycol [65,66], in North America in the mid-1940s by Du Pont chemists searching for new synthetic fibres. In the late 1950s, researchers found a way to stretch a thin extruded sheet of PET in two directions to create PET film, which is now used extensively for video, photo and packaging films. In the early 1970s, the technology was developed for blow-stretch moulding PET into bottles. The PET bottle was patented in 1973. Due to PET's unique properties, it is rapidly becoming the world's preferred packaging material for foods and beverages.

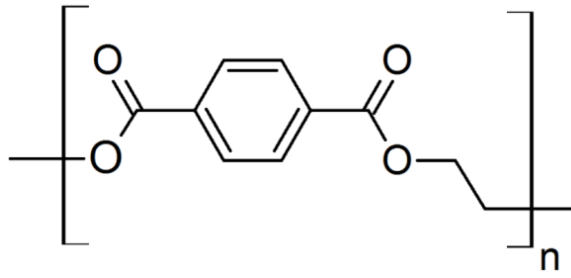


Figure 2. Polyethylene terephthalate (PET) structure scheme

Poly (ethylene terephthalate) (PET) is a general-purpose thermoplastic polymer which belongs to the polyester polymer family. PET is the most used and recommended for engineering purposes [67]. The presence of the aromatic ring in its structure (Fig. 2) gives this material excellent mechanical properties, including good strength, rigidity, high strength-to-weight ratio, transparency, thermal stability, gas barrier property, chemical resistance and formability. Moreover, PET is the most widely recycled plastic which has recently been considered of significant importance from the industrial point of view. For all the above-mentioned reasons, PET is a proper candidate to be further studied in this Thesis.

Although PET films have some excellent bulk properties, their uses are sometimes limited by undesired surface properties, such as low surface free energy, ergo leading to poor wettability and adhesiveness [68]. This is closely connected with the absence or lack of polar surface functional groups resulting in weak mechanical interaction between two contacting surfaces [69]. To address it, the surface energy content needs to be increased prior to its application. As PET is considered to be a heat-sensitive material, a delicate balance must be reached during its surface modification. Thus, the cold plasma treatment seems to be a good route to process it, since it prevents, a priori, any thermal damage of the material.

Considering that PET is one of the most common consumer plastics used [70], many attempts to improve its wettability by cold plasma have been reported in the literature. The processing of PET by cold plasma has been commercially exploited in many different fields, some of the most important applications are:

Food packaging. PET is frequently used as a raw material to manufacture food packaging products, such as bottles and containers, and it is intended to preserve foodstuff

quality along the distribution and storage chain as well as to protect it from deterioration, damage or external pollution. If packaging material is not properly sterilised, it may cause further contamination of the packaged food and consequently it leads to health risks and economic losses [71]. One strategy to solve this issue, is the use of cold plasma treatment of the supports. For instance, Deilmann et al., [72] used oxygen cold plasma treatment for the sterilization of PET bottles, and reported that *Bacillus Atrophaeus* and *Aspergillus niger* spores were significantly reduced after the plasma treatment. Miao and Yun [73] also reported that the air plasma treatment was very effective in *Escherichia coli* sterilization of PET bottles. Furthermore, Koulik et al., [74] applied air cold plasma treatment inside PET bottles and observed over 3 log₁₀ in reduction of microbes and a deodorization effect in a few milliseconds. Muranyi et al., [75] stated that packaging materials such as plastic bottles, lids and films can be rapidly sterilized using this technology, without adversely affecting their bulk properties or leaving any residues. These authors confirmed the workability of plasma for sterilizing PET and multi-layer packages like PET/polyvinylidene chloride (PVDC)/polyethylene (PE). In another research, glass, paper and PET foil have been treated by cold plasma and resulted in the inactivation of *E. coli* O157:H7, *S. Typhimurium*, *S. aureus* [76]. Schneider et al., [77] investigated the practicability of low-temperature plasmas to treat PET foil substrates at industrial level to attract commercial interest, the results obtained suggested the scalability of the approach. Moreover, the cold plasma has also been used to immobilize antimicrobial compounds like lysozyme, nisin, vanillin, or antimicrobial peptides into the packaging materials [71]. The issue of high temperature used to be a main concern in these processes, especially in those involving PET, which is temperature-sensitive material. Such a disadvantage has been overcome by using non-thermal plasma technology. Although the nutritional, sensory and other quality attributes could be affected depending on the residues left after the before-mentioned treatment [78], it is not expected to be a handicap in terms of the nutritional quality of foods [79].

Cleaning. Plasma should not only be universally known as an effective, economical, environmentally safe method for critical cleaning, for instance, dirt particles could be removed by chemical reaction with the ionised gases. But it should also be regarded as remarkable effective material for breaking most organic bonds (i.e., C-H, C-C, C=C, C-O, and C-N) from surface contaminants, which could definitely help to break apart high molecular weight contaminants [80]. The result is an ultra-clean surface. Muranyi and co-researchers reported in their papers [81,82] about the fundamental study of cold plasma treatments for cleaning PET

foils as well as multi-layer packaging based on PET/ PVDC/PE-LD. The group has identified an increase in relative gas humidity as a key factor to achieve a minimum of $2\log_{10}$ inactivation in *Aspergillus niger* and *Bacillus subtilis* for 1 s treatments. A comparative study between plasma and wet chemical cleaning of PET fibres was done by Keller et al., [83]. In this work, different cleaning methods were compared in order to remove contaminants on the fibres. The authors stated that plasma cleaning showed the best performance in terms of efficiency, flexibility, ecology and industrial applicability. Gospodinova et al., [84] used cold plasma treatment for cleaning PET fibres. They studied the evolution of hydrophobic changes at different working plasma treatment regimes. The main results showed a good correlation between the cold plasma treatment and its mechanical properties. Finally, they stated that the PET surface was modified and cleaned by a combined plasma-chemical process. Cleaning by using plasma offers much more intense methods of sterilisation, permitting to operate at a lower temperature. The ability to maintain a lower temperature through sterilization gives companies the opportunity to easily sterilize packaging material sensitive to high heat [85].

Printing. Many polymers do not bond readily to other materials as could be the case of paints and glues, the low surface energy and the chemical inertness of PET lead to poor adhesion of printings and coatings in practice. Surface activation and functionalisation by low temperature plasma enables better adhesion on polymers and fibres, facilitating the printing processes such as labelling PET bottles or containers and sealing packages [86]. For instance, Pankaj et al., [87] reported that the surface functionalization by cold plasma treatment imparted surface energies to polymers which promoted ink adhesion. In similar way, the improvement in printability of air plasma treated PET films was reported by Deshmukh and Bhat [88]. In both cases the authors explained that the increment of the functional groups of the PET surface, achieved an improved ink adhesion. On the other hand, Junkar *et al.*, [89] and Strauss and Pulker [90] agreed that to improve the adhesion and the wettability of PET, the most efficient process is usually the low temperature oxygen plasma application.

Biomedical applications. PET has long been commercially employed in the biomedical field for sutures, vascular grafts or cell adhesion, the plasma treatment leads to an enhancement of the wettability which facilitates cell adhesion. Poletti et al., [91] used the atomic force microscopy to investigate the morphological changes in the surface of PET fibres treated by cold plasma. They reported that the changes in morphology in the cases of air, He and Ar gases, seems to be due mainly to etching effects. PET surface was modified and/or functionalized to

achieve improved biomolecule adhesion. The research group of Hubbell has also reported on the functionalization of several polymers including PET in order to improve cell attachment [92,93]. Previous studies showed the potential benefits of the incorporation/grafting of nanoparticles on the activated PET polymer. For instance, Kasálková et al., [94] studied the cell adhesion and proliferation on the modified polymers-grafted to make them more suitable for cell cultivation. The plasma treatment leads to an enhancement of the wettability, which facilitates the cell adhesion. These findings are crucial for many medical and biological applications like tissue engineering or delivery vehicles among others. Švorčík et al., [95] proposed an interesting way of making use of polymeric materials through tissue engineering applications, where the activation of PET and grafting with Au nanoparticles resulted in an alteration of the surface hydrophilicity, roughness and morphology, producing a significant improvement on the materials intended for cell adhesion and proliferation.

It is interesting to point out that even in short terms of treatments, the surface properties of PET are affected by the plasma application. This is the case of the work by Junkar et al., [89] who studied the surface modification of PET films through the changes in wettability and surface chemistry, using different times of treatment with N₂ /and O₂ cold plasmas. The wettability enhancement was set to be crucial for the deposition of biologically active compounds.

Let us emphasize that, although much work has already been done in the use of cold plasma to characterize PET polymer, there are still some issues that have been relatively poorly studied. In the next chapter, some of these research gaps will be identified with the aim of justifying the research done in this Thesis.

I.4 Biopolymers-hydrogels

If civilizations are remembered for what they leave behind, our time might be labelled as the Plastic Age. Plastic is everywhere and it is famous for being long-lasting. Thus, for environmental reasons, many efforts are being made by academics and researchers in an attempt of replacing plastic with others more eco-friendly materials. Biopolymers are, for example, the optimal solution for organic waste bags, compostable fruit and vegetable bags, compostable food packaging or agricultural mulch films. Thus, biopolymers can make an important contribution to *Circular Economy*, which it most aims put emphasis on using products in the

best way possible across the entire value chain, on keeping them in usage for as long as possible and to recover them at the end of their service life. However, for some specific applications, many biopolymers are still at an experimental phase, being their industrial uses often restricted because of concerns regarding relatively poor mechanical properties, such as in the medical field, where much work is still required for developing biocompatible coatings [96]. These limitations can be overcome by developing efficient methods for producing biopolymers with a good mechanical performance.

Biopolymers are a kind of innovative polymers produced by natural sources, either chemically synthesized from a biological material or entirely biosynthesized by living organisms. Due to their abundance, biocompatibility, price and unique properties, they are very promising materials which have received recent attention in research, considering that they provide a great opportunity for developing green chemistry in industries. Biopolymers-based hydrogels represent one of the most fascinating developments in material science. They are defined as three-dimensionally cross-linked networks of polymers chains capable of holding large amounts of water [97]. Softness, smartness, and the capacity to store water, make them unique materials [98]. Hydrogel structure and thus hydrogel properties are very closely related to the conditions under which the hydrogels are formed, i.e., the cross-linker concentration, the monomers' initial degree of dilution and the chemistry of the units building the network structure. A convenient approach to produce novel materials for specific applications, with the desired functional properties, is met by the manipulation of different biopolymers [99]. Among the proteins, gelatin-based films have shown strong potential for commercial applications. In some areas, especially the food industry, gelatin tends to replace the conventional polymers due to its biodegradable properties. The estimated world usage of gelatin is 200,000 metric tons per year [100]. Gelatin is a water-soluble protein obtained by hydrolysis from renewable sources like collagen connective tissue of muscles, skin and bones of animals. Some of its most attractive properties are biodegradability, excellent biocompatibility, non-antigenicity, plasticity or adhesiveness [101]. Gelatin is one of the most popular gelling agents widely used in biomedical or pharmaceutical industries and home kitchen. For instance, gelatin is used as the main ingredient of the hard capsules shells used in the pharmaceutical industry [102]. Additionally, gelatin scaffolds have been developed as an injectable material for tissue engineering applications due to its biocompatibility [103]. Nevertheless, improving mechanical properties of the biomaterial for these applications is crucial. For instance, the hydrogel scaffold mode of action depends on their physical properties (e.g. mechanics, degradation, gel formation), mass transport properties (diffusion) and biological properties (cell adhesion and

signalling) which can all be carefully engineered [104]. To fulfill the commercial requirements, many experts and engineers have carried out researches to improve the functional properties of gelatin products [105]. Chemically induced and enzymatic-induced cross-linking, as well as mixing with other biopolymers, have been used in developing the functional properties of gelatin gels [106]. One established way to change functional properties of gelatin is to incorporate additional double bonds in the collagen chains using glutaraldehyde [107] or formaldehyde [108]. Other methods include the use of enzymes [109], or a mixture of gelatin and carrageenan, [110] and the use of additional salts [111,112]. Coughlan et al., [113] reported that combinations of proteins and hydrocolloids resulted in more effective functional properties than when working individually. Protein-hydrocolloid interactions may thus enhance the stabilization of interfacial layer thickness, leading to the stability of foam against bubble coalescence [114]. A series of studies [105,115,116] have shown that gelatin films combined and blended with other proteins would improve the physical and mechanical properties of the mixed gel produced. In light of the above, the protein-based hydrogels will be built by manipulating the proportions of gelatin with egg white albumin protein - EWA, which are representative natural polymers that help to produce natural hydrogels.

Chicken egg is one of the perfectly preserved biological items that is easy to be found in nature and it is also considered as the best source of protein, lipids, vitamins, and minerals. Moreover, EWA is one of the most popular gelling protein. The efficiency of the functional properties of EWA protein may be adversely or favorably affected by the addition of other hydrocolloids. Building mixed gels of egg white albumen with other hydrocolloids can create practically unlimited possibilities for gels with different textures, viscoelasticity and surface properties [117]. Badii and Howell [118] found out that a mixture of gelatin and EWA (3:10) gave greater storage modulus value than the ones expected from just adding the values from the singular gels. Moreover, Ersch et al., [119] also reported that mixing gelatin with egg protein resulted in stronger gels in terms of rigidity. EWA is a system built out of numerous globular proteins, it has a protein content of about 11 % and comprises about 58 % of the entire egg mass [120]. It also has multiple functional properties and, among them, foaming, emulsification, gelation, microencapsulation and adhesion are the most significant [121-123]. The understanding of the formation mechanism of hydrogels under various experimental conditions is of great interest to predict their physical properties. Tomczyńska-Mleko and Mleko [124,125] have studied the aptitude of EWA protein gels as matrices for active ingredients release. Taheri et al., [126] used nanoparticles of EWA protein as drug carrier systems. Rosenzweig et al.,

[127] used EWA to develop and characterize floating, stomach-retentive matrix tablets, which would deliver polyphenols in a controlled release manner.

As already stated, the combinations of proteins and hydrocolloids can allow the design of new biomaterials with enhanced mechanical properties, which are fundamental from the industrial viewpoint. However, extensive research is still needed on new methods for gelatin-based films formation to improve the final properties and their potential applications. Development of biopolymers by means of some physical or chemical routes is a necessary tool to overcome frequent limitations concerning surface properties. In particular, low surface energy would affect surface wettability and other related properties, and it could be a handicap, as it would lack specific requirements in applications, such as drug delivery systems, intelligent packaging or tissue scaffold preparation. Among the current methods for surface modification, cold-plasma treatment is an emerging technique offering many potential benefits in treatments of biomaterials. It enables us not only to improve adhesion [128] and to modify wetting properties but also to enhance functionality and biocompatibility of biomaterial surfaces [129].

The surface state of a medical device is crucial for its biocompatibility, and bacterial adhesion onto it. For instance, treating cell scaffolds with plasma increases hydrophilicity and improves cell adhesion [130]. The roughness can also be controlled using plasma technology; surface roughness of the gels can influence their application as matrices for active ingredients [131]. A rougher surface implies that scaffold disappears faster by hydrolyzing enzymes acting, and faster is, thus, the release of drugs and active ingredients in active packaging systems [132]. These modifications allow us to design well-suited materials for reaching the industrial target.

1.5 Surface Thermodynamics: Surface Free Energy (SFE)

Having a deep knowledge on polymers interfaces in a wide range of technologies and application must be considered of utmost importance. Surface free energy (SFE) is one of the quantities describing the properties of solids, being a powerful tool to predict the behaviour of the polymers at the interfaces. It is a physical phenomenon which is originated by the intermolecular interactions. Three interphases are involved in the solid-liquid interactions: liquid/vapour, solid/liquid and the solid/vapour interfaces. The intermolecular interactions include van der Waals, London dispersive, Debye inductive and Keesom orientation forces; other interactions are hydrogen bonding, Lewis acid–base, and energetically homogeneous and

heterogeneous interactions [133]. The sum of all of these interactions determines the SFE. The spherical shape of the liquid drop is changed to spread across the surface of a solid and, consequently, makes the surface wet. The work needed per unit area (J/m^2), in order to change the drop shape, is called surface free energy. This term describes the excess energy that the surface has compared to the bulk of the material; the main use of SFE is to determine the work of the adhesion between the liquid and the solid. For this purpose, there are different models, which differ mainly in the interpretation and calculation of these interfacial interactions. In the next chapter, the approaches chosen for the calculation of SFE, will be further developed.

In view of the above, a good understanding of the surface properties of a material can be obtained from the analysis of the SFE; for instance, Białopiotrowicz and Bronisław [134] studied the wettability of the gelatin films; they conclude that the values of the SFE components and parameters calculated from the contact angles of glycerol, ethylene glycol, and diiodomethane allowed them to predict the wetting behaviour for other studied organic liquids. Precise estimation of the SFE value is of significant importance in many industrial areas. Busscher et al., [135] calculated the SFE of bacterial cell surfaces and studied its relevance for adhesion; they concluded that bacterial adhesion was energetically unfavourable. Katsikogianni et al., [136] observed an increase in the free energy of PET surfaces treated with He and He/O₂ plasmas, which significantly reduced the adhesion of a specific strain of *S. epidermidis*. Notably, the wetting of a surface is not only determined by the magnitude of its surface energy, but also by the free energy of adhesion between the solid and liquid as well as the surface tension of the liquid [137].

Interactions between solid and liquid are important in many processes that determine the adhesion between the two phases. When the SFE of the solid is high, it is usually easily wetted by any liquid, such as paints. Metal and glass are examples of high SFE materials where wetting is not problematic. In contrast, some commonly used polymers are hydrophobic, having very low SFE, causing poor wetting; for instance, the SFE of common polymers is around 30-40 mJ/m^2 at 25°C [138] (see appendix 5); it is known that when a polymer has a SFE lower figure than approximately 60 mJ/m^2 , a good adhesion is not possible and consequently, there is a problem with the spreading of liquids upon the surface [139]. This is mainly due to the fact that the surface is not capable of forming strong bonds, so there is little energetic reward for the liquid to break the bulk bonding, in order to favour surface interaction. This is undesirable for many commercial applications and certain pre-treatments are applied to improve their wettability. Scientists tried to improve the wettability of mainly hydrophobic polymers, by

using cold plasma technology [140-142]. For instance, Jurak et al., [143] used air plasma to improve PET surface properties for biomedical applications; the authors reported that the surface activation significantly enhances its polarity, evidenced by the increase of the SFE value. It improves the PET biocompatibility, making it more suitable for cardiovascular devices. In the work by Yang et al., [144] the low-temperature air plasma was applied to improve the intrinsic low SFE of a PET film for technical applications. The authors also reported that the polar groups appearing after the activation, make the surface more hydrophilic as compared to the untreated one.

II. JUSTIFICATION

Taking the literature review of the previous section as a starting point, in this section the research done will be put in context and its main aims emphasized. This thesis is focused mainly on two issues: i) surface characterization of PET by cold plasma treatment; and ii) surface and bulk characterization of EWA/gelatin gels formed onto PET and glass treated plates, using the so-called ‘‘indirect plasma treatment’’. In the first case, the influence of cold plasma treatment of PET plates under several conditions is studied. This is an important step in the research because the knowledge obtained will be later on used in the second part of the research work, in which pre-treated PET plates are used as ‘activated support’. In the second part of this work, a novel methodology for gel-type biopolymers activation is developed; then, the scope and possibilities of this methodology are evaluated, which allows not only tuning the surface of the bio-layers, but also their rheological characteristics. In the next section the motivation of these studies is briefly discussed.

II.1 Research focus

First part of the research: Surface characterization of PET by cold plasma treatment

It is generally agreed that the changes of the surface properties of PET are produced by the incorporation of polar groups after the plasma treatment. For instance, Levine et al., [145] reported that the most important aspect in the modification of polymer surfaces was the chemical changes which resulted in an increase in polarity and SFE. In a research made by Pelagade et al., [146], the authors studied the modification of PET using Ar-cold plasma. The wettability of the surface was studied in terms of the changes in the SFE, using Van Oss approach; as a result, they reported that the SFE of the PET increased with Ar plasma treatment due to an increase in polar component. However, in this study, the authors did not compare the results of SFE using others approaches. In a work carried out by El-Saftawy et al., [147], the wetting behavior of the polymer (PET) was studied by employing the contact angle method and the SFE analysis. In this case, three different approaches (Neumann, Owens and Wendt (OW)

and Van Oss, Chaudry and Good (vOCCG)) were used to calculate the SFE of the film. The authors reported that the differences observed among the used approaches were mainly due to the intermolecular interactions in the liquid/polymer surface system. In that paper, however, the treatment used for the polymer surface was not the cold plasma, but electron-beam induced plasma. It is interesting to note that when comparing results obtained using different approaches, the differences in the SFE values were not caused by the experimental errors, since the same values of contact angles were used for all calculations. The differences are attributed to the several assumptions made in the approaches used for SFE calculation, and to the differences in the intermolecular interactions between the probe liquids and the examined material. Therefore, in the above-mentioned works, although the authors have studied the effect of plasma treatment of PET polymer using different approaches to determine the SFE, however, the discussion concerning the importance and limitations of the different methods used is still due [148]. In this study, the models have been selected based on specialized literature reports [149], and the real potential of this field of research will be used; in order to do so, a careful study of the wettability of PET plates has been performed, using contact angles measurements with several probe liquids, and theoretical approaches for the SFE determination. The most frequently used methods are those proposed by Fowkes [150, 151], Van Oss, Good, Chaudhury [152-154], Owens and Wendt [155], as well as those based on the “state equation” given by Neumann [156-159].

In the present work, the surface modifications of PET after induced cold plasma activation will be evaluated by comparing the SFE values obtained using the “Hysteresis method” [160-162] vs. Van Oss model [152-154]. Although different models include different interactions for calculation of the SFE, it is hypothesized that these models could be directly comparable. The contact angle hysteresis approach (CAH) is one of the latest methods for calculating the ‘apparent’ SFE of polymeric materials, elaborated in the Department of Interfacial Phenomena by Prof. E. Chibowski [160-162]. It is based on the determination of the contact angle hysteresis, which is the difference between advancing and receding contact angles. This method was successfully applied for the calculation of SFE independent of its surface character, that could be hydrophilic [163], hydrophobic [164] and even superhydrophobic [165].

The Lifshitz–Van der Waals/Acid–Base approach (LWAB) [152-154] is based on the partition of the SFE quantity into independent components, which enables to learn better the examined phenomena, especially the interfacial acid-base interactions. Multicomponent

theories play an important role for the prediction of solid-liquids adhesion work and SFE of solid surfaces. This method is undoubtedly one of the recent achievements in the studies on the SFE of polymeric materials and it will be briefly described, as well as the CAH model, in section III.4. Thus, the wettability study based on the SFE values obtained by various methods and with the use of different measuring liquids, will be one of the very consistent and precise issues considered in this work.

Additionally, it is known that surface modifications induced on the polymers after plasma activation are not sustainable over time and may change during sample storage. The loss of beneficial attributes derived from plasma processing of polymers over time is often called “ageing”. The surfaces modified by the plasma suffer changes during storage, which basically consist of a decrease in their hydrophilicity, manifested by a decrease in the value of surface free energy and an increase in the water contact angle [166]. This undesirable phenomenon is called ‘hydrophobic recovery’. The evaluation of ageing time represents a fundamental step to plan the insertion of the plasma process inside the industrial system. The mechanism of ageing as well as approaches to delay the hydrophobic recovery, is a subject of active research and an issue still open for discussion.

Concerning cold-plasma treatments, the mechanisms of surface modification differ, depending on the type of gas used [167,168]. The main difference between reactive and noble gas plasmas is the lack of chemically active species in the latter. Noble gases are inert and do not react with the treated samples or alter their surface chemistry, so they do not incorporate new chemical species on the surface during exposure in the chamber. However, transfer reactivity, giving rise to bond breakage and the subsequent origin of free radicals, which may undergo many reactions with the atmospheric molecules, are the responsible for the surface changes. Thus, the main mechanisms are selective bond breakage (scissoring) and possible desorption of short chain species (cross-linking) [169]. However, treatment with other than noble gases, like O₂ or air, results in the incorporation of the active species contained in plasma onto the sample surface under treatment (functionalization) [170]. They may remove organic contaminants by chemical reaction with highly reactive oxygen radicals and through ablation by energetic oxygen ions, promote surface oxidation and hydroxylation (OH groups) and there is also an increase of the surface wettability [171]. With this in mind, the treatment processes have been designed using both, an inert (Ar) and a reactive (air) gas, and then the results evaluated. The alterations in the surface chemistry of the cold-plasma treated PET plates will be analysed using the XPS technique.

The first part of this study is aimed to cover the following scientific problems:

1. The study of the effect of cold plasma treatment (air and Ar) on the wetting properties of PET plates. For this purpose, experimental measurements of contact angles, optical surface-profilometry and XPS analysis of the surface chemical composition of the PET plates, have been conducted.
2. The study of the time storage effects on the surface properties of the plasma-treated PET plates (hydrophobic recovery).
3. The discussion of the wettability enhancement of treated PET plates, in terms of SFE changes obtained by different models.
4. To draw a proper comparison about the efficiency of the different plasma treatments applied, the reactive (air) and inert (Ar) gases used for the activation of the PET plates.

This set of studies is of fundamental importance for the second part of the planned work, since PET is used as ‘activated support’ for biopolymers gelling.

Second part of the research: Surface modification of EWA/gelatin gels by the “indirect plasma treatment” methodology.

Due to the usually poor mechanical properties of biomaterials films, their use to eventually replace the conventional plastics, introduces new challenges. This research features a novel methodology for the preparation of gel-type films with specific chemical/mechanical properties. EWA-gelatin gels is proposed as a good candidate for this study because these proteins are being widely studied by our research group [121,124,125,131,132,172-180]. Enhancing their mechanical properties could turn them into a new generation of bioplastics which would serve as alternatives to conventional petroleum-based plastics.

In the Department of Interfacial Phenomena, current studies are being made on the activation of different types of surfaces by cold plasma technology in order to improve the surface properties of the materials under study. Concerning the study of hydrogels, in our

attempts to activate them by the cold-plasma technique, the films were always damaged, even under several working conditions. It is a requisite that the material under cold-plasma activation must be capable of enduring the operating conditions reached in the chamber. However, in the case of hydrogels, not only vacuum cannot be applied (in cold plasma processes, gas is excited by energy supplied in a vacuum), but also the material is destroyed (hydrogels are extra-heat-sensitive materials). Additionally, the nature of hydrogels precludes the use of some techniques like XPS. In these cases, it is necessary to adopt strategies that yield reliable results. To the best of our knowledge, very little attention has been paid in the case of biomaterials, which cannot be usually exposed to the plasma working conditions. There are only a few experimental works (performed by researchers from our lab) dealing with new methodologies for hydrogels activation. Terpiłowski et al., [172] have used glass plates activated by cold plasma for deposition of ion-induced whey protein gels. The authors reported improvement in the surface properties of gels deposited on cold-plasma activated glass support. However, for certain specific applications further improvements can be required. In this work, an innovative methodology has been proposed, the '*indirect plasma treatment*', in order to design and characterize thermal-sensitive materials, mainly gel-type biopolymers. It is based on sample contact of the biomaterial (a gel in our case) with plasma-activated surfaces, which supposedly modifies their physical properties. In the particular case of hydrogels, they become directly gelled onto the activated support. It is hypothesized that, after the support activation by cold plasma, physical and chemical phenomena should occur on its surface and, indirectly, also on the surface of any other phase in contact with the activated plate. In this way, after detaching the gels from the activated support, the contacted surface should be '*indirectly activated*', and their surface properties should reflect it. Figure 3 shows a graphical representation of the scheme according to the steps followed in the methodology.

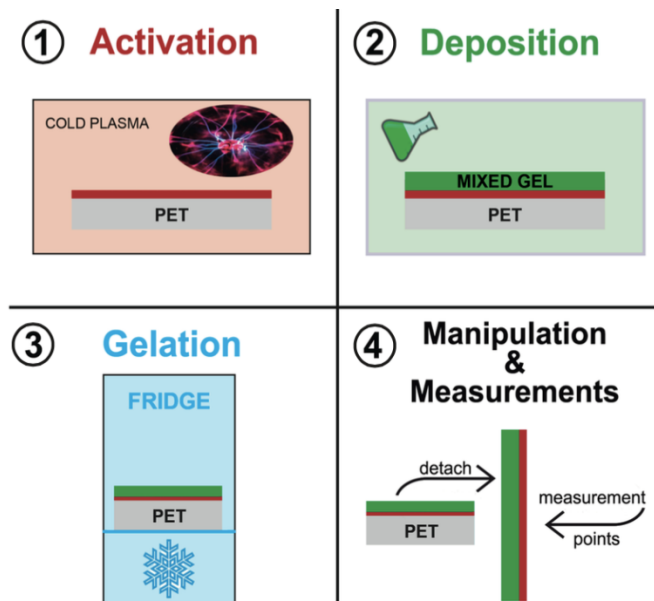


Figure 3². Graphical representation of the indirect plasma treatment working methodology.

On the other hand, cold plasma activation is usually cited as “not altering the bulk properties of the materials” [181]. However, it is still an open question whether in the case of the gels formed in the way above described, properties other than surface chemical ones, are indeed changed. In particular, it could be thought that the strength to deformation and the viscoelastic properties of these materials could be altered after their contact with treated plates. It was our hypothesis in this work that the bulk properties of the hydrogels would be affected under indirect activation.

In order to develop this methodology, it is necessary to prove it by using activated supports of different nature. It will allow to evaluate not only its validity but also its scope. For that purpose, glass, along with PET, has been widely studied by our team [165,172,182-185], and now both used as activated supports where the gels will be formed. Thus, the second part of this dissertation will describe the study on the surface characterization of EWA/gelatin gels by indirect plasma treatment, gelled on i) glass and ii) PET activated plates.

² Graphical Abstract; Pérez-Huertas et al., *Plasma Processes and Polymers*, 17, 3 (2019) 1900171.

The aims covered in the second part of this Thesis are as follows:

5. Study of the impact of indirect cold-plasma activation on the hydrogel functional properties using different gases (air, Ar and O₂). Hydrogels were prepared at different concentrations of gelatin in the EWA dispersions. The characterization of the resulting films will be done by measuring the contact angles, roughness and the calculation of SFE using CAH approach.
6. Determine whether there is some kind of interaction between the gelatin and the EWA particles. For this purpose, microscopic observations are conducted, which allow gaining information on the aggregation state of the samples.
7. Rheological characterization of the hydrogels. For this purpose, dynamic oscillatory measurements were done, and the moduli were characterized, which enabled to check the mesoscopic changes.
8. Make a comparison between the results obtained for hydrogels produced on PET and glass activated support.

As a conclusion, it is expected to get results which will provide valuable guidance for the proper design and manufacturing of hydrogels-based biomaterials. It is the will of this research to get working methodologies that could be used in industrial processes, as those producing intelligent ingredients, scaffolds or tissue engineering films.

III. MATERIALS AND METHODS

III.1 Poly (ethylene terephthalate) (PET) and glass plates

0.5 mm thick commercial PET (Axpert Standard, Bayer AG) plates were used. The material was cut into 20 mm × 20 mm pieces. Before the experiments, the samples were washed in ethanol in the ultrasound bath for 15 min and then rinsed with Milli-Q water to remove their surface contamination. The samples were dried at room temperature in a desiccator. The methodology regarding the study of aging phenomena (hydrophobic recovery) of PET, is given in appendix 2 [138].

Glass microscope slides 76 x 26 x 1 mm with composition (%): 72.6 SiO₂, 13.9 Na₂O, 8.4 CaO, and 5.1 metal oxides were used (Comex, Wroclaw, Poland). Before the experiments, glass plates were cleaned in solution of methanol/water (1:1) in the ultrasounds bath for 15 min and then rinsed with Milli-Q (18.2 mΩ) water and dried in the dryer for 1 h at 50 °C. After drying the glass plates were kept in the desiccator at room temperature.

III.2 Egg white albumin and gelatine

Egg white albumin (EWA) protein, low mineral, high gel type (88.1% protein) was provided by Kewpie Corporation (Tokyo, Japan). Protein concentration was determined by the Kjeldahl method [186]. Gelatin from the porcine skin (type A) was obtained from Dr. Oetker (Gdansk, Poland). According to the specification, it has “platinum strength” with a 250 Bloom and ca. 60,000 mean molecular mass. To perform the Bloom test on gelatin, a 6.67% gelatin solution was kept for 17–18 hours at 10 °C prior to being tested. The higher a Bloom value is, the higher the melting and gelling points of a gel are, and the shorter its gelling time is too.

III.2.1 Hydrogels films preparation

EWA protein dispersion was made by hydrating the protein in distilled water and then heating in a water bath for 30 min at 80 °C. Gelatin solution was added to obtain mixtures with

3% (w/w) EWA protein, and 3%, 4%, 5% or 6 % gelatin concentration. In the preliminary tests, the mixed gels with gelatin concentrations ranging from 1-6% were prepared. At low gelatin concentration (1-2%), the water content was too large to manipulate without damaging the films; thus, only gels containing 3% or higher gelatin concentration were used onwards. The mixed solutions were stirred for 30 min at 80 °C and poured on the support's plates placed on Petri dishes to obtain a 1.0 ± 0.1 mm thick gel layer (checked using a micrometer screw gauge). The Petri dishes were levelled using a self-levelling laser IM0201 (Magnusson, Longpont-sur-Orge, France). In total, 4 samples with different gelatin contents were gelled on each plasma-treated plate (PET and glass), stored for 20 hours at 7 °C and equilibrated at 21 °C for 2 hours, prior to the determination of their properties. After one day, the gel was detached from the support and studies on its wettability and surface topography were carried out on the inner surface, which had been previously attached by the methods described in the following sections [117, 187]. Moreover, rheological measurements were also performed.

III.3 Materials characterisation

III.3.1 Cold plasma treatment

Plasma activation was performed in a low-pressure plasma system, Pico, from Diener Electronic, Germany. The plates were placed on the sample shelf and the system was adjusted to a pressure of 0.2 mbar. Then, the flow of gas (air, argon and oxygen, from Air Products, Warsaw, Poland) was set at 22 sccm (standard cubic centimetres per minute). The plates were treated with the cold plasma at the electric voltage 400 V for 1 min. The chamber was purged with air for 10 s, so as to remove the existing gaseous products.

III.3.2 Contact angle measurements

Advancing and receding contact angles on the surfaces were measured by making use of the contact angle meter GBX (France), equipped with a temperature and humidity-controlled measuring chamber and digital camera. The measurements were conducted at 20 °C and 50% relative humidity. A 6 μ L droplet from a syringe was gently settled on the sample surface by

means of an automatic deposition system. The advancing contact angle was evaluated from the droplet shape by the computer program Win Drop. Then, 2 μL of the droplet volume was sucked into the syringe and the receding contact angle was calculated by the above-mentioned program. Three liquids of known SFE, distilled water, ethylene glycol and diiodomethane, were selected to cover the range from a very polar liquid to a non-polar one. The advancing and receding contact angles were measured for up to 10 droplets of each probe liquid [184].

III.3.3 Topography. Optical profilometer

Topography of the gel surface was obtained using an optical profilometer GT Contour Surface Metrology (Veeco, Tucson, USA). Surface roughness was determined numerically from 3D images through Vision64 software (Veeco, Tucon, USA). The parameters were the average roughness (Ra), the quadratic mean of the surface roughness (RRMS) and the maximum roughness height (Rt).

III.3.4 X-ray photoelectron spectroscopy (XPS)

XPS, spectra of polymer samples, were obtained by a multi chamber UHV analytical system. The system, equipped with a monochromatic hemispheric analyser (R4000; VG Scienta), was used under UHV conditions in the analytic chamber, with vacuum at the 5×10^{-9} mbar level. The Casa XPS program was used for the XPS spectra data processing and the quantitative analysis of the surface properties of the examined samples.

III.3.5 Oscillatory rheology

Viscoelastic properties of the gels were determined using a Kinexus Lab+ dynamic rheometer (Malvern Instruments Limited, Malvern, U.K.). Rheological properties were investigated using a serrated parallel steel plate (35 mm diameter, 1 mm gap size) to limit the sliding effects. The gel samples (10 mm diameter and 1 mm thick) were cut and detached from the plate using a scalpel. Oscillatory measurements, with frequency sweeps in the range 0.1-10 Hz, were performed. All the measurements were performed at 0.01 strain in the linear

viscoelastic range determined by the strain sweep. The complex modulus, G^* , as well as the loss tangent, $\tan \delta$, were obtained from the experimental data.

III.3.6 Confocal microscopy

Optical confocal images were captured by means of an inverted light microscope, Eclipse MA200, Nikon (Japan) with confocal attachment. Moreover, rhodamine solution was used as a contrast.

III.4 Surface Free Energy determination

Since it is not possible to measure the surface energy of a solid directly [188], the contact angle is used as an indirect approach for this purpose. Therefore, surface free energy can be calculated from the wettability angles of experimental liquids (with known surface tension) measured on the solid surface. The contact angle is the tangent of the drop profile at the three-phases contact point where the liquid/gas interface meets the solid–liquid interface (see Fig. 4). The contact angle measurement gives an indication of the capability of a solid to be wetted by a liquid, and the SFE is the quantitative measure of the resulting intermolecular forces at the interphase, which is (or at least should be) independent of the liquid used. At the beginning of the 19th century, Thomas Young published his famous work “An essay on the Cohesion of Fluids” [189], which resulted in one of the most famous equation in surface science, commonly known as Young’s equation (Eq. 1). This equation, describes the relationship between the contact angle and the interfacial tension between the interfaces in contact:

$$\gamma_L \cos \theta = \gamma_{SV} - \gamma_{SL} \quad (1)$$

where γ_L is surface tension of a measuring liquid, θ is contact angle between the solid and the measuring liquid, γ_{SV} is the interfacial tension solid/vapor and γ_{SL} is the solid/liquid interfacial tension.

There is a relationship between γ_{SV} and γ_S , the solid surface tension, given by:

$$(2)$$

$$\pi_e = \gamma_S - \gamma_{SV}$$

With π_e , spreading pressure. Usually in low-energy surfaces as polymers are, this term is negligible, so that Young's equation appears as:

(3)

$$\gamma_L \cos \theta = \gamma_S - \gamma_{SL}$$

The Young equation and the measured values of the contact angles are currently used as the basis of many methods to obtain the SFE of polymeric materials. According to this equation, the surface free energy depends on the contact angle of an experimental liquid, its surface tension as well as the solid/liquid interfacial energy. However, regarding the described parameters, the contact angle and the surface tension are the only ones that can be experimentally measured; and the wettability angle must also be measured under special conditions of equilibrium (Fig. 4a), on ideal solid surface, which should be chemically homogeneous, rigid, smooth and without being perturbed by chemical interaction or by vapour or liquid adsorption.

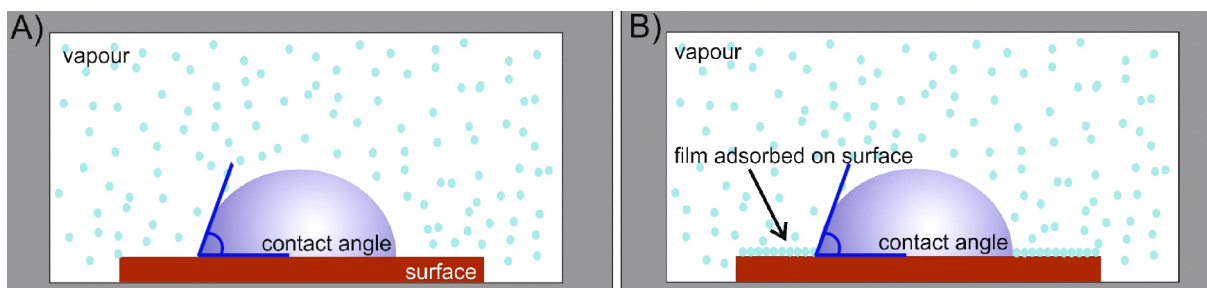


Figure 4³. a) Contact angle in the Young equation meaning b) Contact angle measured under real conditions

Obviously, such conditions cannot be experimentally satisfied, since finding such a flat and chemically homogenous ideal surface is almost impossible and all the practical surfaces deviate from ideality. Therefore, to solve Equation (1), some additional assumptions concerning the relations between γ_S , γ_L , and γ_{SL} should be made. The essence and physical interpretation

³ Terpiłowski et al., Advances in Contact Angle, Wettability and Adhesion, Chapter 14, 332

of these assumptions led to the development of some theoretical approaches for the calculation of the SFE of the solid (γ_S). To be able to understand the different models, the term “work of adhesion” has to be explained. Thermodynamic adhesion is the work required to separate different surfaces into two new surfaces. The equation for work of adhesion can be written as:

(4)

$$W_A = \gamma_A + \gamma_B - \gamma_{AB}$$

where γ_{AB} is the interfacial tension between two phases, γ_A is the surface tension of phase A and γ_B is the surface tension of phase B. Now, if one phase is solid and the other is liquid, the Equations (1) and (4) can be combined into the Young-Dupré equation:

(5)

$$W_A = \gamma_L(1 + \cos \theta_a)$$

This Equation is the basis of SFE theories [190,191]. They all are semi-experimental ones, which is the reason why the used theory must always be stated.

Consequently, the obtained value of SFE will depend, to some extent, on the surface tension of the liquid whose contact angles are measured, and the approach used for its calculation [159]. The specific elements in the development of such approaches are going to be detailed below.

In addition of the two models below described, which are applied for the calculation of the surface free energy, it is possible to calculate the “Young equilibrium contact angle” from Tadmor theory, which contributes to a better description of polymer surface wettability properties. Tadmor proposed the equations 6a-c, which connect the equilibrium contact angle with the advancing and receding contact angles on a really smooth (finitely rigid surface) surface. According to Tadmor’s theory, the advancing contact angle is the largest possible angle on a given surface, being the receding contact angle the lowest and the equilibrium contact angle, somewhere in between them. Associating the line energy (along the three-phase contact line composed of local interfacial energies) with various values of contact angles on the same surface and assuming that heterogeneities on a given surface are of similar nature, Tadmor proposed the following equations [192-196].

(6a)

$$\Gamma_a \equiv \left(\frac{\sin^3 \theta_a}{(2 - 3\cos\theta_a + \cos^3\theta_a)} \right)^{1/3}$$

(6b)

$$\Gamma_r \equiv \left(\frac{\sin^3 \theta_r}{(2 - 3\cos\theta_r + \cos^3\theta_r)} \right)^{1/3}$$

(6c)

$$\theta_{Eq} = \arccos \left(\frac{\Gamma_a \cos \theta_a + \Gamma_r \cos \theta_r}{\Gamma_a + \Gamma_r} \right)$$

Γ_a and Γ_r are, respectively, the advancing and receding angle weight coefficients [185].

These equations allow the calculation of the equilibrium contact angles from the measurements of advancing and receding contact angles on the PET plates. The equilibrium angles will be used in calculations of the SFE by Hysteresis (CAH) approach (equation (8)).

III.4.1 One liquid model: Contact Angle Hysteresis approach (CAH)

This approach is based on the “contact angle hysteresis” (CAH) measurements. The contact angle hysteresis is defined as the difference between the advancing and receding contact angles and it is attributed to the surface roughness and its chemical heterogeneity [197]. CAH approach is based on the measurements of both, advancing (θ_a) and receding (θ_r) contact angles, using the same measuring liquid of a known value of γ_L . The calculated SFE depends somewhat on the physicochemical properties of the liquid used.

The equation used is:

(7)

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

It allows the determination of the “apparent surface free energy” [162], namely γ_s , where γ_L is the liquid surface tension; and θ_a and θ_r are the advancing and receding contact angles, respectively.

Moreover, when the equilibrium contact angles (i.e., θ_{Eq}) are used for the calculation of the apparent SFE, Eq. (7) is transformed into Eq. (8) [162]:

$$\gamma_s = \frac{\gamma_L}{2} (1 + \cos \theta_{Eq}) \quad (8)$$

where the symbols are as above.

III.4.2 Three liquids model: Lifshitz–van der Waals/Acid–Base approach (LWAB)

Van Oss-Good-Chaudhury acid-base theory constitutes one of the most known and successful multicomponent approaches, which is used for the estimation of the SFE of solids [152-154]. According to Van Oss’s approach, the surface tension could be expressed as the sum of several components, due to dispersion, induction and dipole-dipole forces, and hydrogen bonding [152].

In this model, γ_i is splitted into two components; (i) apolar, Lifshitz-van der Waals, γ_i^{LW} , and (ii) polar Lewis acid–base γ_i^{AB} [152-154]:

$$\gamma_i^{tot} = \gamma_i^{LW} + \gamma_i^{AB} \quad (9)$$

The polar Lewis acid–base component, γ_i^{AB} , in turn, is determined by two parameters, electron donor γ_i^- and electron acceptor γ_i^+ . In most cases, the acid-base interaction is mainly due to hydrogen bonding [152]. They assumed that γ_i^{AB} can be expressed as the geometric mean of γ_i^- and γ_i^+ parameters and included the dipole-dipole and dipole-induced dipole (if present) into the γ_i^{LW} component, in which the principal interaction is London dispersion. In fact, experimentally speaking, just this latter interaction is determined from the contact angle

of an apolar liquid, usually diiodomethane. Therefore, for a solid surface, its total SFE can be written as:

$$\gamma_S^{tot} = \gamma_S^{LW} + 2\sqrt{\gamma_S^+ \gamma_S^-} \quad (10)$$

and for solid/liquid interfacial free energy the equation reads:

$$\gamma_{S/L} = \gamma_S + \gamma_L - 2\sqrt{\gamma_S^{LW} \gamma_L^{LW}} - 2\sqrt{\gamma_S^+ \gamma_S^-} - 2\sqrt{\gamma_S^- \gamma_S^+} \quad (11)$$

γ_L is the liquid surface tension; $\gamma_{S/L}^{LW}$, the Lifshitz van der Waals component of solid/liquid free energy of interaction; $\gamma_{S/L}^+$ and $\gamma_{S/L}^-$, are the electron acceptor and the electron-donor parameters, respectively, of the acid-base component of solid/liquid free energy of interaction.

Next, taking into account Eqs. 4 and 5, and using the measured advancing contact angles, it can be assessed that:

$$\gamma_L(1 + \cos\theta_a) = 2\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + 2\sqrt{\gamma_S^+ \gamma_L^-} + 2\sqrt{\gamma_S^- \gamma_L^+} \quad (12)$$

Thus, γ_S^{tot} can be obtained from a series of measurements of advancing contact angles with three selected probe liquids of known surface tension components; one nonpolar and two bipolar liquids should constitute the set of the three measuring liquids. Then, this set of three equations of type (12) can be solved simultaneously and the solid total surface free energy γ_S^{tot} can be obtained from equation (10).

IV. DISCUSSION

In this section, an integral discussion is given about the most important findings, which are interrelated in this set of studies (appendixes 1-4). First, focus is made on the changes of the wetting properties of PET plates induced by cold plasma treatment. Then, an analysis is made on the surface properties of hydrogels, which have been gelled on both activated supports: PET and glass.

A different focus will be given to the findings shown on appendixes 3-4, which deal with the rheological properties found in hydrogels. The possible relationship between the altered bulk and surface properties of gels is considered and evaluated as well. Moreover, a comparison of the surface and bulk properties of the gels which were gelled onto both PET and the glass activated supports, is also carried out.

IV.1 A methodology based on the ‘indirect plasma activation’. Tuning surface and bulk properties of hydrogels

IV.1.1 Surface properties changes induced in treated PET and pre-treated gels by cold plasma.

According to what was mentioned in the previous sections, the analysis of wetting properties is better done from the values of SFE than from the contact angle data, which are the direct consequence of the former quantity; thus, the SFE seems to be the proper analysis tool to carry out this analysis. Fig. 3 displays the SFE of PET prior to and after air- and argon- plasma treatments. The SFE values were calculated from the CAH approach, using the water “equilibrium contact angles” obtained using the Tadmor’s [192-196] derivations.

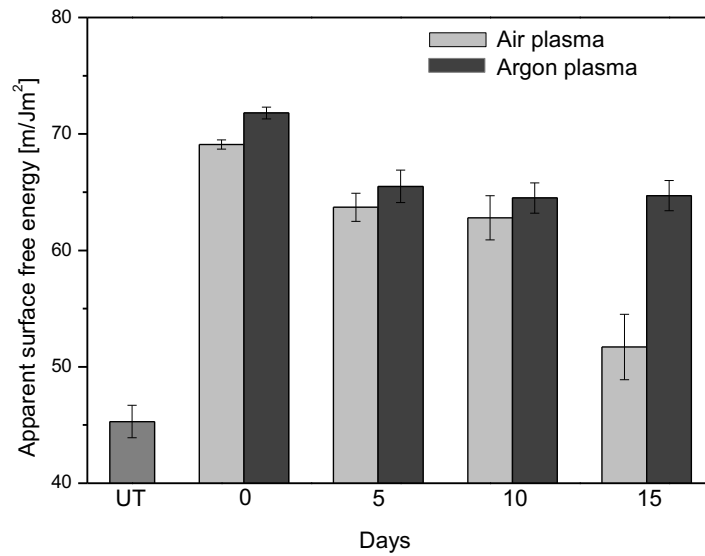


Figure 5. PET surface free energy values based on CAH approach using equilibrium water contact angles in relation to the treatment and storage time UT, untreated

Focusing on the values obtained immediately after the treatment of the PET plates, the SFE increased from $45.3 \pm 3.8 \text{ mJ/m}^2$ (untreated) to $71.2 \pm 5.9 \text{ mJ/m}^2$ after the argon treatment, and to $69.8 \pm 8.2 \text{ mJ/m}^2$ in the case of air. It can be clearly seen that the exposure of PET surface to the air/argon-plasma caused a decrease in water contact angle as a result of the increase in the SFE. During activation, the weakly non-polar bonds of the plastic polymer are replaced by oxygen bonds (cross-linking reactions) which can provide free valence electrons for bonding the liquid molecules. It was corroborated by the changes of the surface chemical composition given by the survey scan XPS spectra. Table 1 provides the percentage contributions of carbon and oxygen atoms on the PET surface. The content of carbon atoms decreased after the plasma treatments. The plasma activation generates polymer chain-breaking for some bonds, resulting in a disruption in the polymer chain, which is proved by the decrease in the percentage of C content. The percentage contribution of oxygen increased significantly from 19.1% up to 30.0% and to 28.1% after ar- and air- plasma treatment respectively, indicating the formation of induced oxidized functional groups. Similar results were obtained by Slepicka et al., [198] who studied the effect of argon cold plasma on the surface chemistry of PET. They found that new functional groups were formed on the polymer surface after plasma treatment. It was reported

that the formation of oxygen rich functional groups at the polymer surface, was due to the decrease in C content.

Table 1. Elemental surface chemical compositions of non-treated and plasma-treated PET samples, as calculated from the XPS survey spectra.

Component (%)	UNTREATED	AIR-plasma	ARGON-plasma
O	19.1	28.1	30.0
C	79.7	70.3	67.3

Thus, the enhancement of the wettability is attributed to a bond scission and the incorporation of oxygen into the PET surface leading to the formation of additional functional groups, which enables modulation of anti-wetting properties. Consequently, this first part has provided essential mechanisms to explain the enhancement of the surface properties of PET plates after cold plasma treatment. The study of this data, in turn, serves as a basis for understanding the changes in the surface properties of the next part, in which treated PET plate is used as an activated support according to the indirect plasma working methodology. This is important since the nature of the hydrogels precluded the surface chemistry composition methods like XPS. The discussion of the effects of time storage, known as the ‘hydrophobic recovery’ phenomena, on the surface properties of plasma-treated PET is given in appendix 2.

Concerning the hydrogels, Fig. 6 represents the SFE as a function of the gelatin concentration and the type of plasma pre-treatment. The formation of gels on PET activated support has a significant effect on their wetting properties. All the pre-treatments improved the wettability of the gels (regardless its concentration), as the SFE increased.

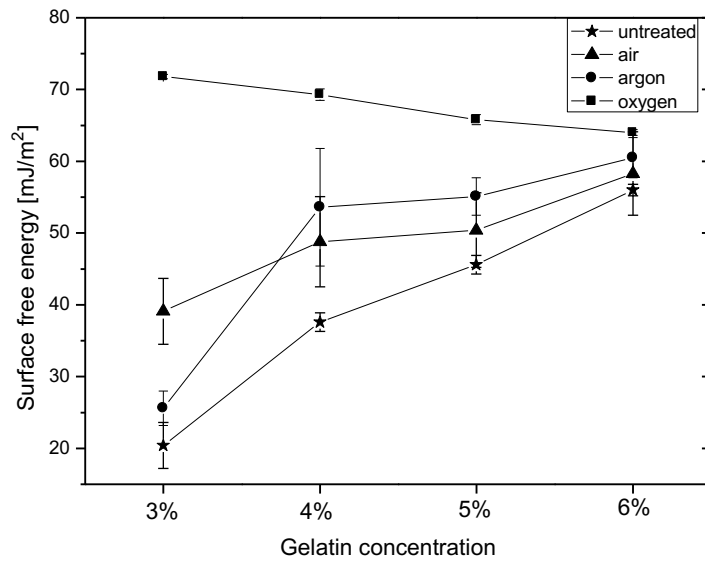


Figure 6. Surface free energy for the hydrogels gelled on PET treated plates; SFE calculated by CAH approach.

It is shown that the largest change of SFE was obtained with oxygen-plasma at the lowest concentration of gelatin, from 20.4 ± 3.2 mJ/m² to 71.8 ± 0.3 mJ/m². In the case of the air-plasma treatment, the change was up to 39.1 ± 2.6 mJ/m² and to 25.6 ± 2.4 mJ/m² in the case of argon-plasma. It was demonstrated that after the support activation, physical and chemical phenomena occur on its surface and, indirectly, also on the surface of other phase in contact with the activated plate (a gel in our case).

Let us now try to explain how it could be possible that even when the plasma gas is not acting directly onto the surface, good results can be obtained. The answer seems to lie in the functional groups appearing after the plasma treatment. The changes of the SFE result from a series of surface chemical changes induced by the plasma treatment, which is usually corroborated by the XPS technique. Unfortunately, the nature of the hydrogels precluded the use of such techniques, being not possible to carry out the analysis of surface chemistry composition, as was highlighted in the introduction. However, the use of the XPS results of the treated PET plates allows to overcome this limitation. In accordance with the working methodology, immediately after the support activation, the hydrogels were deposited very quickly on the treated PET. Thus, the gelation took place on the excited external layer, consisting of oxygen reactive species (among others) which appeared after being treated, as was

corroborated by XPS spectra (Table 1). These functional groups interacted with the hydrogels surface during their gelation, providing them with a more hydrophilic character.

The analysis of the roughness parameters of the treated PET and those obtained for the gel formed on activated PET, suggests that, in spite of the different nature of PET and hydrogels, the plasma-acting mechanisms resulted in quite similar effects. In fact, the most effective gas was oxygen-plasma, followed by argon and air (Fig.7). It also suggests a similarity in the contributions of functional groups on the surfaces after the activation in case of PET and the pre-activation in case of gels.

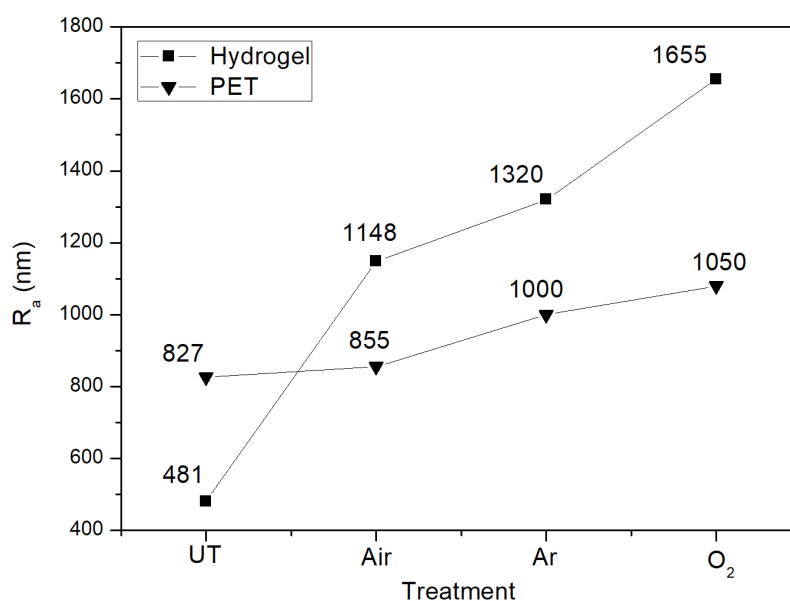


Figure 7. R_a average roughness parameters of PET and hydrogels produced on PET 1) untreated, 2) air-plasma, 3) argon-plasma and 4) oxygen-plasma treated supports; scanned area 1.3 mm × 0.95 mm

Considering that the surface roughness is related with the SFE [199-202], these increments can also be connected with the polar groups originated, in the activated support, by the plasma treatments. When the gel comes into contact with the treated support, the induced oxidized functional groups are ‘transferred’ to allow for subsequent reactions during the gelation. These groups interact with the surface of the substrate and modify its characteristics.

In the same way, the analysis of the surface properties of the gels produced on the treated glass support, draws very similar conclusions. Regardless of the comparison between the modified surface properties of untreated and pre-treated gels that could be done, the results once

again demonstrate that the indirect treatment induced great changes in their properties. It is becoming increasingly clear that the methodology tested here seems to be valid. What is more, not only this methodology allows to pre-treat biomaterials which cannot be exposed to the plasma working conditions, but also it is quite effective regardless the nature of the support (plastic or glass). The comparison of the results obtained using hydrogels gelled on PET and on glass plates, shows a strong similarity in the tendency of SFE lines (Fig. 8); moreover, the roughness parameters are also proportional, the treatment efficiency follows the sequence:

$$Untreated < Air < Argon < Oxygen$$

as it can be seen in Table 2, which means that our methodology works accurately, even when using different supports, corroborating hence their workability.

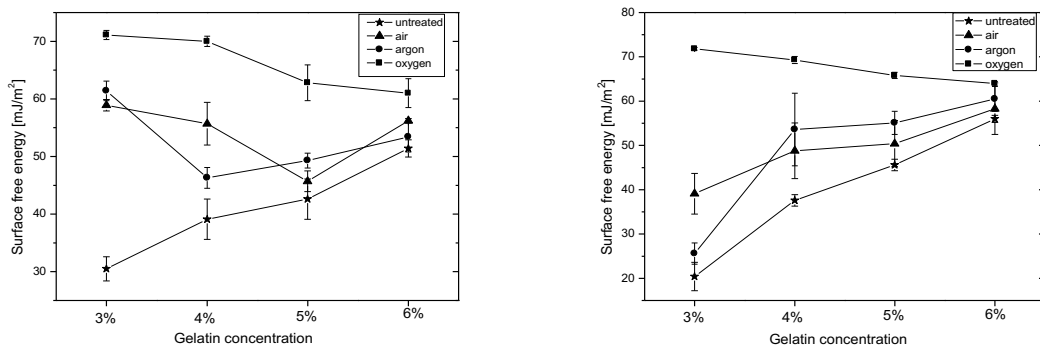


Figure 8. Surface free energy for the hydrogels gelled on treated glass plate (left) and on PET treated plates (right); surface calculated by CAH approach

Table 2. Topography parameters of the EWA gelatin gels formed on PET and glass activated plates by different plasma gasses. Surface: 0.9x1.3 mm. Ra – average roughness.

Parameters (nm)	UNTREATED	AIR-plasma	ARGON-plasma	OXYGEN-plasma
R _a (GLASS-GEL)	164,3 ± 27,9	297,1 ± 50,9	325,8 ± 59,6	995,2 ± 198,7
R _a (PET-GEL)	481 ± 80	1148 ± 233	1320 ± 290	1655 ± 163

The formation of polar groups on the activated support, their interaction with the gel during its gelation and the soft nature of the hydrogels, they all jointly may explain the greater enhancement of the surface properties using the indirect methodology.

IV.1.2 Rheological behaviour

In this Dissertation, the premise that cold plasma is usually cited as “without altering the bulk properties” [181] has been outlined many times. However, as there are no results so far for rheological properties of gels formed on activated supports, it is still an open question whether the bulk properties could be as altered as the surface properties are. This study finally provides the answer.

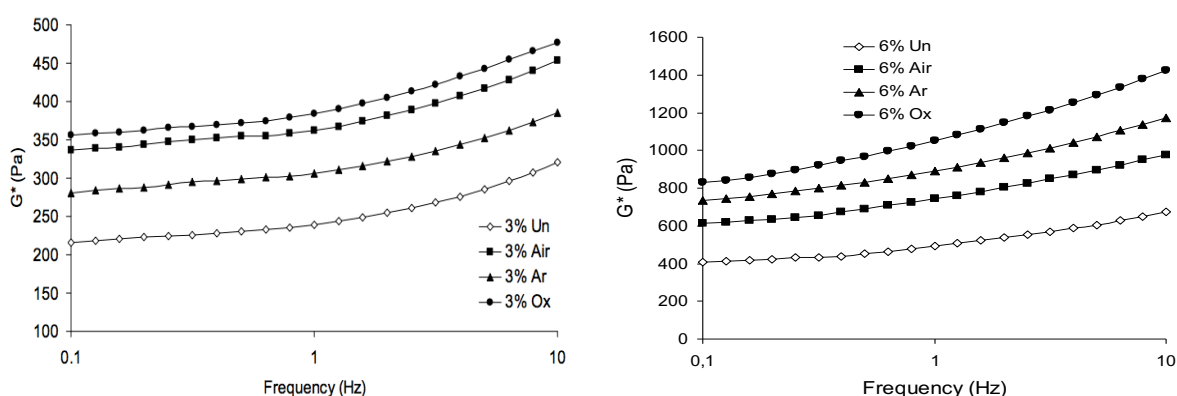


Figure 9. Influence of sweep frequency on the values of complex modulus G^* for hydrogels gelled on glass (left) and on PET (right) plates previously treated with different plasma gases; (Un-untreated, Ar-Argon plasma, Air-air plasma and Ox-Oxygen plasma); % in the figures; concentration of gelatin in hydrogels.

Fig. 9 shows the complex modulus, G^* , obtained from frequency sweeps for gels gelled on pre-treated glass (left) and PET (right) plates at different gelatin concentration. To cover the whole range, only the charts of the gels with the lowest (3%) and highest (6%) gelatin concentration are shown. The hydrogels produced on activated support presented an unusual modification in their bulk properties, the two rheograms display (Fig. 9) that all treated gels

enhanced their rheological properties in terms of rigidity. The most rigid gels were obtained from those formed onto oxygen treated plates, regardless of the nature of the support. The complex modulus G^* increases about two times when comparing untreated gels vs. oxygen treated gels (Fig. 9). Therefore, as in the case of the surface properties, the chart shows a proportional enhancement when comparing the gels formed on PET and glass activated supports, confirming that the stiffness can be tuned in the same way the surface properties are. It is also remarkable that there are similarities when comparing the SFE, roughness parameters and the complex modulus values.

Taking into account that the plasma treatment incorporates new species onto the surface (XPS analysis), and the gelation could be enhanced by an increased number of hydrogen bonds, the probability for a chain to be bound to another one would be higher after exciting the surface, resulting in additional bridging. This means that plasma activated support caused remodelling of the gel bonds not only on its surface, but also at a deeper level, enhancing its viscoelastic properties. One possible explanation could be in its thickness, the sample thickness was small (1 mm), therefore the obtained samples can be treated as composite materials consisting of a bulk phase with different, modified surface layer. Hence, the bulk modification may be the consequence of the appearance of specific components resulting of their migration or diffusion from the surface into the bulk. The soft nature of the hydrogels could also allow that such species may be inserted in a deeper layer than the topmost one. Thus, it can be concluded that ‘indirect plasma treatment’ is an interesting way to adjust the bulk properties. This finding supposes an original contribution with respect to the standard meaning of the cold plasma treatment, based on just altering the surface properties.

Finally, it is worth noting the similarity found between the modified surface and the bulk properties of both systems. The results shown in Fig. 8 are correlated with those in Fig. 9. In order to clarify this idea, both figures are combined (Fig. 10). From that figure, it can be observed that the modifications of the rheological properties follow the same patterns than those of the surface properties; for instance, oxygen-plasma pre-treatment caused the most significant changes in wetting and rheological properties of the gels, or the similar trends which air and argon treatment provoke in the gels surface and bulk properties (see all charts given in the papers). All this jointly support the idea that the reactive groups incorporated on the surface, due to the activation, migrate into the bulk, having proportional consequences. Hence, it can be concluded that there is a strong relationship between the origin of the changes induced on the surface and the bulk properties when using this methodology.

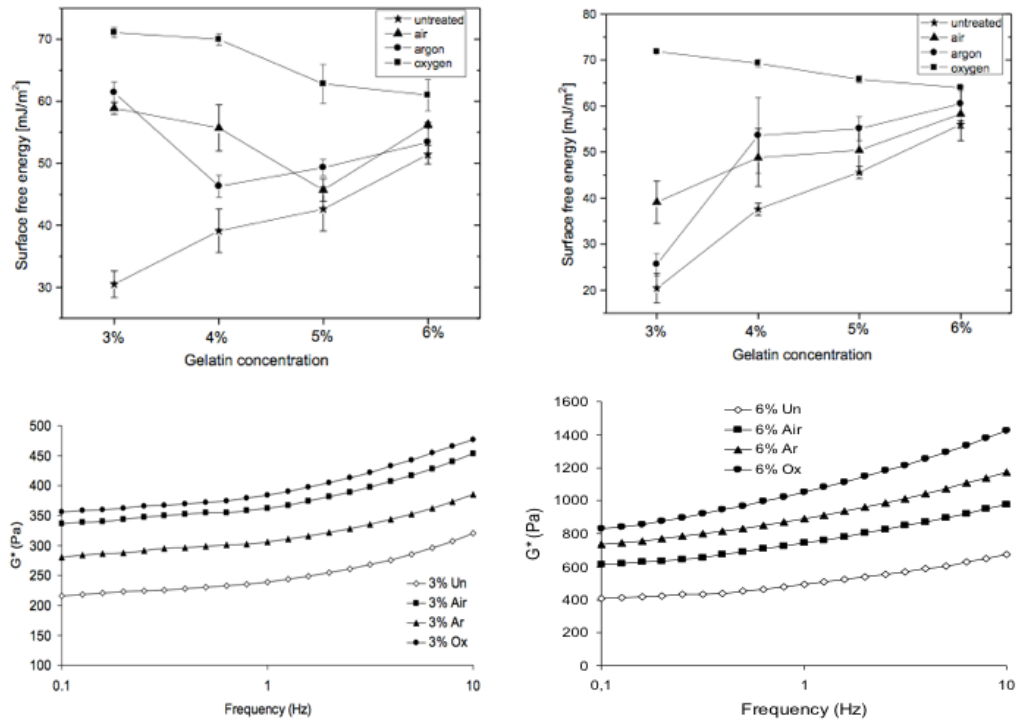


Figure 10. Fig. 8 (up) and Fig. 9 (down). See figure legends above.

To conclude this section, it could be said that this methodology shows the potential modification induced in the surface and the bulk properties of the hydrogels. Being able to adjust such properties provides new possibilities to design hydrogels with enhanced functional properties, which may be interesting to be applicable in industrial applications, such as active packaging or drug delivery system, as it was mentioned in the introductory section.

V. CONCLUSIONS

In this Thesis, two types of modifications induced by cold plasma have been studied: i) surface chemistry of PET, and ii) surface and bulk changes of EWA/gelatin gels induced by *indirect plasma treatment*. In the first case, the influence of cold plasma treatment on the surface properties of PET plates, as well as the effects of time storage on their properties have been considered. A set of conclusions can be drawn from the comparison in between the sequencing of changes of contact angles, SFE, topographic images and XPS surface chemical data.

The main conclusions that can be drawn from the *first part of this work* are the following:

- i. Plasma treatment leads to dramatic changes in the surface polarity (wettability) of the polymer. The study reveals that after the air/argon-plasma treatment, the polymer surface becomes more hydrophilic and, consequently, the contact angles are lower. The increment of the SFE caused by argon-plasma treatment is greater as compared to the air-plasma treatment, which indicates stronger interactions of the probe liquid with the PET surface. The wettability enhancement is provoked by the formation of polar groups on the polymer surface upon reaction with plasma generated reactive species, increasing the polar component and finally the total SFE of the PET plates. Moreover, it was found that the average total SFEs obtained by CAH and LWAB approaches are comparable, which indicates that these methods are complementary.
- ii. PET surface becomes rougher with plasma treatment, no regard which gas was used. The roughness parameters show that argon-plasma activation induced the most significant changes again, thus the topography study agreed with the SFE results.
- iii. Cold plasma treatment increased the surface oxygen content of the activated polymer by increasing the amount of oxygen-containing polar groups, such as C-O-H and C=O on the film surface. The plasma treatment generates polymer chain-breaking in some of the polymer bonds, which is proved by the decrease in the percentage of C content and the increment in the percentage of O₂ content, shown by X-ray photoelectron spectroscopy (XPS) analysis. This chemical modification, leading to the incorporation of functional groups onto the PET surface, is mainly responsible for the changes of the surface polarity,

which affects the surface properties of the material. The final result is the increased wettability on the PET polymer.

- iv. PET surface chemical composition changes as a function of time, indicating that there are mechanisms contributing to the loss and further recovery of its hydrophobicity. Interpretation of the effects of ageing after plasma activation must take into account both, the extent of the increase of SFE after plasma treatment and the nature of the molecules in contact with the surface during the ageing process. There is a complete loss in the case of air-plasma activation after a period of 15 days. However, when PET is treated in argon environment, the surface (mainly manifested not only by the values of its SFE but also by the XPS data) becomes stable after a short period of time, retaining its wettability enhancement and being activated in comparison with its previous, non-treated, state. The plasma activation created active sites on the surface, that depend on the nature of the gas used in the treatment. These sites, in turn, interact with air and deactivate the surface at different rates; this depends on the type of the functional groups created during the activation process, as well as the ability of the resulting surface groups to rearrange into a more stable configuration.

The following are some of the mechanisms involved in the process of activation and deactivation of PET surface during air/argon-plasma treatment and further ageing in open atmosphere: Appearance of functional groups involving the C, O and N atoms; reorientation of polar species of the surface; diffusion of those groups into the bulk, and reaction of free radicals arising from the activated surface with the molecules of the air. They all jointly explain the surface properties studied in this work.

Therefore, the first part of this work has provided essential mechanisms to explain the enhancement of the surface properties of PET plates after cold plasma treatment and the so-called hydrophobic recovery. The study of these data, in turn, has allowed to understand the processes described in the second part of the work, in which plasma treated PET plates are used as an activated support, in accordance with the indirect plasma working methodology.

Concerning our second issue, the indirect plasma treatment has been featured as a way of surface and structural bulk characterization for gel-type biopolymers, which cannot be exposed to the plasma working conditions. This study, in turn, has two different configurations: characterization of EWA/gelatin gels gelled on: i) glass and ii) PET activated supports.

The most relevant conclusions derived from this *second part of our study* are as follows:

- v. It has been particularly successful the development of a novel methodology for the activation of gel-type biopolymers via indirect cold plasma treatment, which imparts desirable effects for their efficient functionality. Compared with the untreated hydrogels, the pre-treated ones exhibit an enhanced version in terms of mechanical properties. There is a significant modification of the surface, as well as of the structural bulk conformation of gel films formed in situ on both PET and glass plates pre-treated by cold plasma. The effect of plasma pre-treatment to the gels is to provide a more hydrophilic character to their surfaces, which is manifested by the SFE increment. The SFE of the gels was shown to be a function of the gelatin concentration in the EWA protein gels and the type of plasma pre-treatment. The most effective pre-treatment was obtained by the oxygen cold plasma, which induced the higher hydrophilicity of the samples. The gelation onto activated supports strongly altered the surface polarity, which enables modulation of anti-wetting properties. Hydrophilic bonds/groups appeared, creating intensive hydrophilic interactions at the activated gel surface/water interface, which resulted in a wettability enhancement.
- vi. The surface roughness can be tailored by indirect plasma treatment. Gel surface becomes rougher with the plasma treatment of supports, no regard which gas was used. The roughness parameters show that oxygen-plasma induced the most significant changes again, thus the topography study agreed with the SFE results. The wetting properties was affected by the formation of micro-pores on the gel surface and characterized by the huge increment of the roughness parameters of the gel.
- vii. Cold plasma pre-activation caused deprotonation of the sulfhydryl groups of albumin and formation of a larger number of disulphide bonds, which results in a higher aggregation (shown by confocal microscopy images). Such a structure leads to a reduction in the value of water wetting contact angles, since albumin is more hydrophilic than gelatin.
- viii. These studies also demonstrated that the rigidity of gels can be tuned too, as manifested by the oscillatory rheological technique. The surface composition was changed and modified by the indirect plasma treatment and, consequently, the bulk modification was

achieved by the presence of the specific components resulting of their migration from the activated surface into the bulk. This means that plasma activated support caused remodelling of the gel bonds not only on its surface, but also at a deeper level, enhancing its viscoelastic properties. Moreover, increasing the concentration of gelatin in the gels produces stronger gels by strengthening the interactions into the gel network.

- ix. The results obtained for the gels gelled on both PET and glass plates show a strong similarity in the patterns followed by the SFE values, the roughness parameters and the complex rigidity modulus, corroborating that our methodology works accurately in activated supports of different nature.

To conclude, it could be said that we were able to make progress in the characterization of a thermal-sensitive biomaterial, which could not be treated directly by cold plasma without causing any damage. It has been elucidated a methodology that allows the control of the hydrogels surface and their rheological properties and also gives some keys for the enhancement of the technological performance of gel-type biopolymers. From a practical point of view, the inclusion of gels would enable the preparation of cost-competitive and field-responsive biomaterials, which is always a concern for the applicability of these materials.

V.1 Avenues for future research

If the “indirect plasma treatment” methodology gets recognition, it would open new trials coming from many different areas of technological interest. Thus, exploring the efficiency of indirect plasma treatment using other biopolymers is highly recommended. In the Department of Interfacial Phenomena, there is current research involving the optimization of this methodology, by testing not only new biomaterials but also activated supports of different nature. A better understanding of the interactions between plasma reactive species with the structural components of hydrogels and a proper identification of the specific biofilm targets, will allow further optimization of plasma treatments towards more effective and less damaging activation processes, as it has been required in the fields of food production or biomedicine.

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VII. APPENDIXES

Appendix 1

Surface Properties of Whey Protein Gels (first paper of the “compendium”)

Surface Properties of Whey Protein Gels

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(Received on 30th July 2018, accepted on revised form 7th March 2019)

Summary: Surface properties of whey protein gels are reviewed based on traditional microscopic techniques and new methods, as optical profilometer and contact angle measurements. Optical profilometer is an instrument allowing measurement of surface roughness and contact angle measurements to determine the surface wettability behavior (hydrophobicity/hydrophilicity) of the gels. Investigation of surface properties of whey protein gels is very important, as it can transform this product to a new level of application. It could be used as a matrix for an active ingredient release, material for tissue engineering, e.g. scaffolds, i.e. temporally structures biodegraded in the human organism.

Keywords: Microscopy, Wettability, Contact angle, Roughness, Surface hydrophobicity, Spectroscopy, Rheology.

Introduction

Whey proteins are becoming very popular as functional ingredients in foods. Their application in food products will increase in the future, as consumers pay more and more attention on valuable sources of exogenous amino acids. It is connected with increasing interest of population in sport and recreation. Whey proteins are generally marketed in three forms: whey protein concentrate, whey protein isolate and whey protein hydrolysate [1]. The concentrate contains fat and lactose along with the quintessential proteins (29–89%); the isolate is made of 90% protein and the hydrolysate is the semi-digested form of the protein. Whey protein enriches foods in the most valuable amino acids and shapes their texture, mouthfeel, water and flavor holding capacity [2, 3]. Gelation is the most important functional property of whey protein and it is a key process to generate food texture. Properties of the gel depend on the ionic strength of the solution, temperature and the time of heating. Heating of whey protein solution above the denaturation temperature (greater than 65° C), causes unfolding and aggregation of proteins. After cooling down, at low ionic strength, a thick solution can be obtained. Addition of ions will result in electrical shielding of charges and formation of a gel [4, 5]. Whey protein gel microstructure has been observed to change from fine stranded to particulate with increasing salt concentration [6]. The properties of whey protein

gels are greatly modified by the presence of dispersed filler particles, for example oil droplets, within the protein gel matrix, due to interfacial filler particle gel matrix interactions [7 – 10]. The gelling ability of proteins provides important textural and water-holding properties in many foods [11 – 13]. The mechanism of heat-induced gelation of whey proteins is not completely understood. Former investigations interpreted the gelation as a two-phase process consisting of unfolding of the globular structure, and subsequent aggregation of protein chains into a three-dimensional network. Today, gelation of whey proteins is basically considered as a four-phase process consisting of unfolding of the native structure, aggregation of the unfolded protein molecules, string formation of the aggregates, and linkage of the strings to a three-dimensional network [14]. Partially stable intermediates of the three-dimensional structure of whey proteins, called the “molten globule state”, are of particular importance during gelation [12, 15]. The formation of heat-induced whey protein gels, mainly due to disulfide bridges and hydrophobic interactions, is irreversible [14, 16, 17]. The structure and texture of the gels depend on protein concentration, ion strength and type as well as on pH value, temperature and degree of denaturation [14, 16 – 19]. Furthermore, the origin of the whey (rennet casein cheese or acid casein) as well as the operations used for concentrating,

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isolating and/or fractionating proteins is particularly relevant for the resulting properties of the whey protein products [14, 20].

The cold gelation process itself is induced by adding salt or acid to the heat-treated protein solutions [21 – 24]. Comparative studies on the compositional, physicochemical and functional properties of whey protein concentrate (WPC) and whey protein isolate (WPI) are widely described in the literature [20, 25 – 28]. In this review different techniques of investigating surface properties of whey protein gels are presented. Due to growing interest in the study of biopolymers, this paper aims to review up-to-date publications on the surface properties of whey protein gels investigated by traditional and the recently applied technologies.

Methods/Techniques for the analysis of the surface properties of whey protein gel

Traditional Microscopic Methods

Traditionally the surface of the gels is observed using different types of microscopy. For the examination of the gel structure different types of microscopy (scanning electron microscopy (SEM) [5, 29 - 33], atomic force microscopy (AFM) [29, 34 - 38], transmission electron microscopy (TEM) [39 - 41] or confocal laser scanning microscopy (CLSM) [42 - 48] are applied. Some of them require a special sample preparation. The combination of microscopy with other techniques allows the direct study for the investigation of gel structure. The choice of the adequate method for the examination of the structure of the gels is essential for a successful research in this field. A broad variety of microscopic methods has been applied for whey protein gels and it is beyond the scope of this review to discuss them. We are focused on some interesting examples from research conducted by our research group, especially aerated gels.

Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) is very suitable for observing the structure of gels without finer details such as casein micelles or protein clusters [49 – 51]. It enables to observe gel structure without any preparation procedure, as the laser beam penetrates the sample at a desired depth, without changes in the gel properties [52]. The microstructure of aerated gels was investigated using confocal laser scanning microscopy [53]. At different pH, a very interesting structure of air bubbles was observed, larger bubbles contained smaller ones and

these contained other smaller bubbles. This microstructure resembled a fractal structure. Ikeda *et al.* [54] reported a fractal nature of whey protein gels. Similarly, the fractal nature of the whey protein gels surface was investigated by Chen and Dickinson [55]. Tomczyńska-Mleko and Mleko [56] showed that different microstructure of the ion induced aerated gels was obtained at different ions. For Ca^{2+} induced gels a particulate gel was formed and for Fe^{2+} and Mg^{2+} fine-stranded microstructure was noticed. The aerated gel induced by Fe^{2+} ions was the most transparent. Air bubbles were composed of fractal structures as larger bubbles contained smaller ones in the same geometrical pattern. Kharlamova *et al.* [46] studied whey protein isolate gels formed by acidification of fractal aggregates with different sizes. The microstructure was analyzed by CSLM and it was found, that varying the protein composition, the microstructure changed drastically. The images showed more homogeneous structure when the protein content increase. Similar microstructure was observed by Ju and Kilara [57] for gels formed from whey protein isolate by adding GDL or CaCl_2 .

Scanning electron microscopy (SEM)

Comparing this technique with light microscopy, the resolution is notably improved with electron microscopy. This technique has been used to study representative surface structure of whey protein gels and to assess their homogeneity. The microstructure of ion induced whey protein aerated gels has been investigated by Tomczyńska-Mleko [5]. It was reported that at higher calcium concentration more porous structure of particulate whey protein gel was obtained. It resulted in higher syneresis. An opposite effect of syneresis was observed for increased protein concentration. It was caused by more packed gel structure with higher number of bonds between protein molecules. Fine-stranded gel microstructure produced at pH values far from the isoelectric point is characterized by lower porosity with lower syneresis [58]. Similar results were observed by Liu *et al.* [33]. They investigated the influence of Ca^{2+} and Na^+ ions on the properties of whey protein isolate/lotus root amylopectin composite gel system. The SEM images showed a high number of pores in the network of WPI-LRA gels without salt ions, however the addition of Ca^{2+} (0.05M-0.1M) resulted in a denser and more homogeneous microstructure, in case of Na^+ the images showed a more compact gel microstructure than without Na^+ (0.5M). They concluded that an appropriate amount of Ca^{2+} or Na^+ results in a more compact and stable gel network structure with compact cavities. In SEM images, the addition of salt

ions in low concentration resulted in a denser and more homogeneous gel network, however large cavities and irregular structure in the gel network appeared increasing the salt concentration.

Aerated whey protein isolate gels were obtained at different pH using reversibility of heat-induced gels [53]. SEM micrographs indicated that at different pH (3.0, 9.0, 10.0) a smooth gel microstructure was observed. At these pH values, repulsive electrostatic force between charged protein chains is effective [22]. Tomczyńska-Mleko *et al.* [59] observed aerated whey protein gels induced by calcium ions on SEM micrographs. They showed that increased ions concentration causes higher aggregation of protein matrix and more porous microstructure is present at the interface of the gel and air. Similar changes were observed by Croguennec *et al.* [60]. This is in an agreement with results presented by Tomczyńska-Mleko *et al.* [61], which demonstrated that at higher magnesium ions concentrations, stronger ionic interactions were possible with more magnesium bridges being formed. This resulted in a more aggregated structure. Maltais *et al.* [62] observed fine-stranded microstructure for a gel at low salt concentration (10 mM of calcium chloride) and a particulate, unordered gel structure was noticed at higher calcium chloride concentration. At higher salt concentration, the surface microstructure becomes rougher. Nayebzadeh *et al.* [63] observed natural, not-dried mixed whey protein/xanthan gels using a modified, steam filled column method of scanning electron microscopy. Microscopic images were used for calculation of roughness these mixed gels surface. It was noted that xanthan has a surface smoothing effect on the heat-set whey protein gels.

Transmission electron microscopy (TEM)

Transmission electron microscopy allows observing the microstructure of the gels at very high magnitude. For a new high-resolution transmission electron microscopy, it is possible to obtain above 50 million times magnification with a resolution of 1 Ångström. The problem is with the preparation of the sample which must be cut into ultrathin section less than 100 nm thick. It is possible to observe the surface section of the gel, but usually this technique is used to observe protein gels in terms of the degree of aggregation and the shape of protein aggregates. Tomczyńska-Mleko *et al.* [61] showed denser microstructure of the gels with increased magnesium ion concentration which coincided with more elastic behavior. Increased elasticity of the gels was caused by a higher number of bonds between whey protein molecules. A higher concentration of divalent

magnesium ions probably caused a stronger screening effect, which facilitated more powerful hydrophobic interactions [61]. Recently Jiang *et al.* [39] used the transmission electron microscopy to compare the microstructure characteristics of polymerized whey protein isolate and concentrate. The TEM images revealed that the network of the isolate form was more homogeneous, stable and denser than the concentrate form. Uzun *et al.* [41] proposed an interesting way for developing delivery system. The morphology of dried WPI gels containing lutein droplets was observed by TEM. The images of gels confirmed that the process preserved the original size of lutein droplets.

New methods

Optical profilometer

Optical profilometer is an instrument allowing measurement of surface roughness. Microscopic methods do not allow for quantification of this property. There are very scarce research studies on whey protein gel surface using optical profilometry.

Optical profilometry is a non-contact technique which uses a light source to investigate the surface. The key component to this technique is directing the light in a way that it can detect the surface in 3D. Optical profilometry is faster than contact profilometry with sacrifices in lateral resolution. It is completely non-destructive to samples that are not sensitive to light and very soft surface can be scanned. Based on the images, the computer program calculates the parameters determining the surface properties. The gels roughness is described using three different parameters: Ra, average roughness, is the main height as calculated over the entire measured length or area. It is useful for detecting general variations in overall profile height characteristics. Rq is a quadratic mean of the surface roughness and is given by the standard deviation of the vertical values for the gel area. The root means square index RMS (Rq) is the best parameter to compare roughness of the gels, as it is insensitive to local surface topographical heterogeneity. Rt represents the distance between the highest peak and the lowest valley on the measured gel surface. Generally, gels with more aggregated microstructure have surfaces with higher values of roughness parameters. Chen *et al.* [64] noticed that whey protein gel obtained without any salt addition had a very smooth surface with Rq and Ra of 0.20 and 0.18 μm , respectively. For the gel obtained at 200 mM of sodium chloride, a much rougher surface with

large R_q and R_a values were produced. It was caused by increased protein aggregation caused by high concentrations of salt [58]. Higher magnesium ion concentration produced gels with a rougher surface structure i.e. with a higher quadratic mean of the surface roughness R_q and maximum roughness height R_a [65]. Tomczyńska-Mleko and Mleko [56] reported that the roughness of the obtained gel surface depends on the type and concentration of added salt. Higher concentration of cations resulted in gels with a higher quadratic mean of the surface roughness and maximum roughness height. Aerated calcium ion induced gels were used for hydrolysis experiments in an artificial stomach [59]. There was a linear correlation between the quadratic mean of the surface roughness (R_q) and the maximum roughness height (R_t). Similar correlation was observed for the non-aerated egg white gels [66]. Surface roughness of the gels influences different active ingredients release from the gel [67]. Tomczyńska-Mleko and

Mleko [56] found, that microstructure and different surface roughness of whey protein gels influenced contact area of the gels with pepsin, which resulted in different release time of active ingredients. In recent research Terpiłowski *et al.* [68] examined whether activation of glass support can influence surface properties of ion-induced whey protein gels deposited on this support. The gel surface was observed using an optical profilometer. Increasing of ion concentration resulted in the rougher structure of the obtained gel (Fig. 1). It is in line with our previous results on the aerated whey protein gels [61]. Whey protein gel layer is smooth on the untreated glass while for the argon treated sample it is rough with big deep holes on the surface. Gels deposited on the air treated glass plate have also a different structure which is rougher than for untreated glass support (Fig. 2).

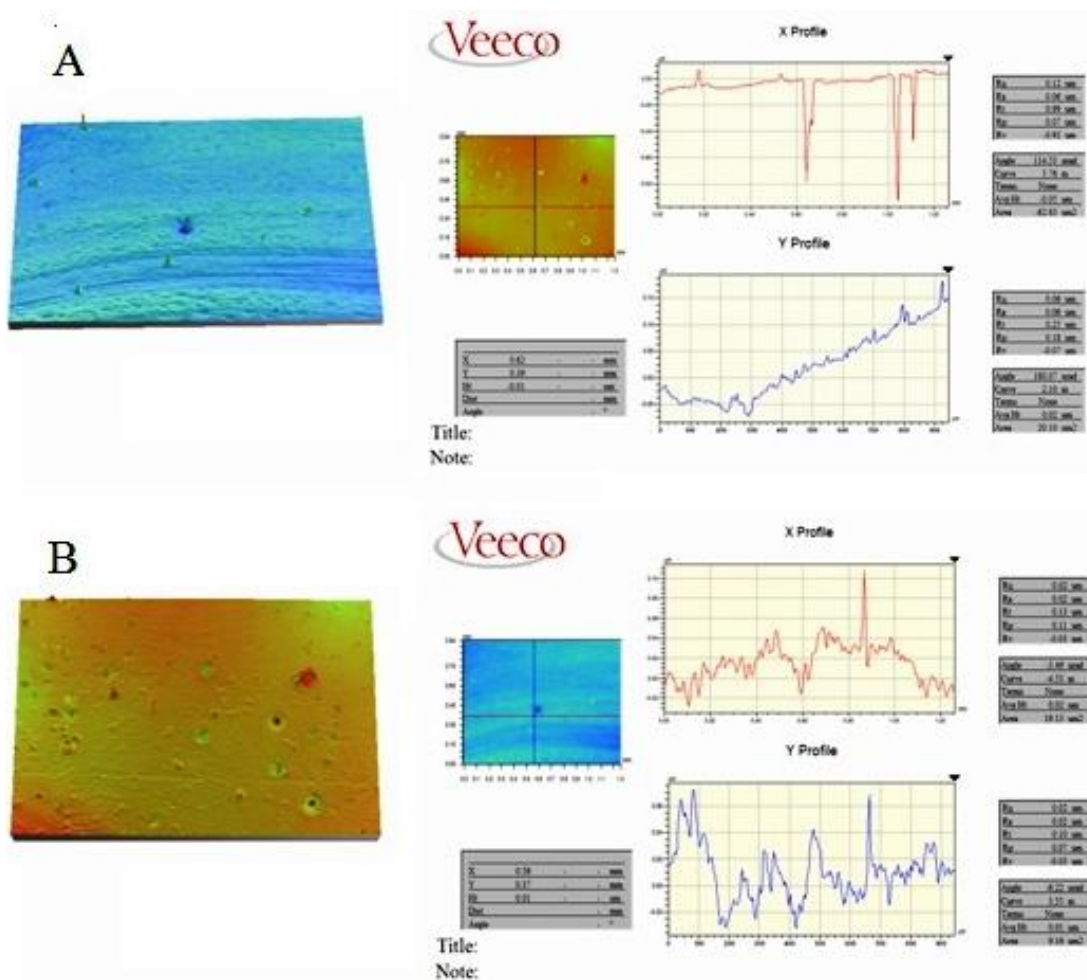


Fig. 1: Optical profilometer images (0.9 x 1.3 mm) and side profiles for whey protein gels deposited on untreated glass surface A: 20 mM Ca^{2+} , B: 30mM Ca^{2+} .

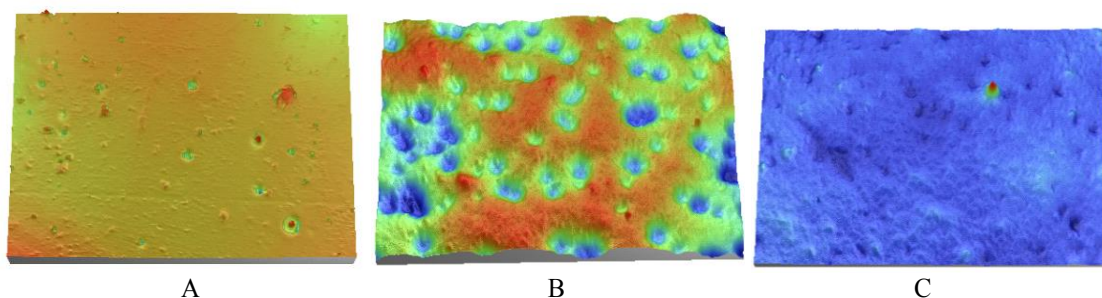


Fig. 2: Optical profilometer images (47 x 62 μm) for whey protein gels induced by 30 mM Ca^{2+} deposited on: A - untreated glass surface, B - Ar plasma activated glass and C - air plasma activated glass.

Contact angles and surface free energy (SFE)

Another important surface property is its wettability. It is a physical property relating to the ability of a liquid drop to spread on a surface of a solid or liquid material. The strength of the attractive or repellant force is closely related to the "contact angle" between the water drop and the surface (Fig. 3). When the solid has a high affinity for water, this kind of material is called hydrophilic (e.g. glass), the contact angle will be less than 90° . Water drop tends to spread out and "wet" the surface. In the opposite case of hydrophobic (e.g. Teflon) surface, the contact angle will be greater than 90° , and instead, forms at equilibrium a spherical cap resting on the substrate with a "contact angle", the water drop tends to bead up on the surface (Fig. 3). Measurements of contact angles require the solid surfaces to be rigid, smooth and homogeneous, so the Young's equation is calculated for the appropriate equilibrium condition. The solid surfaces should be as inert as possible, so that effects such as swelling and chemical reactions are minimized.

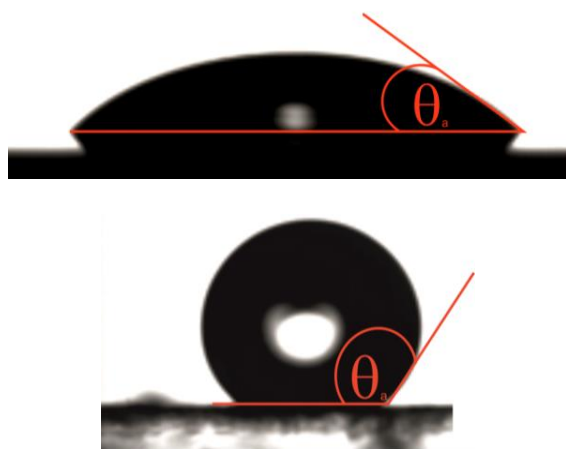


Fig. 3: Different contact angles for a hydrophilic (left) and a hydrophobic surface (right).

Two different theoretical approaches to interfacial interactions are presented for the determination of very useful parameter - surface free energy (SFE), which quantifies the characteristic of the solid surface and its wettability. Acid-base (LWAB) and hysteresis (CAH) approaches are presented below.

In the LWAB approach of Van Oss *et al.* [69] the surface free energy is showed as the sum of two constituents: a polar Lifshitz-Van der Waals γ_i^{LW} and Lewis acid-base γ_i^{AB} :

$$\gamma_i = \gamma_i^{\text{LW}} + \gamma_i^{\text{AB}} \quad \text{Eq. 1}$$

Besides dispersion interactions, the component γ_i^{LW} includes the dipole orientation and the induction ones which were considered to be polar earlier. The component of acid-base interactions γ_i^{AB} can be expressed by the geometric mean:

$$\gamma_i^{\text{AB}} = 2 (\gamma_i^+ \gamma_i^-)^{1/2} \quad \text{Eq. 2}$$

Based on such model the adhesion work can be written by means of the constituents:

$$\gamma_l(1 + \cos \theta) = 2 \sqrt{\gamma_s^{\text{LW}} \gamma_l^{\text{LW}}} + 2 \sqrt{\gamma_s^+ \gamma_l^-} + 2 \sqrt{\gamma_s^- \gamma_l^+} \quad \text{Eq. 3}$$

where: γ_l liquid surface tension, θ -contact angle (advancing), $\gamma_{s/l}^{\text{LW}}$ solid and liquid apolar Lifshitz van der Waals interactions, $\gamma_{s/l}^+$ solid and liquid electron acceptor parameter of surface free energy, $\gamma_{s/l}^-$ solid and liquid electron donor parameter of surface free energy. The quantity of the constituent γ_s^{LW} and the parameters γ_s^+ , γ_s^- of surface free energy can be calculated from Eq. 3 by measuring the wetting angle of three different liquids with known

surface tension constituents: γ_i^{LW} , γ_i^+ and γ_i^- . A set of three equations with three unknown values (γ_s^{AB} , γ_s^+ , γ_s^-) allows to calculate the energy components (γ_i^{LW} , γ_i^{AB}) and finally its total value. Very important in determination of surface free energy of solids is the selection of liquids used for measuring wetting angles [70]. The most used is a set of three liquids of which one is a polar liquid with high surface tension and the other two are polar liquids with differences in the quantities of γ_i^{LW} , γ_i^+ and γ_i^- . The most often the following liquids are used: diiodomethane ($\gamma_i = 50$, 8 mJ/m²) or 1-bromonaphthalene ($\gamma_i = 44.4$ mJ/m²) with formaldehyde and water as polar liquids [71].

The other approach was proposed by Chibowski [51 – 53] and is based on the contact angle hysteresis (CAH). It relates γ_s to the surface tension of the probe liquid γ_L and CAH, which is defined as the difference between the advancing θ_a and receding θ_r contact angles:

$$\gamma_s = \left(\frac{\gamma_L(1 + \cos \theta_a)^2}{(2 + \cos \theta_a + \cos \theta_r)} \right) \quad \text{Eq. 4}$$

where γ_s is apparent surface free energy, γ_L is liquid surface tension, θ_a is advancing contact angle, and θ_r is receding contact angle.

The apparent surface free energy can be calculated from Eq. 4 using the liquid surface tension and advancing and receding contact angles. The calculated free energy value depends on the physicochemical properties of the used liquid. For the equilibrium contact angles used for calculation of apparent surface free energy, Eq. 4 transforms into:

$$\gamma_s = \frac{\gamma_L}{2} (1 + \cos \theta_{Eq}) \quad \text{Eq. 5}$$

where γ_L is liquid surface tension and θ_{Eq} is equilibrium contact angle.

Changes in the gel surface topography causes the changes in the wettability and the differences in the value of apparent surface free energy. For the hydrophilic surface the wettability can be described by the Wenzel model and for hydrophobic surface by the Cassie-Baxter model [72]. Nature of structure of gels can be explained by contact angle measurements. Białopiotrowicz [75] concluded that starch gel surface maintains maximal hydrophobic character with polar domains created by the functional glucose groups with the branched chain of amylopectin directed into air [76].

Tomczyńska-Mleko *et al.* [61] applied contact angles measurements to characterize surface properties of the aerated whey protein gels. It was reported that with increasing the concentration of the MgCl₂, the surface became more hydrophobic. Increasing the concentration of the FeCl₂ had a contrary effect: the surface became hydrophilic. So it was found that the obtained surfaces were influenced not only by the type of the added salt but also by their concentration. The pendant drop method was applied to determine the contact angle of whey protein gels. Moreover, the surface free energy was determined by hysteresis (CAH) and acid-base (LWAB) approaches in order to have more info about energetic changes on the surface. In CAH approach a decrease of SFE was observed with the addition of MgCl₂, however with the addition of FeCl₂ an increase of SFE was found. In the other approach (LWAB) the SFE was confined only to the dispersion components which increased after MgCl₂ addition, however a significant decrease of electron donor parameter was found. They conclude that the difference in SFE is caused by the change in surface topography. In a recent publication by Terpiłowski *et al.* [69] the contact angle values were assessed to determine the surface wettability behavior (hydrophobicity/hydrophilicity) of the ion-induced whey protein gels deposited on plasma activated glass support. For increased contact angles, an increase in surface roughness was observed. This relationship depended on the type of ion used for the gelation induction. Besides, wettability properties were affected by the electron donor parameter of energy obtained using (LWAB) approach and contact angles measurements, which value increased for the samples obtained on the plasma activated supports, especially the air treated ones. Kokoszka *et al.* [77] studied the wetting properties of whey protein isolate/glycerol mixed gels influenced by varying their proportions. It was found that the surface with higher plasticizer content showed more hydrophilic behavior. They concluded that the presence of the plasticizer decreased the protein-protein interaction and increased the chain mobility improving the water absorption. Similar results were obtained by Ramos *et al.* [78], but in this case for the whey protein concentrate/glycerol mixed gels, once again, the surface became more hydrophilic after the addition of glycerol. These results are consistent with the claim by Sobral *et al.* [79], who reported that increasing concentrations of glycerol facilitate water absorption and transport within the films.

Conclusion

Modification of surface properties of whey protein gels can transform this product to a new level of application. It could be used as a matrix for an

active ingredient release, material for tissue engineering, e.g. scaffolds, i.e. temporary structures biodegraded in the human organism. Some traditional techniques, like microscopy are used for investigation of protein gels for many years, but other methods, like optical profilometer and contact angle measurements reveal other very important properties of whey protein gels.

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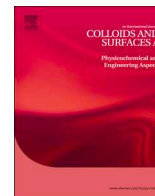
Appendix 2

Time-based changes in surface properties of poly(ethylene terephthalate) activated with air and argon-plasma treatments (second paper of the “compendium”)



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Time-based changes in surface properties of poly(ethylene terephthalate) activated with air and argon-plasma treatments

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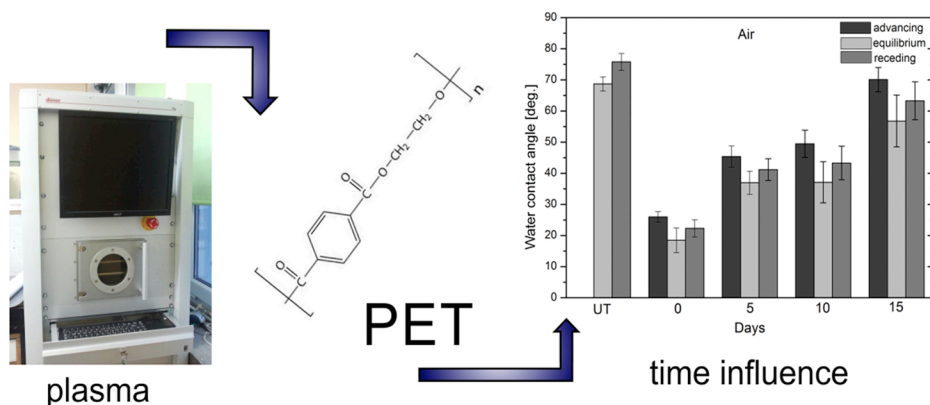
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GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Poly(ethylene terephthalate)
PET
Plasma
Surface free energy
Contact angles
Wetting

ABSTRACT

This paper analyses the effects of time storage on the surface properties of plasma-treated poly(ethylene terephthalate) (PET) plates. For this purpose, optical profilometry-surface topography and surface composition (XPS technique) of the samples were studied before and after plasma treatment. Contact angles were measured using water, ethylene glycol and diiodomethane as liquid probes. Tadmor's theory was used to calculate the equilibrium contact angles. Apparent surface free energy (SFE) was determined using two approaches, the hysteresis CAH approach and the acid-base LWAB. The results showed a significant increase in wettability after plasma treatment, especially in the case of argon-plasma activation. Surface roughness and the polar component of the SFE increased after activation, while the nonpolar component of the SFE did not change. It can be concluded that PET surface roughness is increased by air-plasma activation due to the polar groups originated by the treatment. The surface is not stable during storage time and becomes more hydrophobic because of surface layer reorganization. In the case of argon-plasma activation, the effects are stable over time, which was discussed using two different apparent surface free energy calculation approaches.

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<https://doi.org/10.1016/j.colsurfa.2018.08.026>

Received 23 May 2018; Received in revised form 4 August 2018; Accepted 9 August 2018

Available online 11 August 2018

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1. Introduction

Poly(ethylene terephthalate) (PET) is now one of the most recommended polymers for engineering purposes, due to its excellent properties (i.e. transparency, mechanical strength, gas permeability and chemical resistance) [1], which are useful in many areas of technological interest. Unluckily, PET is not always suitable due to its low surface free energy, which affects its wettability and thus any process involving surface interactions, as in printability, biocompatibility and adhesion [2–4]. This is an important issue, bearing in mind the role played by the wetting behaviour of polymers in a wide range of potential applications, not only industrial (e.g., nanoparticles grafting), but also biomedical areas (e.g., tissue engineering) [5–14]. The key role of wettability has been highlighted in the literature dealing with these topics. Thus, Rimpelova et al. [7] show the need of significantly increasing the hydrophilicity of polyethylene plates as a requisite to improve the adhesion and growth of fibroblasts. Other papers (see Kasálková et al [9]) endorse the potential benefit of grafting of nanoparticles onto the activated polymers.

To overcome the problem brought to the fore above, the surface energy content of any polymer, PET plates included, needs to be increased without changing other bulk properties of the material. A myriad of scholarly papers has tried to improve wettability of a number of mainly hydrophobic polymers [15–17]. In addition to current methods of surface modification, as chemical, thermal, mechanical or electrical treatments [18,19], plasma treatment has become a popular method with an advantage over the traditional wet chemical procedures. Apart from being an environmentally friendly technique because it neither needs water nor chemicals [9], plasma treatment is also a very versatile working method that can be used with polymer surfaces of different nature and shape [20–26]. The efficiency of plasmas is based on the ability of active species such as ions, electrons, atoms or free radicals to induce chemical and physical modifications on the surface [27]. The so-called cold, or low-temperature plasmas, have been found to be most suitable to modify surface properties of polymers of interest in industry, such as adhesivity, hydrophobicity, oleophobicity and hydrophilicity [28,29]. Although the success of oxygen plasma is obvious [23–25,30,31], air and noble gases as well as plasma of different gases and vapours like O₂, N₂, NH₃, H₂O, CO₂ [27,32–34] can also be used. The mechanisms of surface modification after plasma treatment differ depending on the type of gas [35,36]. In case of noble gases, they do not incorporate new chemical species on the surface, and the main mechanisms are selective bond breakage and possible desorption of short chain species. During the treatment with gases other than noble gases, the predominant mechanism is functionalization (i.e., surface formation of new chemical groups) [37]. In any case, it is known that surface modifications induced on the plates after plasma activation are not sustainable over time and may change during sample storage. Wetting properties undergo important modifications, manifested by changes in surface free energy, which, in turn, determine the values of the contact angles of liquid drops placed onto the surface [38–40]. Ageing after plasma activation is also related to the undesirable effect of the so-called hydrophobic recovery of polymers. This makes the study of the ageing effect on the surface properties of plasma-pretreated polymer samples an issue open for discussion. Morent et al [41] found, that the effect of film storage time was the smallest for the argon-plasma treated polymers and the largest for the air plasma. It was explained by the highest crosslinking effect caused by argon plasma treatment. There is no paper in the literature which would explain this problem by changes in surface free energy using two different calculation approaches. So, the novelty of this paper lies in its methodology: both advancing and receding contact angle of probe liquids are measured and applied to calculate surface free energy by means of two different approaches; they explain the specific role played by the different plasma gases used for treatment of PET plates.

The present study aims to understand the effects of ageing of PET

plates, previously activated with air- and argon- low-temperature plasmas, on their roughness and surface free energy. For this purpose, we have carried out experimental measurements of contact angles of selected liquids, XPS analysis of surface chemical composition and optical surface-profilometry. The findings are discussed in the light of recent theoretical approaches.

2. Experimental and theoretical approaches

2.1. PET samples

We used 0.5 mm thick commercial PET (Axpert Standard, Bayer AG) plates. The material was cut into 20 mm × 20 mm pieces. Before the experiments, the samples were washed in ethanol and distilled water to remove their surface contamination, and then dried at room temperature into a desiccator.

2.2. Plasma treatment

Plasma activation of PET samples was carried out in a low pressure plasma system Pico from Diener Electronic, Germany. The untreated polymer plates (UT) were placed in the apparatus chamber at a pressure of 0.2 mbar. The gas flow was set at 22 sccm (standard cubic centimetres per minute). The plates were treated by the plasma for 60 s (convention used, PT-treatment time, PT-60). The plasma power was 400 W.

2.3. Contact angle measurements

Advancing and receding contact angles of the probe liquids onto PET surfaces were measured using a contact angle meter (GBX, France), equipped with a measuring chamber provided with temperature and humidity control, and a digital camera. The measurements were conducted at 20 °C and 50% relative humidity. A 6 μL droplet from a syringe was gently settled on the sample surface by means of an automatic deposition system. The advancing contact angle was evaluated from the droplet shape by the computer program Win Drop ++. Then, 2 μL of the droplet volume was sucked into the syringe and the receding contact angle was calculated by the above mentioned program. The advancing and receding contact angles were measured for up to 10 droplets of each probe liquid [42]. Three liquids of known surface energy, distilled water (W), ethylene glycol (G) and diiodomethane (D) were selected to cover the range from very polar liquid (water) to non-polar liquid (diiodomethane). The values of the contact angles used in this paper are the average of at least 10 measured values at different places on the surface of each sample.

2.4. Apparent surface free energy (SFE) determination

Equilibrium contact angles were calculated from measurements of advancing and receding contact angles [43,44] on the PET plates. For this purpose, Eqs. (1a)–(1c) from Tadmor theory were used [45–47].

$$\Gamma_a \equiv \left(\frac{\sin^3 \theta_a}{(2-3\cos\theta_a + \cos^3\theta_a)} \right)^{1/3} \quad (1a)$$

$$\Gamma_r \equiv \left(\frac{\sin^3 \theta_r}{(2-3\cos\theta_r + \cos^3\theta_r)} \right)^{1/3} \quad (1b)$$

$$\theta_{Eq} = \arccos \left(\frac{\Gamma_a \cos \theta_a + \Gamma_r \cos \theta_r}{\Gamma_a + \Gamma_r} \right) \quad (1c)$$

Γ_a and Γ_r are, respectively, the advancing and receding angle weight coefficients [48].

The SFE of solid surface, γ_s , was calculated using two different approaches. The first one is due to van Oss et al. (LWAB) [49–52], and

γ_s , is determined from a series of measurements of advancing contact angles with three selected probe liquids of known characteristics. The following equation is then applied:

$$\gamma_l(1 + \cos\theta_a) = 2\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + 2\sqrt{\gamma_s^+\gamma_l^-} + 2\sqrt{\gamma_s^-\gamma_l^+}$$

γ_l is the liquid surface tension; θ_a , the (advancing) contact angle; $\gamma_{s/l}^{LW}$, the Lifshitz van der Waals component of solid/liquid free energy of interaction; $\gamma_{s/l}^+$ and $\gamma_{s/l}^-$, are the electron-acceptor and the electron-donor parameters, respectively, of the acid-base component of solid/liquid free energy of interaction.

Another approach used in this research is the one proposed by Chibowski [53–55], based on the contact angle hysteresis (CAH). The equation used:

$$\gamma_s = \left(\frac{\gamma_L(1 + \cos\theta_a)^2}{(2 + \cos\theta_a + \cos\theta_r)} \right) \quad (3)$$

allows the determination of the apparent surface free energy [55], namely γ_s , where γ_L is the liquid surface tension; and θ_a and θ_r are the advancing and receding contact angles, respectively.

When the equilibrium contact angles (i.e., θ_{Eq}) are used for calculation of the apparent SFE, Eq. (3) is transformed into Eq. (4) [55]:

$$\gamma_s = \frac{\gamma_L}{2}(1 + \cos\theta_{Eq}) \quad (4)$$

2.5. Topography investigations

Surface roughness of the untreated, and air- and argon-plasma-treated PET surfaces was investigated using an optical profilometer (Contour GT-K1, Veeco). The optical system allows the characterization of surface topography with a very high accuracy in the range of sizes from the sub-nanometer to the 10 mm. The experiments were done on three different samples (untreated, immediately plasma-treated and after two-week ageing of polymer) and repeated 3 times for each sample. The following surface roughness parameters were determined: average roughness (R_a), root-mean-squared roughness (R_{RMS} or R_q), and peak-to-valley difference (R_t). All surface topography parameters were calculated over the entire measured array and they jointly characterize the roughness of the surfaces in a quantitative way. Vision software (Veeco) [55–57] was used for the analysis of the data.

2.6. XPS (X-Ray photoelectron spectroscopy)

XPS spectra of PET polymer samples were obtained by a multi chamber UHV analytical system. The system, equipped with a monochromatic hemispheric analyser (R4000; VG Scienta), was used under UHV conditions in the analytic chamber, with vacuum at the 5×10^{-9} mbar level. The Casa XPS program was used for the XPS spectra data processing and the quantitative analysis of the surface properties of the examined samples.

3. Results and discussion

3.1. Contact angles

The changes in wetting properties of the PET surface were monitored before and after air- and argon-plasma treatment by means of measurements of advancing and receding contact angles of the three probe liquids above mentioned. After plasma activation, the PET samples were stored into a desiccator and the ageing effect was studied by measuring the contact angles after 5, 10 and 15 days of storage time. Equilibrium contact angles, as defined by Eq. (4), were calculated for their use in the discussion of the hysteresis approach.

The untreated PET surfaces showed advancing water contact angles of the order of $83.5^\circ \pm 3.3^\circ$, which are in agreement with those

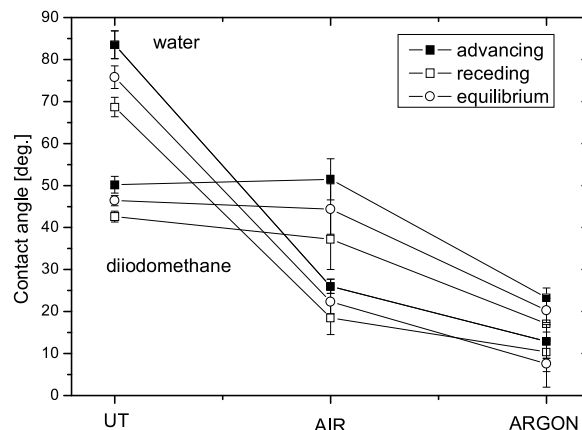


Fig. 1. Water and diiodomethane advancing, receding and equilibrium contact angles measured on the PET plates treated with air and argon plasma.

reported by Yang et al. [58]. Air-plasma activation induced changes on the PET samples which manifested by a huge decrease of advancing water contact angles (measured immediately, after 1 min air-plasma treatment) up to values of $26.0^\circ \pm 1.7^\circ$ (Fig. 1). The same effect was found for ethylene glycol, although less pronounced (not shown here). Thus, as reported for other polymer surfaces [26,59,60], it is observed that also in the case of PET samples, the activation by air-plasma seems to induce appearance of surface polar groups that made them more hydrophilic. The values of the water contact angle prior to and after air-plasma treatment at various storage times are presented in Fig. 2. There is an increase in the contact angles with the storage time, going from the initial $26.0^\circ \pm 1.7^\circ$, to $45.4^\circ \pm 3.4^\circ$ (after 5 days), $52.1^\circ \pm 3.2^\circ$ (after 10 days), and almost the initial value, $72.8^\circ \pm 1.9^\circ$ (after 15 days), which seems to mean a loss of the activation efficiency. Similar trend of changes of contact angles for the other polar liquid (ethylene glycol) was also found. These findings demonstrate that the air-plasma PET surface activation causes an increase in its surface polarity. This increase could indicate the presence of induced oxidized functional groups. Nevertheless, the surface, modified by plasma, is clearly not stable. The rate of change of contact angles is, as mentioned above, greater during the first few days of ageing, while after about 15 days the surface gets stabilized (Fig. 2). The tendency to go back to the initial conditions of contact angle values, and thus to surface hydrophilicity, is an indication that, during sample storage, the surface layer is deactivated; one plausible explanation is that there are possible reaction of surface groups with atmospheric molecules and further molecular

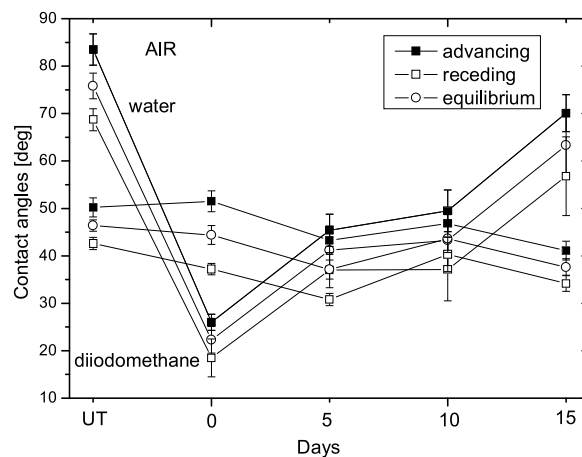


Fig. 2. Water and diiodomethane advancing, receding and equilibrium contact angles measure on the PET plates treated with air plasma in relation to the storage time.

reorganization inside the surface layer [61,62]. Vandecasteele and Reniers [63] explain that, during the storage time of “plasma modified polymer surfaces” under air atmosphere, the polar groups that were formed on the surface of the material out of activation slowly disappeared in the sample bulk.

In the case of nonpolar diiodomethane as probe liquid, the advancing contact angle on PET samples remained unchanged after air-plasma treatment (Fig. 1). The advancing contact angle is $50.2^\circ \pm 2.0^\circ$ and, after the plasma treatment, $51.5^\circ \pm 4.9^\circ$. As diiodomethane interacts with the surface by only London-dispersive forces, hence the changes in the contact angle after air-plasma treatment are not significant. During the next days after plasma treatment, the dispersion interactions remained almost unchanged over time; the change of diiodomethane contact angle is slight and equals only about $8.9^\circ \pm 2.0^\circ$ (Fig. 2), which indicates the main role of polar interactions for the air-plasma treated PET surface.

For further evaluation of the effects of plasma activation on wetting properties, the PET plates were treated using argon under the same conditions. The application of low temperature argon gas plasma led to a significant reduction of the water contact angle, from the initial value of untreated sample $83.5^\circ \pm 3.3^\circ$, to $12.9^\circ \pm 4^\circ$ (see Fig. 1). The reduction is greater as compared to the effect of air-plasma activation, which indicates stronger interactions of this probe liquid with the PET surface. For PET modified with argon-plasma, a loss of activation efficiency was already observed after 5 days of ageing the (advancing) water contact angle increased from the initial (after plasma treatment) 13° to about 44° , and then remained stable; the water contact angle did not show any tendency to reach the initial value of 83.5° ; in other words, the hydrophilic properties remained also beneficial after 15 days. It was thus proved that the working gas has a significant effect on the change in water contact angle during the sample storage. There were obtained similar results with the other polar liquid (ethylene glycol) and, again, the changes during storage time were not significant (no shown).

In the case of diiodomethane with the argon-plasma treatment, the advancing contact angle decreased to $23.3^\circ \pm 2.3^\circ$ (Fig. 1). This is in contrast with the results obtained for the air-plasma treatment under the same conditions. After 5 days, the contact angles reach the initial values and then remain stable (Fig. 3); this suggests that the dispersive interactions become stable after this short period.

If we compare the effects of the different kinds of plasma gas modification, it is clear that argon-plasma treatment makes the greatest changes in surface wettability. In the case of air-plasma treatment, the value of advancing water contact angle is 26.0° ; after argon-plasma activation, it is 12.9° . Similar results were obtained for ethylene glycol.

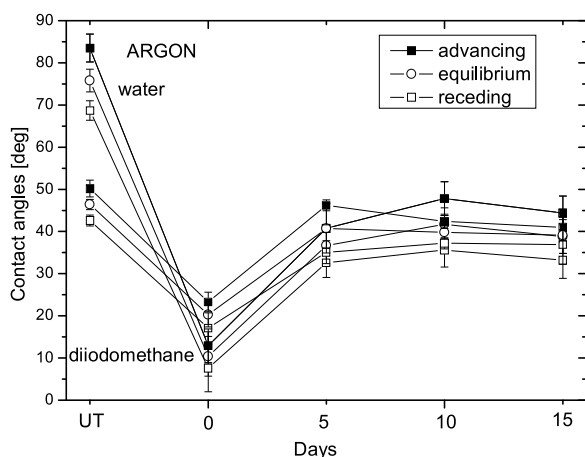


Fig. 3. Water and diiodomethane advancing, receding and equilibrium contact angles measured on the PET plates treated with air plasma in relation to the storage time.

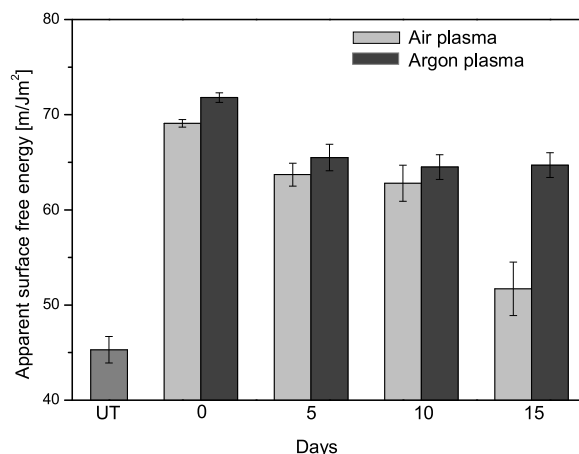


Fig. 4. Surface free energy values based on CAH approach for plasma activated PET plates using an equilibrium water contact angles in relation to the treatment and storage time.

The smallest changes of contact angle values for all the PET samples under analysis were observed for nonpolar diiodomethane (Fig. 1). This proves that the London-dispersion interactions do not change to a large extent [64].

3.2. Apparent surface free energy (SFE)

Measured advancing and receding contact angles were used to calculate the SFE of PET samples treated with air- and argon-plasmas. This was done by using both the SFE components LWAB [49–52] and the contact angle hysteresis (CAH) [53–55] approaches. The analysis of polymeric wetting properties is better done from the values of SFE than from contact angle data, which are the direct consequence of the former quantity. Furthermore, the comparison of the values of the SFE obtained from the two above approaches provides more information about energetic changes occurring on the surface as an effect of plasma used.

Interesting data can be obtained by using only the calculated equilibrium water contact angle and CAH approach. Fig. 4 shows the values of SFE of the PET samples calculated from the CAH approach expressed as the equilibrium contact angles of water obtained using the Tadmor's [45,46] derivations. It was found that the largest change in the SFE resulting from activation of the PET surface layer was obtained with argon-plasma. SFE increased from 45.3 mJ/m^2 to 71.2 mJ/m^2 after the argon treatment, and to 69.8 mJ/m^2 in the case of air. It can be clearly seen that the exposure of PET surface to the air/argon-plasma caused a decrease in water contact angle as a result of the increase in the SFE given by the CAH approach.

Regardless of ageing time, the total SFE shows different trends during the studied period of ageing. In the case of air-plasma treatment, the largest reduction was observed; after 15 days of storage, the SFE decreased from 69.8 mJ/m^2 to 52.7 mJ/m^2 , which is closer to the untreated value. Interpretation of effects of ageing after plasma activation must take into account both the extent of the increase of surface energy after plasma treatment, and the nature of the molecules in contact with the surface during the ageing process. Obviously, activation by plasma treatment creates active sites on the surface that depend on the nature of the gas used in the plasma. These sites, in turn, will interact with the air of the storage place and deactivate the surface at different rates; this will depend on the type of the functional groups created during the activation process as well as the ability of the resulting surface groups to rearrange themselves into a more stable configuration [65]. In particular, the polar groups created after the air-plasma treatment may play a key role in determining post-treatment increased hydrophilicity of the polymer surface. As shown in Fig. 4, given the high reactivity of these groups against air molecules, this effect is not permanent and

gradually disappears with storage time. Argon-plasma treatment, however, causes a different activation mechanism other than air-plasma treatment; permanent surface alteration is provoked by polymer chain-breaking induced by argon activation. Thus, the value of SFE of about 65 mJ/m^2 after 15 days of storage is quite close to the initial value after argon-plasma treatment (Fig. 4).

As it can be seen in Fig. 6, the average total SFEs obtained by CAH and LWAB approaches are comparable, which indicates that these methods are complementary. It is worth mentioning that the total SFE calculated from the CAH approach in this case is expressed as an arithmetic mean of the values assessed separately from the contact angles hysteresis of water, ethylene glycol and diiodomethane. By comparing those approaches (Fig. 6), it was found that the largest change in the SFE was obtained with argon activation in the case of the CAH approach. The SFE of the untreated PET surface is $39.4 \pm 0.9 \text{ mJ/m}^2$ (CAH) and $36.1 \pm 0.7 \text{ mJ/m}^2$ (LWAB). Using the CAH approach, the calculated values after activation were $50.0 \pm 0.8 \text{ mJ/m}^2$ (air) and $55.9 \pm 0.4 \text{ mJ/m}^2$ (argon); using the LWAB approach, the results were $34.4 \pm 1.7 \text{ mJ/m}^2$ and $46.7 \pm 0.8 \text{ mJ/m}^2$. Comparing the initial values with those after the plasma treatment, using LWAB, almost no change was observed in the case of air-plasma.

Useful information about time dependency of SFE can be obtained from the values of the estimated electron-donor parameter of the SFE acid-base component, γ_s^- [54]. Fig. 5 shows a large increase of this parameter after plasma activation, more than threefold, from $20.6 \pm 1.8 \text{ mJ/m}^2$ to $69 \pm 0.9 \text{ mJ/m}^2$, in the case of air-plasma, and to $64 \pm 0.8 \text{ mJ/m}^2$, in case of argon-plasma treatment. Those values suffer an important decrease with the storage time, although after a period of observation of 15 days, one can conclude that PET samples activated by air-plasma recover the values prior to the treatment, which indicates an almost complete deactivation of the polymer surface. Contrary to this, samples activated by argon-plasma show a moderate decrease of γ_s^- , which is maintained in a value close to 50 mJ/m^2 . This behaviour shows again, as discussed above, that ageing affects in a very different way PET samples treated by argon- or air-plasma. In the first case, the surface remains activated after plasma treatment and later storage for moderate time. In the second case, the nature of the active sites on the surface allows the deactivation of the surface after its exposure to atmosphere. In some way, γ_s^- measures the extent of oxygen-containing moieties in the surface after plasma treatment; plasma treatment increases the number of polar groups (Fig. 5), thus the SFE also increases (Fig. 6) and the surface becomes more hydrophilic (Figs. 2 and 3).

Based on the above considerations, we can conclude that the changes of the SFE of samples result from a series of surface chemical changes induced by plasma treatment, which become modulated by the interactions with the atmospheric molecules during storage. There are

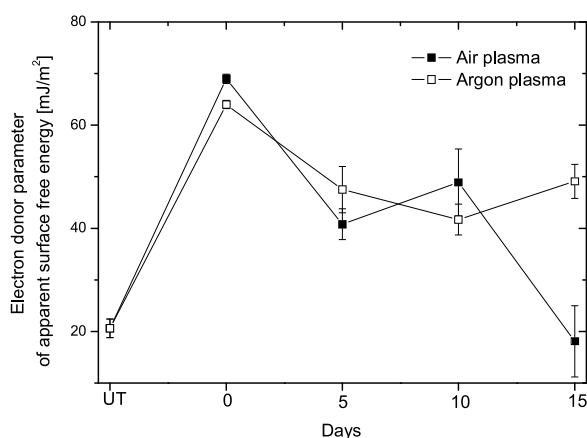


Fig. 5. Electron donor parameter of apparent free energy of air and argon plasma activated samples in relation to the treatment and storage time.

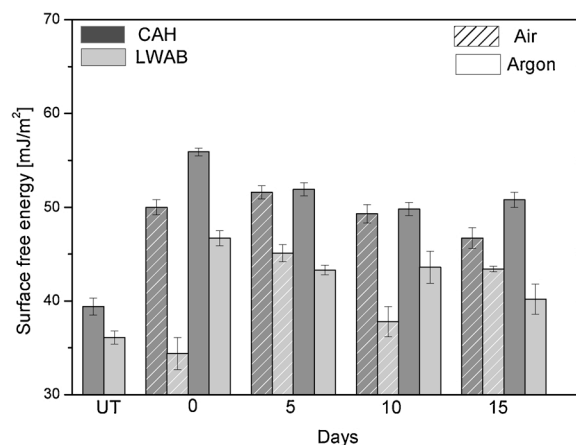


Fig. 6. Surface free energy calculated using an equilibrium contact angles for PET samples based on the CAH and LWAB approaches in relation to the treatment and storage time.

two immediate consequences: surface topography as well as surface chemical composition should reflect these changes. Below we will pay attention to both phenomena.

3.3. Surface roughness

Plasma activation usually results in an increase in roughness in every treatment [65]. R_{RMS} (R_q), or root mean square index, is the preferred parameter to compare roughness of the samples, because it is insensitive to the local topographical heterogeneity of the surface. Other parameters, cited above, are average roughness (R_a) and peak-to-valley difference (R_t). Table 1 displays the values of surface roughness parameters for untreated samples of PET as well as for samples immediately plasma-treated and after two weeks of activation. The general rule is that the surfaces become rougher with any plasma treatment. In the case of air-plasma, the average roughness (R_{RMS}) for the untreated surface is $1000.7 \pm 101.4 \text{ nm}$; after plasma treatment, this rises to $1060.54 \pm 143.5 \text{ nm}$. A similar increase is observed in the case of R_a and R_t values (Table 1). Significant changes in roughness were also observed by Jorda-Vilaplana et al. [66], who used the same plasma treatment but for a different material, i.e., polylactic acid (PLA). The activation of our PET samples with argon-plasma resulted in increased surface roughness. The increase of all surface roughness parameters after argon-plasma treatment was significantly higher than after air-plasma treatment (Table 1). A similar pattern was found with contact angles, where argon-plasma activation induced the most significant changes (see Fig. 1). The huge increase in roughness in the case of argon treatment can be clearly seen from the R_{RMS} value, from $1000.7 \pm 101.4 \text{ nm}$ in untreated samples to $1328 \pm 150 \text{ nm}$ in treated samples. In both plasma treatment cases the results of the

Table 1
Topography parameters of the PET surface, before and after plasma modification.

Parameters	Untreated	Air-plasma, immediate	Air-plasma 2 weeks ageing
R_a (nm)	827.7 ± 61.4	855.9 ± 122.9	810.8 ± 61.4
R_{RMS} (nm)	1000.7 ± 101.4	1060.5 ± 143.5	1050.7 ± 101.4
R_t (nm)	1560.4 ± 242.2	1685.0 ± 109.7	1724.3 ± 246.2
Parameters	Untreated	Argon-plasma, immediate	Argon-plasma 2 weeks ageing
R_a (nm)	827.7 ± 61.4	1000.0 ± 119.19	1260.0 ± 61.4
R_{RMS} (nm)	1000.7 ± 101.4	1328.0 ± 150.2	1780.7 ± 101.4
R_t (nm)	1560.4 ± 242.2	1940.8 ± 109.7	2416.4 ± 246.2

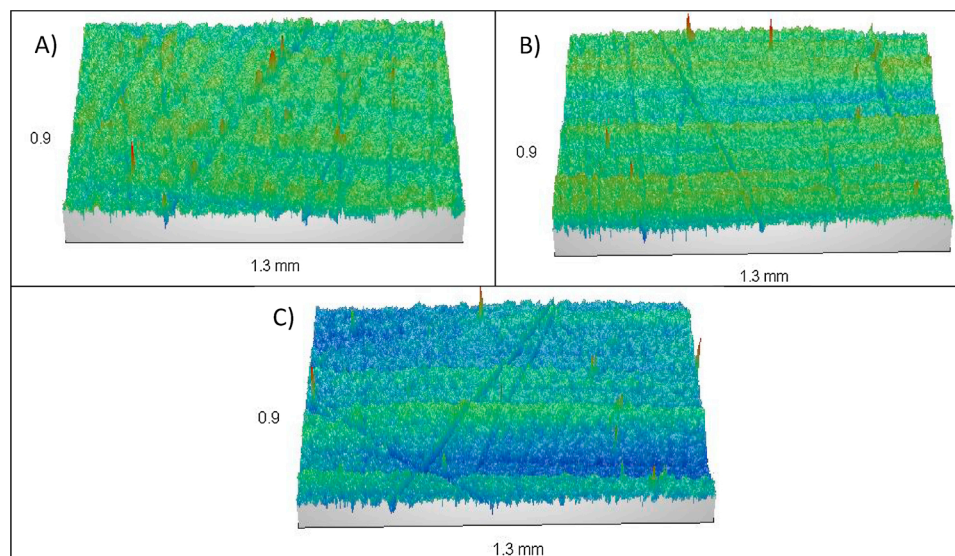


Fig. 7. Surface topography from optical profilometry for a) untreated, b) air treated and c) argon treated PET samples.

surface roughness parameters after 2 weeks showed a different tendency (Table 1). After 2 weeks, R_a is 810 nm in the case of air-plasma treatment, which is very close to the initial values; however, in the case of argon-plasma treatment, the R_a remains almost the same as the values after treatment, which is 1260 nm. Regarding storage time effect, this behaviour is in agreement with what happens with contact angles, SFE and electron-donor parameters. To get a better understanding of the surface structures, Fig. 7 shows the images obtained by using optical profilometry (the size of the surface is $0.94 \times 1.3 \text{ mm}^2$). Higher magnification images were taken from the profilometer. Air/argon-plasma activation of the PET surface results in increased surface roughness; furthermore, the untreated surface on PET exhibits a more homogeneous structure in comparison with the plasma-treated PET surface, which is non-uniform.

3.4. XPS analysis

X-ray photoelectron spectroscopy (XPS) analysis is the usual technique to probe for the identification of chemical grafted groups. XPS measurements were conducted to gain insight into the changes in the chemical composition of treated and untreated PET surfaces. It is important to note that, during the XPS analysis, only a 3–6 nm depth was analysed and the results were the mean of the information collected from this thin surface layer of the material [38]. The results obtained from the survey scan XPS spectra are given in Table 2. The survey spectra show that the elements identified are carbon (C1s), oxygen (O1s) and nitrogen (N1s). Table 2 also provides the percentage

Table 2

Elemental surface chemical compositions (expressed in percentages) of non-treated and plasma-treated PET samples, as calculated from the XPS survey spectra.

Component	UNTREATED	AIR, immediate	AIR 2 weeks ageing
C	79.7	70.3	71.0
O	19.1	28.1	26.8
N	1.2	1.6	2.2
Component	UNTREATED	ARGON, immediate	Argon 2 weeks ageing
C	79.7	67.3	61.8
O	19.1	30.0	29.3
N	1.2	2.7	2.6

contributions of carbon and oxygen atoms on the PET surface. The content of carbon atoms decreases just after the air-plasma treatment from 77.9% to 70.3%, whereas the percentage contribution of oxygen increases significantly from 19.1% up to 28.1%. After plasma treatment, some nitrogen atoms appear. The ratio of oxygen to carbon concentration increases considerably from 0.24 to 0.4 as a result of oxygen increase after plasma treatment [67,68]. PET plasma treatment generates polymer chain-breaking for some of the polymer bonds, which is proved by the decrease in the percentage of C content. On the basis of the results, we can infer that the air-plasma surface treatment changes the content of oxygen moieties, which happen to be responsible for the changes of the surface wetting properties (see above). In the case of argon treatment, the content of carbon atoms decreases to 67.3% and the oxygen amount increases to 30%. In this case, the ratio of oxygen to carbon concentration rose to 0.45; compared with the air treatment, this is much greater.

Table 3 shows the amounts of differently bonded carbon atoms obtained from the deconvolution of the C1s spectrum; this reveals the changes induced by plasma treatment of the surface compositions. The component C–C decreased from 75.7 to 69.5 after air-plasma treatment and, at the same time, C=O appeared, and the oxygen containing polar groups C–O and O=C–O increased (Table 3, Fig. 8). These results show that C–C/C–H bonds decrease significantly after plasma

Table 3

Concentration of the different chemical bonds in PET surface, for untreated (UT) and plasma-treated samples, at different storage times.

Component	Structure	Concentration on the surface [%]				
		UT	AIR, immediate	AIR 2 weeks ageing	Argon	Argon 2 weeks ageing
C	C1+C2	75.7	69.5	67.1	61.2	63.2
	C3	13.7	13.9	14.6	16.9	15.2
	N1	–	–	–	–	–
	C4	10.6	12.0	12.7	18.2	17.8
	C5	–	4.6	5.6	3.6	3.7
O	O1	47.8	66.5	67.2	61.0	63.3
	O2	52.2	28.8	28.9	37.0	34.8
N	N2+N3	–	32.4	67.0	28.0	22.4
	N4	–	56.6	–	53.9	47.9
	N5	–	–	11.9	–	–
	N6	–	11.0	21.1	18.1	29.7

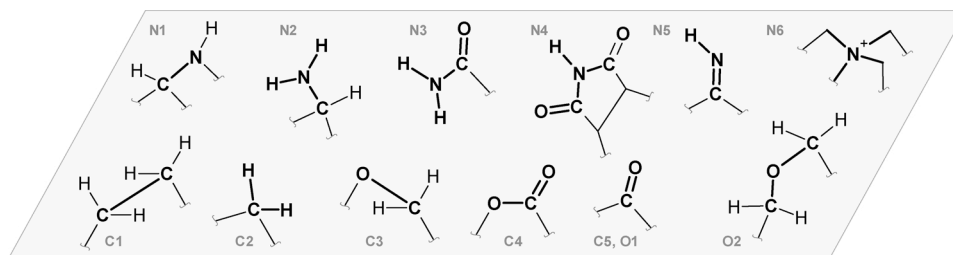


Fig. 8. Schematic illustration of functional groups before and after the plasma activation.

treatment and that the concentration of oxygen- and nitrogen-containing polar groups (i.e. $-\text{OH}$, $\text{C}-\text{O}$, $\text{O}=\text{C}-\text{O}$, $\text{C}=\text{O}$ and $\text{N}-\text{CO}-\text{N}$) increase on the plasma treated polymer surface. Air-plasma can break some of the $\text{C}-\text{C}$ bonds in the polymer surface, which can get recombined with oxygen atoms (Fig. 8). This chemical modification leading to incorporation of functional groups onto the PET surface is mainly responsible for the changes of the surface polarity, which affects the wetting properties of the material.

Argon-plasma activation helps to increase the concentration of $\text{C}-\text{O}$ and $\text{O}-\text{C}=\text{O}$ groups, and decreases the number of $\text{C}-\text{C}$ and $\text{C}-\text{H}$ functional groups (Table 3). As follows from the results, during activation, the surface energy balance is disturbed and, therefore, different kinds of functional groups can be readily attached (Table 3, Fig. 8). The changes are consistent with the previous conclusions drawn from the analysis of contact angles (lower contact angles and increased wettability, see Fig. 1). In addition to the O element, the N element also improves surface wettability by introducing, by the action of the air-plasma, nitrogen-containing functional groups like amine, amide or quaternary nitrogen (Fig. 8). The results also showed that new hydrophilic bonds appeared (Fig. 8) and that some hydrophobic bonds ($\text{C}-\text{C}$) of PET samples were modified during plasma treatment. All these facts resulted in a significant effect on the chemical composition and properties of PET samples.

Finally, the stability of the activated PET surfaces was studied by placing the samples in a desiccator for 2 weeks. After 2 weeks of storage, the carbon content slightly decreased to 71%, in the case of air-plasma treatment; experiments involving the sample storage for fewer than 14 days were not performed. The oxygen content was still slightly higher than the initial oxygen concentration, which remained close to the theoretical value of pure PET (Table 2).

The reported results demonstrate that the oxygen-to-carbon ratio slightly decreases with the time of storage compared with the measurements just after air/argon-plasma treatment.

4. Conclusion

The experimental results obtained by measuring contact angles in PET samples, before and after surface activation with air- and argon-plasmas allowed the calculation of PET surface free energy. A study was then performed to cater for the influence of sample storage time on surface properties. Some important conclusions can be drawn from the comparison of the sequencing of changes of contact angles, topographic images and XPS surface chemical data. After the air/argon-plasma treatment, the polymer surface becomes more hydrophilic and, consequently, the values of contact angles are lower. Additionally, this effect is intensified because of the increased polar interactions of the surface (see polar component of SFE, Fig. 5). When PET surface is treated, its roughness also increases (Table 1). These findings can be interpreted not only by the modifications of surface roughness (plasma etching), but also by the changes of the surface chemical composition (plasma cleaning of the surface and insertion of oxygen containing surface groups). The impact of the activation of PET samples by plasma treatment on wettability and the electron-donor parameter of SFE is due to various post-treatment oxygen species formed on the PET surface.

Regarding the ageing effect on the properties of plasma-treated surfaces, the results show a loss of the initial activation originated by plasma treatment. There is a complete loss in case of air-plasma activation after a period of 15 days. However, when PET is treated with argon plasma, the surface (mainly manifested by the values of its SFE) becomes stable after a short period of time and becomes activated in comparison with its previous, non-treated, state. The following are some of the mechanisms involved in the process of activation and deactivation of PTE surface during air/argon-plasma treatment and further ageing in open atmosphere: Appearance of functional groups involving the C, O and N atoms; reorientation of polar groups of the surface; or diffusion of those groups into the bulk, reaction of free radicals arising from the activated surface with the molecules of the air. They all jointly explain the surface properties studied in this paper.

Acknowledgements

The research was carried out with the equipment purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Programme (contract no. POIG.02.01.00-06-024/09 Centre for Functional Nanomaterials).

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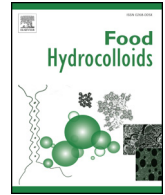
Appendix 3

Surface and rheological properties of egg white albumin/gelatine dispersions gelled on cold plasma-activated glass (third paper of the “compendium”)



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Surface and rheological properties of egg white albumin/gelatin dispersions gelled on cold plasma-activated glass

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ARTICLE INFO

Keywords:

Cold plasma

Wettability

Roughness

Rheology

Egg white protein

Gelatin

ABSTRACT

Thin layers of egg white albumin/gelatin gel have been formed on glass plates previously treated by cold plasma, using oxygen, air and argon gases. After gelation, the gels were detached from the support and the inner surface previously attached to the plate was investigated. Water contact angles were measured on the detached films and their wettability studied in terms of surface free energy changes induced by the contact with the glass support treated with different gases cold plasma. The effect of increasing concentration of gelatin in the gel was also studied. Pre-treatment with oxygen plasma manifested the most significant changes of wetting properties of the films. A novel method was applied to obtain samples with the same thickness and the viscoelastic properties were measured. As expected, increasing gelatin concentration caused the formation of stronger (higher G^*) gels. Gels formed on oxygen plasma treated glass support were the strongest. Optical profilometry was used for the characterization of the gels surface topography. It was found that the surfaces become rougher with the plasma treatment of glass support, no regard which gas was used. Increased roughness of the gels caused a decrease in the contact angles. This paper shows, for the first time in the literature, that even when the gel is not treated directly by cold plasma, but it is obtained on pretreated support, it is an effective method to change its surface and rheological properties.

1. Introduction

Gelatin is one of the most popular gelling agent widely used in food, biomedical or pharmaceutical industries. It is a protein obtained by hydrolysis from collagen connective tissue of muscles, skin and bones of animals. Gelling temperature of gelatin is about 40 °C. The formed gels are stabilized by hydrogen and electrostatic bonds, with additional hydrophobic interactions (Tadeo, Lopez-Mendez, Castano, Trigueros, & Millet, 2009). Some of its most attractive properties are biodegradability, excellent biocompatibility, non-antigenicity, plasticity and adhesiveness together with the ability to detach the gel from the support (Pieracci, Crivello, & Belfort, 1999). Hence, this biopolymer was chosen as a proper candidate to be mixed with another protein to build the desired mixed gels. Egg white proteins, in the form of boiled eggs, are the most known heat-induced gels. Egg white protein is treated as nutritional standard of protein with excellent amino acids composition

(Nyemb, Guérin, Pézenec, Jardin, Briard-Bion, Cauty, 2016). Egg white is composed of different proteins: ovalbumin (54%), ovotransferrin (12%) and lysozyme (3.5%). All of them can be transformed into gels via heating process. The most important interactions in heat-induced gelation process of egg white proteins are: electrostatic, hydrophobic and formation of disulfide bonds (Gu, Su, Zhang, Zheng, Zhang, McClements, 2017). Egg white albumen has been used as matrix for different active ingredients release (Somchue, Sermrsri, Shiowatana, & Siripinyanond, 2009; Su et al., 2015). Mixed gels of egg white albumen with other hydrocolloids created practically unlimited possibilities for gels with different textures, viscoelasticity and surface properties (Hu, Liang, Xu, Wang, An, Yan, 2016; Liu, Zhu, Ye, Wan, Wang, Wang, 2013; Su et al., 2015; van den Berg, Jara, Pilosof, & Renata, 2015). Badii and Howell (2006) found, that a mixture of gelatin and egg albumen (3:10) gave greater storage modulus value than expected from just adding the values from the singular gels. In such techniques as

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Received 18 December 2018; Received in revised form 26 March 2019; Accepted 17 May 2019

Available online 19 May 2019

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phase contrast microscopy and differential scanning calorimetry they showed interactions between egg white albumen and gelatin.

Cold plasma may refer to a kind of surface “activation”. Depending on the plasma gases and conditions used, some of the most important consequences of the activation are: change in surface properties without affecting the bulk material, organic contaminants removal and the formation of functional groups on the surface (Chan, Ko, & Hiraoka, 1996; Chu, Chen, Wang, & Huang, 2002). These changes result in increased bonding, chemical inertness or adhesion improvement (Yamamoto, Okubo, Imai, & Mori, 2004). Glass is widely used as support in many applications. It has low price, it is amorphous and heat resistant. Furthermore, it is frequently found in contact with the food-stuff, in particular in the packaging industry; so, we decided to choose this material in our study as support for gels forming. Glass has been used previously as a support for biological (Chibowski, Jurak, Hołysz, & Szcześ, 2014), surfactants (Szymczyk & Janczuk, 2008) or superhydrophobic layers (Chibowski, Hołysz, & Terpiłowski, 2006; Hołysz, Terpiłowski, Zarko, & Chibowski, 2013; Terpiłowski, Rymuszka, Goncharuk, Sulym, & Gun'ko, 2015; Terpiłowski, 2015). The influence of cold plasma on glass support has been studied in detail, and it is known that after the activation, the surface becomes “smoother” and its wettability and adhesion properties are deeply modified (Terpiłowski & Rymuszka, 2016). After glass activation, physical and chemical phenomena should occur at the surface of the contacting phases. No research was done so far to show these changes on a gel surface contacting with the pre-activated support. Changes of surface properties of films are usually observed in terms of their wettability. It is very important in many applications, as the contact angle is an indication of hydrophobicity/hydrophilicity character of the film (Adam, 1968, pp. 1–16; Butt, Graf & Kappl, 2013; de Gennes, Brochard-Wyart, & Quere, 2004). The measurement of the advancing and receding contact angles of different probe liquids, allows the calculation of the surface free energy (SFE) using different approaches. In this paper the SFE is determined by contact angles hysteresis (CAH) approach which was developed in our laboratory (Chibowski, 2002, 2005; Chibowski & Terpiłowski, 2009).

The roughness of gel surface can affect the properties of hydrogels, especially in the applications in scaffolds engineering, drug delivery systems and active packaging materials. A rougher surface implies that scaffold disappears faster by hydrolyzing enzymes acting, and faster is the release of drugs and active ingredients in active packaging systems (Tomczyńska-Mleko & Mleko, 2014). Recently, Terpiłowski, Tomczyńska-Mleko, Nishinari, and Mleko (2017) have used glass activated by cold plasma for deposition of ion-induced whey protein gels. The activation of glass support increased not only the roughness but also the hydrophobic properties of the deposited layers. In that study, however, the gel layers were not detached from the support. In the research presented in this paper, a new methodology was used: by a special procedure we obtained thin layers of mixed gels on non-activated and plasma-activated glass plates with exactly the same thickness. It allowed for measurement and comparison of the rheological properties of the gels. After gelation, the gels were detached from the support and the surface properties of the inner surface, previously attached to the plate, were measured. No results were obtained so far for rheological properties of gels formed on surfaces pre-treated with cold plasma. This study aims to elucidate the extent of the changes induced in the surface and viscoelastic properties of egg white isolate/gelatin mixed gels by gelling on plasma activated glass. These modifications can create a new material, with enhanced possibilities for application as a matrix for active ingredients release, scaffolds and active packaging material.

2. Materials and methods

2.1. Materials

Egg white albumen “low mineral, high gel type” (88.1% protein) was a gift from Kewpie Corporation (Tokyo, Japan). Protein concentration was determined by the Kjeldahl method (AOAC, 1995, pp. 7–9). Gelatin from porcine skin (type A) was purchased from Dr. Oetker (Gdańsk, Poland). According to the company specification, it has “platinum strength” with a 250 Bloom and ca. 60,000 mean molecular mass.

2.2. Glass support preparation and plasma treatment

Microscope slides $76 \times 26 \text{ mm}$ were used (Comex, Wrocław, Poland). Before the experiments, glass plates were cleaned by methanol in the ultrasounds bath for 15 min and then rinsed with Milli-Q water and dried at 50°C . After drying the glass plates were kept in the desiccator at room temperature. Glass plates were activated by cold plasma using three different gases: air, argon or oxygen. Plasma activation was performed in the low-pressure plasma system Pico (Diener Electronic, Germany). The plates were placed on the sample shelf and the system was adjusted to a pressure of 0.2 mbar. Then the gas flow (Air Products, Warsaw, Poland) was set at 22 sccm (standard cubic centimeters per minute). The plates were treated with the plasma at the electric voltage 160 V (400 V max) and frequency 13.56 MHz for 60 s. In order to remove the gaseous products, the chamber was purged with air for 10 s.

2.3. Preparation of mixed gels

Egg white protein (EWP) dispersion (3% of protein in all samples) was made by hydrating in distilled water. After that, the dispersions were heated in water bath for 30 min at 80°C and then cooled down immediately. Gelatin solution was added to egg white dispersion to obtain mixtures with 3% egg white albumen and 3%, 4%, 5% and 6% gelatin concentration. After adding the gelatin, solutions were mixed for 30 min at 80°C using an electric heater with magnetic stirring, and some volume of the dispersion was poured on cold plasma activated glass plates leveled on the Petri dishes to obtain a $1 (\pm 0.1) \text{ mm}$ thick layer (it was checked using a micrometer screw gauge). The Petri dishes were leveled using a self-leveling laser IM0201 (Magnusson, Longpont-sur-Orge, France). The plates were stored for 20 h at 7°C . Immediately after removing from the refrigerator, the gels were detached from the glass support, equilibrated at 21°C for 2 h and subjected to determination of their properties. The gels were gently detached by passing a curved-tip metallic spatula around the perimeter of the gels; under used gelatin concentrations, the gels stuck to some extent to the glass, but they were elastic and firm enough to manipulate them without fracture or any other damages.

2.4. Dynamic oscillatory measurements

Viscoelastic properties of the gels were evaluated using a Kinexus Lab + dynamic rheometer (Malvern Instruments Limited, Malvern, U.K.). Rheological properties were investigated using a serrated parallel steel plate geometry (35 mm diameter, 1 mm gap size) to limit the sliding effects. The gel samples (10 mm diameter) were cut using a scalpel. The thickness of the samples was the same (1 mm) due to described previously method to form the gel. Oscillatory measurements with frequency sweeps in the range 0.1–10 Hz were performed. Complex modulus G^* and tangent delta were measured. All the measurements were taken at 0.01 strain, which was in the linear viscoelastic range determined by the strain sweep.

2.5. Contact angle measurements

Advancing and receding contact angles of the probe liquid on the gel surfaces were measured using the contact angle meter GBX (France), equipped with a temperature and humidity-controlled measuring chamber and digital camera. The measurements were conducted at 20 °C and 50% relative humidity and based on the sessile drop method (Kwok & Neumann, 1999) with water. A 6 µL droplet from a syringe was gently settled on the sample surface by means of an automatic deposition system. The advancing contact angle was evaluated from the droplet shape by the computer program Win Drop. Then 2 µL of the droplet volume was sucked into the syringe and the receding contact angle was obtained from the above mentioned program. The advancing and receding contact angles were thus measured for up to 10 droplets.

2.6. Surface free energy determination

Equilibrium contact angles were calculated from measurements of advancing and receding contact angles (Holysz et al., 2013; Terpiłowski, Rymuszka, Holysz & Ilnicki, 2017) on the glass plates. For this purpose, equations (1a) and (1b), and (1c) from Tadmor theory were used (Tadmor, 2004, 2008; Tadmor & Yadav, 2008).

$$\Gamma_a \equiv \left(\frac{\sin^3 \theta_a}{(2 - 3 \cos \theta_a + \cos^3 \theta_a)} \right)^{1/3}; \quad (1a)$$

$$\Gamma_r \equiv \left(\frac{\sin^3 \theta_r}{(2 - 3 \cos \theta_r + \cos^3 \theta_r)} \right)^{1/3}; \quad (1b)$$

$$\theta_0 = \arccos \left(\frac{\Gamma_a \cos \theta_a + \Gamma_r \cos \theta_r}{\Gamma_a + \Gamma_r} \right) \quad (1c)$$

Γ_a and Γ_r are, respectively, the advancing and receding angle weight coefficients (Terpiłowski, 2015), θ_a - the advancing contact angle, θ_r - the receding contact angle and θ_0 - the equilibrium contact angle.

The surface free energy (SFE) of solid surface, γ_s , was calculated using the hysteresis approach proposed by Chibowski (Chibowski, 2002, 2005; Chibowski & Terpiłowski, 2009), based on the contact angle hysteresis (CAH). The equation used:

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

allows the determination of the “apparent surface free energy”, γ_s , with γ_L , the liquid surface tension, θ_a the advancing contact angle, and θ_r the receding contact angle.

When the equilibrium contact angles, θ_{Eq} , are used for calculation of the apparent surface free energy, Eq. (2) transforms into:

$$\gamma_s = \frac{\gamma_L}{2} (1 + \cos \theta_{Eq}) \quad (3)$$

2.7. Gel surface topography

The gel surface was observed using an optical profilometer GT Contour Surface Metrology (Veeco, Tucson, USA). The optical system allows the characterization of surface topography with a very high accuracy in the range of sizes from the sub-nanometer to the 10 mm. Surface roughness was determined using Vision64 (Veeco, Tucon, USA) (Horcas et al., 2007). Optical profilometry uses light instead of a physical sensor. The optical profilometer scans the tested material vertically. The material in the field of view passes through the focal plane, which creates interference. Each level of height in the material under test achieves optimal sharpness at other times. With a calibrated optical profilometer, accuracy below the nanometer is possible.

3. Results and discussion

3.1. Dynamic oscillatory measurements

Fig. 1 A–D show the complex modulus obtained from frequency sweeps for gels with 3% egg white albumen and different gelatin concentration (3, 4, 5 and 6%). G^* increased with the gelatin content in the gel, the effect being more appreciable with the gels formed on cold plasma-activated glass support. Badii and Howell (2006) found, that the addition of gelatin (3% or more) to 10% egg white albumen enhanced both the storage and loss moduli values. Increasing frequency caused an increase in complex modulus. Investigated material was characterized as a physical gel with tangent delta at all measured frequencies in the range 0.16–0.26 (chart not shown). Activation of glass support by cold plasma caused forming stronger gels with higher G^* . The most effective was plasma generated from oxygen, then air and the least from argon. The mechanisms explaining the different behavior of the gels formed on the surface activated by the cold plasma must take into account the type and number of surface functional groups appearing at the surface after its treatment, as they determine the protein adsorption processes and its gelation. As usually in this kind of processes, different interactions are involved: electrostatic, van der Waals, hydrophobic, and covalent. Thus, for example, electrostatic attraction between hydroxyapatite powder and bovine serum albumin (Kollath et al., 2015) was promoted after plasma activation of the surface, which was used to graft functional groups on the support surface. The material used (dry hydroxyapatite powder) facilitated the experiments design allowing to explain the attraction between hydroxyapatite powder and bovine serum. Wiącek, Gozdecka, Jurak, Przykaza, and Terpiłowski (2018) found that air plasma treatment of a glass surface induced stronger interactions of this support with hyaluronic acid and alginate. There, the role played by the –OH groups appearing in the activation process of the support was emphasized and shown to determine the properties of the films deposited on it. Their conclusions were supported by exactly the same experimental methods we used in our surface properties research presented in the following sections, however the thickness of the sample prepared was notably smaller (glass surface covered by 45–300 µm film vs. 1 mm thick gel). Our study shows that not only surface (see next sections) but also the rheological properties are modified. It is very difficult to choose a special experimental design to explain why the changes in the rheological properties of investigated gels were observed. We have planned our experiments based on the nature of the samples. We could not use a high vacuum for our gel samples to carry out the test for the XPS method. When trying to apply IR-ATR or RAMAN techniques, we could not obtain the interpretable test results. Drying the gels would allow to perform some spectral methods, however, the drying itself changes the gel (in fact we don't have a gel anymore) so we would not be able to observe the effect of the plasma. We can only conclude, that because of small thickness of the sample (1 mm) we probably obtained samples, which can be treated as composite materials consisting of a bulk phase with different, modified surface layer.

3.2. Contact angles and wetting behavior

Fig. 2 shows the water advancing contact angle on mixed gels as a function of the gel composition and the plasma gas used for glass activation (vertical bars show standard deviations). It can be seen that the increase of gelatin concentration of mixed gels previously formed on non-plasma activated glass support (“untreated sample” in figure legend of Fig. 2), produces a progressive decrease in water advancing contact angles, going from $109.9^\circ \pm 7.9^\circ$ (3%) to $76.4^\circ \pm 2.7^\circ$ for the sample with highest gelatin content (6%). So, increasing gelatin concentration resulted in a more hydrophilic surface. Similar results were found by Velu, Muruganandam, and Arthanareeswaran (2015) in polyethersulphone (PES) membranes, which hydrophilicity was

increased with the increase of gelatin concentration in their composition. Moreover, it is well known that the physicochemical and functional properties of the gelatin can be specially altered by the amino acid composition. Previous researches (Arnesen & Gildberg, 2007; Gilsenan & Ross-Murphy, 2000) report that the most abundant amino acids in gelatin are glycine and proline (mainly non-polar, hydrophobic surface) and arginine (mostly polar). This set of amino acids should thus determine the extent of the changes of hydrophilicity/hydrophobicity of the gel surface induced by the addition of gelatin. Fig. 3 shows that, by using different gases, the contact of mixed gels with plasma-activated glass has an effect on their wetting properties. It can be seen that such activation resulted in lower contact angles in all gel samples. The oxygen plasma caused the highest changes in the contact angles, increasing in a remarkable way the hydrophilic character of mixed gels. For the gel with 3% gelatin concentration, the contact angle dropped from $109.9^\circ \pm 7.9^\circ$ to $19.9^\circ \pm 5.9^\circ$ in case of oxygen-plasma activation. It is already known (Terpiłowski & Rymuszka, 2016) that the plasma-treated glass surface becomes more hydrophilic after plasma activation due to the increase in the strength of polar interactions. The incorporation of new polar groups by the action of the oxygen treatment of glass surface would make possible the “transfer” of these functional groups to the mixed gel surface formed on the treated support, making it more hydrophilic. In a similar way, the water contact angles of mixed gel (3% gelatin concentration), dropped from $109.9^\circ \pm 7.9^\circ$ to $52.5^\circ \pm 2.4^\circ$ in the case of argon plasma and to $60.6^\circ \pm 4.0^\circ$ in the case of air plasma. Similar trend (within $\pm 5.0^\circ$) as discussed for the 3% gelatin-mixed gel was found for the contact angles measured on surfaces with different gelatin content (4%, 5% and 6%, see Fig. 2). It could, however, be noticed that the “efficiency” of the activation of the glass support (making it much more hydrophilic) is diminished by the increase of gelatin concentration in the gel: the differences between all inactivated- and activated-samples are progressively diminished, having the lowest change at the highest gelatin concentration (Fig. 2). On the other hand, increasing the concentration of gelatin in the mixed gel, produces “stronger gels” (see Fig. 1), hence strengthening the interactions into the gel network. This could affect the introduction of polar groups on the surface by the plasma treated glass surface, causing progressively smaller differences in the contact angles between untreated mixed gel and treated by plasma activation. Note that similar behavior was obtained for the receding contact angles, used for the calculation of surface free energy γ by the CAH approach, but not shown here for brevity.

3.3. Surface free energy (SFE)

Useful information about energetic changes occurring on the surface of mixed gels, after being formed on both untreated- and cold plasma-treated glass surface, can be obtained from the surface free energy (SFE) of these systems. For this purpose, SFE has been obtained from the contact angle hysteresis (CAH) approach, using equilibrium contact angles of water obtained using Tadmor's (Tadmor, 2004, 2008; Tadmor & Yadav, 2008) derivations. SFE is a useful parameter, which allows to quantify the characteristics of the solid surface, its wetting properties in particular.

Fig. 3 shows the values of SFE of the mixed gels at different gelatin concentration as a result of surface activation. It was found that the largest change in the SFE was obtained for the lowest concentration of gelatin (3%), increasing from $30.5 \pm 2.1 \text{ mJ/m}^2$ to $71.1 \pm 0.8 \text{ mJ/m}^2$ for oxygen treatment, to $61.4 \pm 1.7 \text{ mJ/m}^2$ for argon treatment and to $58.9 \pm 1.0 \text{ mJ/m}^2$ for air treatment. Regardless of plasma treatment, the total SFE of mixed gel increased in all studied samples as we expected, the decrease of contact angles produced by surface activation confirming an increase of the surface free energy given by the CAH approach. In any case, it must be kept in mind that the increase of the surface free energy results from the incorporation of new polar groups on mixed gel surface during its contact with the treated support. These

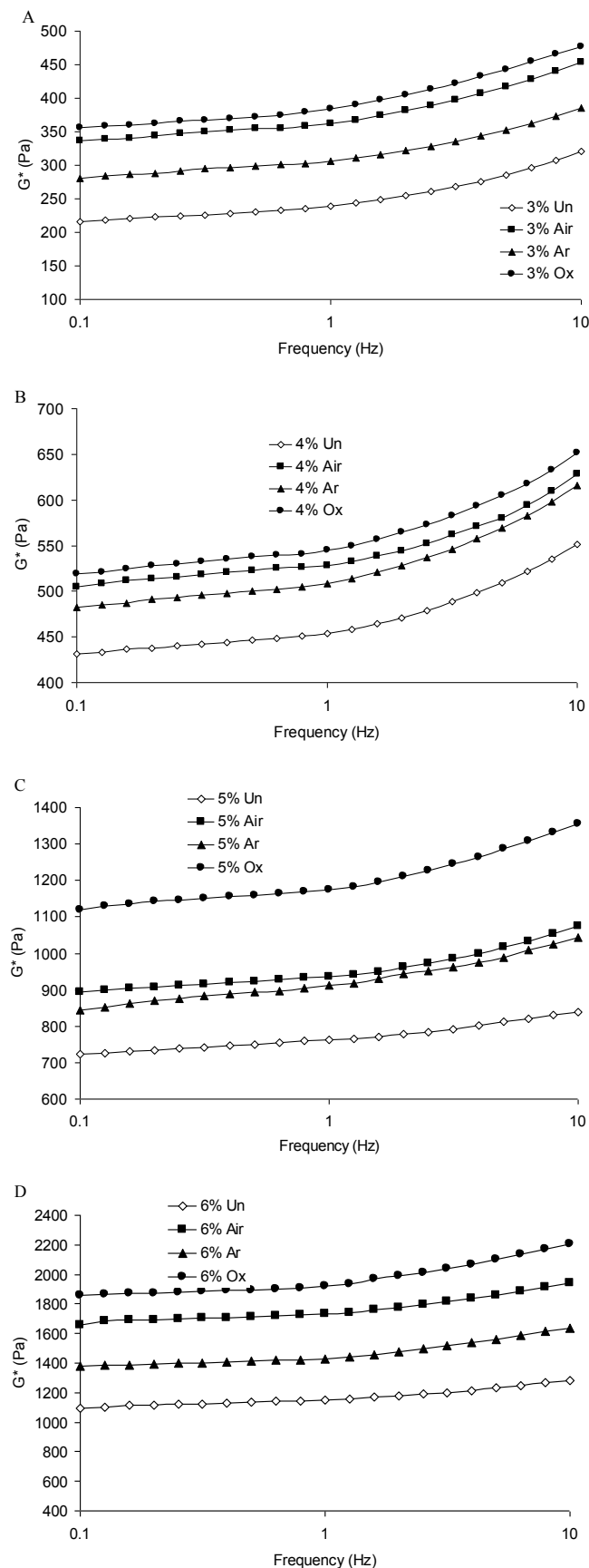


Fig. 1. A-D. Influence of the frequency on the complex modulus for gels obtained at different gelatin concentration detached from glass support which was non-activated (Un) or cold plasma activated using different gasses (Ar - Argon, Air and Ox - Oxygen).

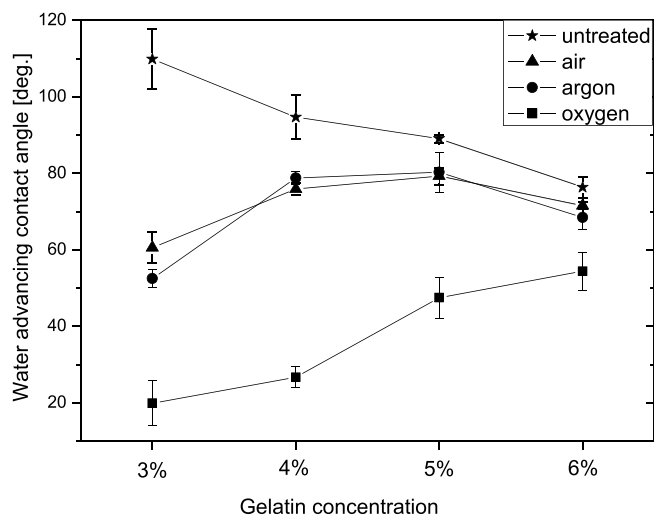


Fig. 2. Influence of gelatin concentration on water advancing contact angles on the gel surface formed on non-activated and activated glass support by air, argon and oxygen plasma.

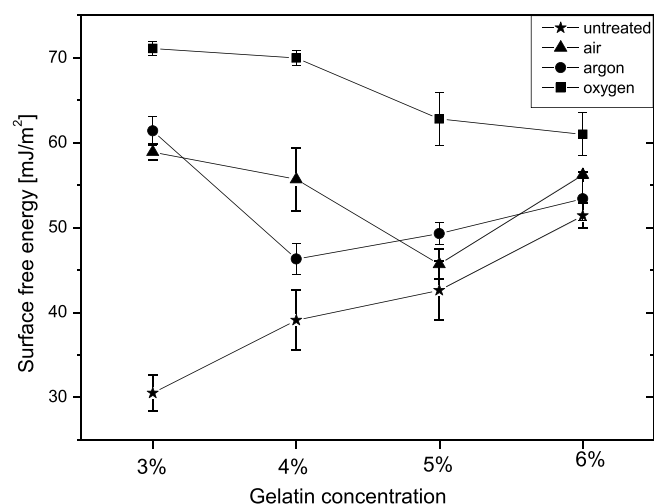


Fig. 3. Influence of gelatin concentration on surface free energy values based on CAH approach for the gel surface formed on non-activated and activated glass support by air, argon and oxygen plasma.

findings are of great interest for the industry as a way to activate gel surfaces that cannot be directly exposed to the plasma treatment, due to the damage that would occur by the exposition to conditions reached in the cold-plasma chamber. If we consider now the highest concentration of gelatin in the mixed gel (6%), the SFE only slightly increases, as compared with the 3% gelatin samples; the difference between “untreated” sample and the sample formed on oxygen-activated glass was less than 10 mJ/m^2 ; about 5 mJ/m^2 for air treatment and 2 mJ/m^2 for the argon treatment. Understanding of these differences needs of additional information about surface topography: it should reflect these changes more clearly, so we will pay attention to it in the next section.

3.4. Gel surface topography

To find a relationship between topography and wettability the analysis of surface roughness was carried out. For that purpose, as we explained in the previous SFE section, it was chosen the sample with the highest concentration of gelatin (6%); it seems to be the best choice due to the small differences in surface energy between the untreated and treated samples. Recurrence of the contact angle values and the topography analysis make it possible to complete characterization of the

Table 1

Topography parameters of the mixed gel formed on glass surface, untreated and after different gasses plasma activation (surface $0.9 \times 1.3 \text{ mm}$).

Parameter	Untreated	Air	Argon	Oxygen
R_a (nm)	164.3 ± 27.9	297.1 ± 50.9	325.8 ± 59.6	995.2 ± 198.7
R_{RMS} (nm)	197.9 ± 31.4	353.8 ± 57.5	397.8 ± 57.5	1270.0 ± 190.8
R_t (μm)	1.1 ± 0.2	1.6 ± 0.2	2.1 ± 0.4	15.5 ± 2.6

R_a – average roughness, R_{RMS} – quadratic mean of the surface roughness, R_t – maximum roughness height.

surface because the contact angle strictly depends on both composition as well as roughness of the film surface (Terpiłowski, Wiącek & Jurak, 2017; Jurak, Wiącek & Terpiłowski, 2016; Wiącek, Terpiłowski, Jurak & Worzakowska, 2016a, 2016b).

The surface roughness parameters are displayed in Table 1. Three different parameters are given; for the purpose of roughness comparison, the root mean square index R_{RMS} (R_q) is the most frequently used, as it is insensitive to the local topographical heterogeneity of the surface. It is clearly seen (Table 1) that the surfaces become rougher with the plasma treatment, with no regard which gas was used. The R_{RMS} for the untreated surface is $197.9 \pm 31.4 \text{ nm}$, increasing to $353.8 \pm 57.5 \text{ nm}$ (air), to $397.8 \pm 57.5 \text{ nm}$ (argon) and to $1270.0 \pm 190.8 \text{ nm}$ for the oxygen treatment. Plasma treatment causes formation and deposition of new functional polar groups on the plasma-activated support and they are the responsible of the changes in roughness of the attached mixed gels.

Comparing the results of contact angles and SFE (Figs. 2 and 3) with those of the topography, we can verify that these results have a strong relationship; it may be concluded that the wettability of the studied gels is affected by their roughness, influencing the specific values of SFE. When the roughness is gradually increased, the contact angles decreased. A surface with low roughness has higher contact angle values and the surface is less water-repellent than the untreated surface which was smoother.

We found in this work is, that oxygen-plasma treated glass support caused the most significant changes in wetting and rheological properties of the gels, but also a similarity in the properties of samples gelled on argon and air plasmas treated glass (see all charts). The profilometer images (Fig. 4) shows, that the surfaces contacted with non-treated glass support exhibit more homogeneity than the ones with oxygen-plasma treated glass support, which is non-uniform. As we hypothesized, the gel surfaces formed on both air- and argon-plasma treated glass support show very similar profiles. Previous research in our laboratory (Terpiłowski & Rymuszka, 2016) showed that after air- and argon-plasma treatment of glass support, the R_{RMS} values were nearly identical, $0.3 \pm 0.05 \text{ nm}$ and $0.3 \pm 0.01 \text{ nm}$ respectively; similar transference of modifications induced in the glass support by plasma treatment to gel formed on it, could explain this behavior.

4. Conclusions

An innovative technique has been used, based on the modification of gel properties by indirect cold-plasma treatment. It has been shown that formation of gel on glass previously treated by cold plasma, affects their surface as well as their rheological properties. The nature of the gas used to form cold plasma (oxygen, air or argon) influenced the extent of the modifications of surface and viscoelastic properties of the gels. The most effective was plasma generated from oxygen, then air and the least from argon. Regardless of gas type plasma treatment, the total SFE of gels increased in all studied samples. The decrease of contact angles produced by surface activation caused an increase of the surface free energy given by the CAH approach. Gel surface becomes rougher with the plasma treatment of glass support, no regard which gas was used. Increased roughness of the gels caused a decrease in the

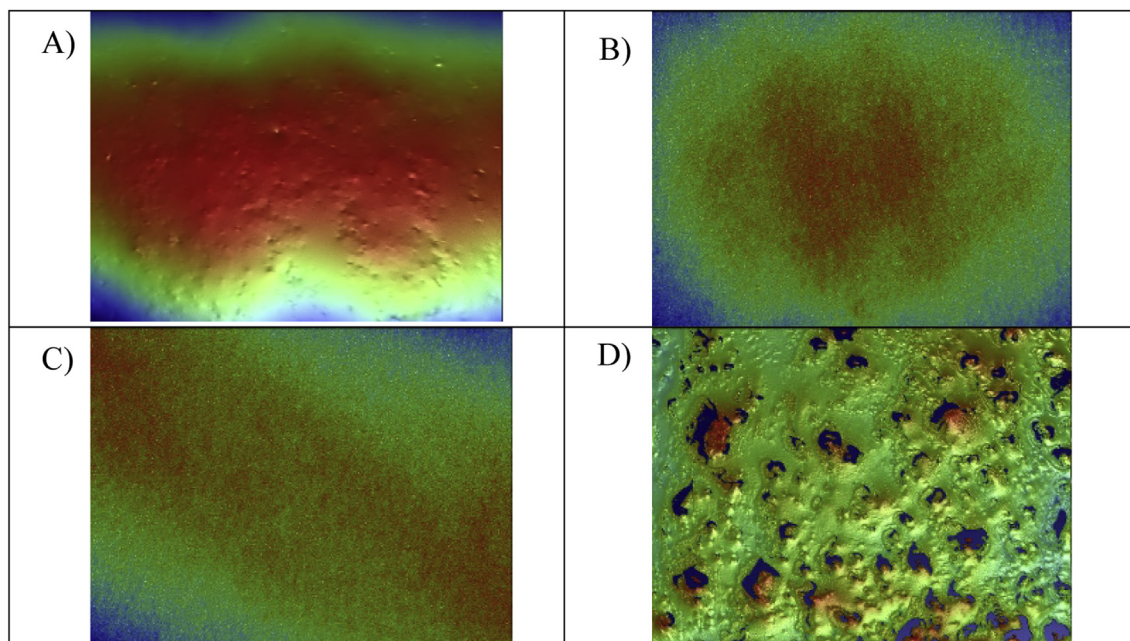


Fig. 4. Surface topography of gels formed on a) non-activated glass support and activated by b) air, c) argon and d) oxygen plasma treatment (shown surface is 0.9×1.3 mm).

contact angles. Changes in these properties increases functionality of the gels in many applications. The paper shows, that even when the gel is not treated directly by cold plasma, pre-treatment of the support is an effective method to cause changes in surface and rheological properties of the gel obtained on such surfaces. These findings could help to create new materials, especially in the applications in scaffolds engineering, drug delivery systems and active packaging. Increased roughness of the gel surface increases ability of the gastrointestinal enzymes to decompose the gels (Tomczyńska-Mleko & Mleko, 2014). Also increased surface roughness can change the ability of an active ingredient to be adsorbed on the surface of the active packaging system.

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Appendix 4

Surface modification of albumin/gelatin films gelled on low temperature plasma-treated poly (Ethylene Terephthalate) plates (fourth paper of the “compendium”)

FULL PAPER

Surface modification of albumin/gelatin films gelled on low-temperature plasma-treated polyethylene terephthalate plates

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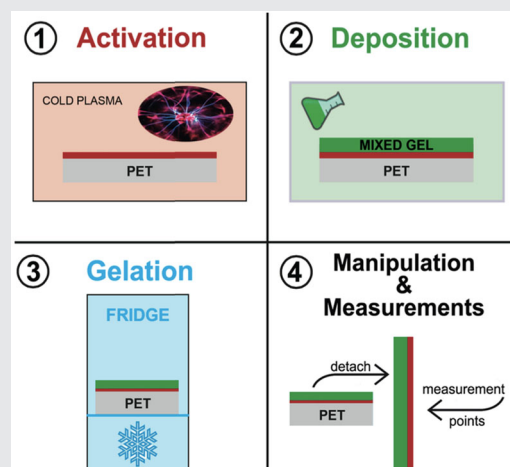
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Abstract

Albumin/gelatin gel (AG) properties are studied with an innovative cold-plasma-treatment-based technique. Gel films are produced in situ on polyethylene terephthalate (PET) plates previously treated by gas (O₂, air and Ar) cold plasma. After detaching the films from the surface, the contact angles of probe liquids are measured and wettability studied, based upon the induced changes of AG surface free energy. The effect of gelatin concentration in the AG is analysed. Oxygen-cold-plasma-treated PET plates induce outstanding changes in wetting properties. Optical profilometry is used for the characterisation of surface topography. Dynamic oscillatory measurements help check for mesoscopic changes. The latter technique allows shaping of gel sample stiffness. The findings support a new methodology for biomaterials/bio-polymer activation when unable to be exposed to the plasma working conditions.

KEYWORDS

biopolymers, cold plasma, contact angles, rheology, thin films



1 | INTRODUCTION

Protein gels are three-dimensional (3D) networks, which are formed when the viscous liquid of the protein solution is transferred into a viscous-elastic matrix.^[1] The quality and suitability of the network depend not only on the physicochemical conditions of the medium^[2] but also on its physicochemical characteristics,^[3]

such as surface hydrophobicity and net charge of proteins.^[4–6]

Egg white albumin (EWA) is probably the most popular gelling protein. It has a protein content of approximately 11% and comprises approximately 58% of the entire egg mass.^[1] EWA has multiple functional properties, with foaming, emulsification, gelation, microencapsulation and adhesion being the most significant.^[7] Ovalbumin, ovotransferrin and

lysozyme are the constituents mainly responsible for the formation of heat-induced gels^[8,9] and their properties determine the gelation of EWA protein. The functional groups of these constituents become exposed upon protein denaturation to form intermolecular linking, yielding gel structures.^[8–10] Tomczyńska-Mleko and Mleko^[11,12] have studied the characteristics of EWA protein gels as matrices for active ingredient release. Taheri et al.^[13] used nanoparticles of EWA protein as drug carrier systems. The efficiency of the functional properties of EWA protein may be adversely or favourably affected by the addition of other hydrocolloids. Coughlan et al.^[14] reported that combinations of proteins and hydrocolloids resulted in more effective functional properties than when working individually. Protein-hydrocolloid interactions may thus enhance the stabilisation of interfacial layer thickness, leading to the stability of foam against bubble coalescence.^[15]

Gelatin is a water-soluble protein, which may be obtained from renewable sources, for instance, waste products from food industries. The growing popularity of this biopolymer in, for example, packaging, medicine or pharmaceutical industries is mainly due to their inherent properties, such as nontoxicity, relatively low cost, eco-friendliness or high availability. A convenient approach to produce novel materials for specific applications with the desired functional properties is by the manipulation of different biopolymers.^[16] The combination of gelatin with egg albumin proteins has been described as leading to a uniform, strong gel structure.^[17] The use of these biopolymers could allow us to create composite biomaterials which may serve as intelligent packaging agents or controlled drug carriers. Thus, we hypothesise that the manipulation of the proportions of gelatin in an EWA-based gel can yield composite gels for these applications.

Modification of biopolymers by means of some physical or chemical routes is a necessary tool to overcome frequent limitations concerning surface properties. In particular, low surface energy would affect surface wettability and other related properties and could be a handicap, as it would lack specific requirements in applications such as drug delivery systems, intelligent packaging or tissue scaffold preparation. Among the current methods for surface modification, cold-plasma activation is an emerging technique offering many potential benefits in treatments of biomaterials. It enables us not only to improve adhesion^[18] and to modify wetting properties but also to enhance functionality and biocompatibility of biomaterial surfaces.^[19]

Polyethylene terephthalate (PET) is one of the most recommended materials for this purpose.^[20,21] Numerous pieces of research have shown the influence of cold plasma on PET.^[22] However, this technology has some limitations. In particular, the material must be capable of withstanding

the operating conditions reached in the chamber. In our attempts to activate the gels, we could catch sight of the fact that they were noticeably damaged, so further methodology development appeared to be needed to overcome this issue. In addition, activating the gels by “indirect plasma treatment” is proposed throughout this paper. After the activation of a PET plate by cold plasma, physical and chemical phenomena should occur on its surface, and, indirectly, also on the surface of any other phase in contact with the plate. This methodology is applied here to AG gels to gain understanding about the impact on gel properties of gelling directly onto the preactivated PET.

Cold plasma is usually cited for “without altering other bulk properties”.^[19] However, in the case of biopolymer-gel type, it is still an open question whether other bulk properties, such as strength to deformation and viscous properties, can be as altered as the surface properties are. The present study aims at developing and characterising biodegradable films produced by the technique above described using different concentrations of gelatin, along with EWA protein. An important set of techniques were used for the characterisation of the mixed gels, comprising contact angles measurements, surface free energy (SFE) determination, surface topography and rheological studies by dynamic oscillatory measurements. The analysis of these data, in turn, could be used to design gels with enhanced possibilities of their applications in many industrial processes.^[23,24]

2 | EXPERIMENTAL SECTION

2.1 | Materials

EWA, low mineral, high gel type (88.1% protein) was provided by Kewpie Corporation (Tokyo, Japan). Protein concentration was determined by the Kjeldahl method.^[25] Gelatin from the porcine skin (type A) was obtained from Dr. Oetker (Gdansk, Poland). According to the specifications, it has “platinum strength” with a 250 Bloom and ca. 60,000 mean molecular mass. To perform the Bloom test on gelatin, a 6.67% gelatin solution was kept for 17–18 hr at 10°C before being tested. The higher a Bloom value, the higher the melting and gelling points of a gel are, and the shorter its gelling time is.

2.2 | PET plate preparation and plasma activation

0.5 mm thick commercial PET (Axpert Standard, Bayer AG) plates were used. The material was cut into 20 mm × 20 mm pieces. Before the experiments, the samples were washed in ethanol in an ultrasound bath for 15 min and then rinsed with Milli-Q water to remove their surface contamination.

The samples were dried at room temperature in a desiccator. Plasma activation was performed in a low-pressure plasma system Pico from Diener Electronic, Germany. The plates were placed on the sample shelf, and the system was adjusted to a pressure of 0.2 mbar. Then, the flow of gas (air, argon and oxygen, from Air Products, Warsaw, Poland) was set at 22 sccm (standard cubic centimetres per minute). The plates were treated with cold plasma at the electric voltage of 400 V for 1 min. The chamber was purged with air for 10 s so as to remove the existing gaseous products.

2.3 | Preparation of gel films

EWA protein dispersion was made by hydrating the protein in distilled water and then heating in a water bath for 30 min at 80°C. Gelatin solution was added to obtain mixtures with 3% (wt/wt) EWA protein, and 3%, 4%, 5% or 6% gelatin concentration. In the preliminary tests, mixed gels with gelatin concentrations ranging from 1% to 6% were prepared. At low gelatin concentration (1–2%), the water content was too large to manipulate without damaging the films; thus, only gels containing 3% or higher gelatin concentration were further used. The mixed solutions were stirred for 30 min at 80°C and poured on the PET plates placed on Petri dishes to obtain a 1.0 ± 0.1 mm thick gel layer (checked using a micrometre screw gauge). The Petri dishes were levelled using a self-levelling laser IM0201 (Magnusson, Longpont-sur-Orge, France). In total, four samples with different gelatin content were gelled on each plasma-treated plate, 12 “activated” and 4 “inactivated” surfaces (used as control), stored for 20 hr at 7°C and equilibrated at 21°C for 2 hr before the determination of their properties. After 1 day, the gel was detached from the PET support and studies on its wettability and surface topography were carried out on the inner surface, which was previously attached by the methods described in the following sections. Moreover, rheological measurements were also performed.

2.4 | Oscillatory measurements

Viscoelastic properties of the gels were determined using a Kinexus Lab+ dynamic rheometer (Malvern Instruments Limited, Malvern, UK). Rheological properties were investigated using a serrated parallel steel plate (35 mm diameter and 1 mm gap size) to limit the sliding effects. The gel samples (10 mm diameter and 1 mm thick) were cut and detached from the plate using a scalpel. Oscillatory measurements with frequency sweep in the range 0.1–10 Hz were performed. All the measurements were performed at 0.01 strain in the linear viscoelastic range determined by the strain sweep. The complex modulus,

G^* , as well as the loss tangent, $\tan\delta$, were obtained from the experimental data.

2.5 | Contact angle measurements

Advancing and receding contact angles on the gel surfaces were measured by making use of a contact angle metre GBX (France), equipped with a temperature and humidity-controlled measuring chamber and digital camera. The measurements were conducted at 20°C and 50% relative humidity. A 6 μ l droplet from a syringe was gently settled on the sample surface by means of an automatic deposition system. The advancing contact angle was evaluated from the droplet shape by the computer programme Win Drop. Then, 2 μ l of the droplet volume was sucked into the syringe, and the receding contact angle was calculated by the above-mentioned programme. Two liquids of known SFE, distilled water and diiodomethane, were selected to cover the range from very polar liquid to the nonpolar one. The advancing and receding contact angles were measured for up to 10 droplets of each probe liquid.^[26]

2.6 | SFE determination

Equilibrium contact angles were calculated from the measurements of advancing and receding contact angles^[27] on the mixed gel samples. For this purpose, eqs. 1–3 from Tadmor’s theory were used.^[28]

$$\Gamma_a \equiv \left(\frac{\sin^3 \theta_a}{(2 - 3\cos\theta_a + \cos^3 \theta_a)} \right)^{1/3}, \quad (1)$$

$$\Gamma_r \equiv \left(\frac{\sin^3 \theta_r}{(2 - 3\cos\theta_r + \cos^3 \theta_r)} \right)^{1/3}, \quad (2)$$

$$\theta_{Eq} = \arccos\left(\frac{\Gamma_a \cos\theta_a + \Gamma_r \cos\theta_r}{\Gamma_a + \Gamma_r} \right), \quad (3)$$

where Γ_a and Γ_r are the advancing and receding angle weight coefficients, respectively, and θ_a , θ_r and θ_{Eq} , the advancing, receding and equilibrium contact angles. The SFE of the solid surface, γ_s , was calculated using the hysteresis approach proposed by Chibowski,^[29] based on the contact angle hysteresis. The equation

$$\gamma_s = \frac{\gamma_l (1 + \cos\theta_a)^2}{2 + \cos\theta_r + \cos\theta_a}, \quad (4)$$

allows the determination of the “apparent surface free energy”, γ_s ,^[29] with γ_l , the liquid surface tension. When θ_{Eq} is used for calculation of γ_s , Equation (4) transforms into:

$$\gamma_s = \frac{1}{2}\gamma_l(1 + \cos\theta_{Eq}). \quad (5)$$

2.7 | Topography

The topography of the gel surface was obtained using an optical profilometer, GT Contour Surface Metrology (Veeco, Tucson, AZ). Surface roughness was determined by making use of a Vision64 (Veeco) apparatus. The parameters were the average roughness (Ra), the quadratic mean of the surface roughness (RRMS) and the maximum roughness height (Rt).

2.8 | Confocal microscopy

Optical confocal images were captured by means of an inverted light microscope, Eclipse MA200, Nikon (Japan) with confocal attachment. Moreover, rhodamine solution was used as a contrast.

3 | RESULTS AND DISCUSSION

3.1 | Oscillatory measurements

Figure 1a,b shows the complex rigidity modulus, G^* , obtained from frequency sweeps for gels with 3% EWA and different gelatin concentration (3% [a] and 6% [b]) produced on cold plasma untreated/treated PET plates.

From the findings, we could clearly claim that the increase of gelatin concentration resulted in more rigid gels, as shown by the higher G^* values. Data for 4% and 5% gelatin concentration were obtained, although not shown for brevity.

Complementary information can be obtained using the loss tangent parameter, $\tan\delta$, (Figure 2), the quotient between the viscous portion of the viscoelastic properties (loss modulus) and the elastic portion of these properties (storage modulus). Smaller loss tangent values characterise a more elastic-like material.

As shown in Figure 2 the elasticity of the gels was significantly affected by the concentration of gelatin. This means that stronger bridges should appear, which result in a denser network and a more aggregated structure. Xu et al.^[30] also observed variations of aggregate morphology and size caused by the increase of gelatin concentration. This is probably connected with a different microstructure of gels with different gelatin content.^[31]

It is worth noting the different behaviour observed for the nonactivated gels as compared to the activated ones. Gels produced/gelled on activated supports enhanced their rheological properties in terms of rigidity. The most elastic gels (with the lowest loss tangent values) were obtained from those formed onto PET plates activated by oxygen plasma, followed by those in contact with PET treated with argon- and air plasma. A stronger gel network could be the result of the stabilisation of EWA protein by intermolecular linkages (e.g., disulphide cross-link or hydrogen bonds) and formation of polar groups during its gelation.^[32,33] The polar groups obtained on the surface of PET by argon-plasma activation caused a higher impact on the gelation process of the EWA/gelatin mixture, in comparison to the

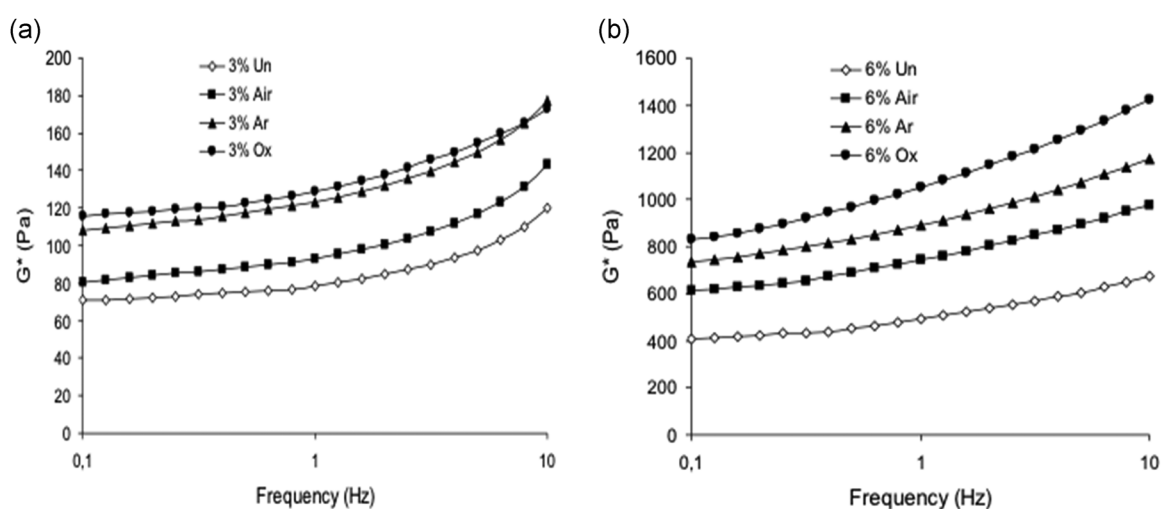
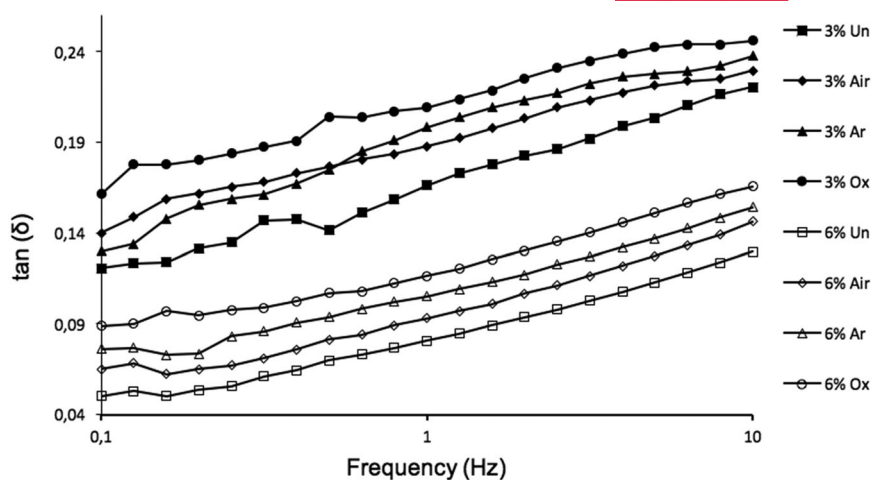


FIGURE 1 Influence of sweep frequency on the values of complex modulus G^* for AG gels produced on polyethylene terephthalate plates previously treated with different plasma gases (Un, untreated; Ar, argon plasma; Air, air plasma; and Ox, oxygen plasma). 3% (a) and 6% (b) gelatin concentration in the mixed gel

FIGURE 2 Influence of sweep frequency on the values of loss tangent, $\tan\delta$, for AG gels produced on polyethylene terephthalate plates previously treated with different plasma gases (Un, untreated; Ar, argon plasma; Air, air plasma; and Ox, oxygen plasma). Percentage in the figures, gelatin concentration in the mixed gel



air-plasma activation. Oxygen-plasma activation produced probably more surface hydroxyl groups, which could cause deprotonation of sulfhydryl groups of albumin proteins and formation of a larger number of disulphide bonds. These processes probably led to a rougher, more aggregated surface of the gels formed on the oxygen-treated PET support (discussed in confocal microscopy section). These findings demonstrate that plasma-activated support causes remodelling of the gel bonds, not only on its surface but also at a deeper level. Viscoelastic properties of the gels are thus changed with a tendency to get a more rigid and elastic structure of the material.

3.2 | Contact angles and wetting behaviour

Figure 3 displays the water advancing contact angles as a function of the gelatin concentration in the mixed gel as well as the influence of different plasma treatments of the PET support.

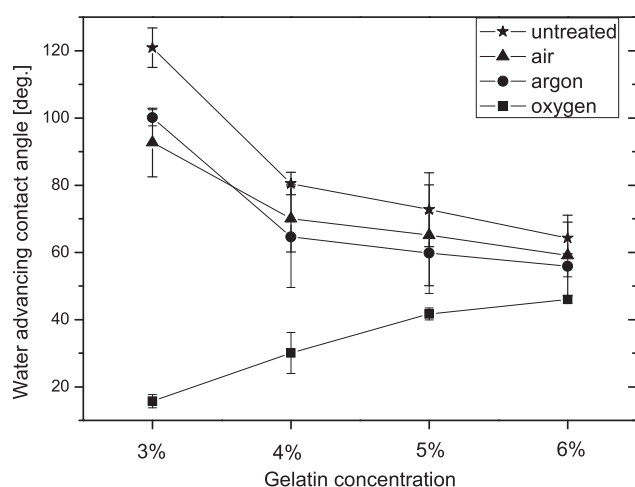


FIGURE 3 Water advancing contact angles measured on mixed gels surface

For the “untreated” gels, the values decrease gradually as the gelatin concentration increases. The water contact angle at 3% gelatin concentration was $121 \pm 8^\circ$, showing the hydrophobic nature of the gel; however, increasing the gelatin concentration up to 6%, the contact angle decreased to $62 \pm 8^\circ$. Thus, we found that the addition of gelatin resulted in a more hydrophilic surface. Gelatin is composed of a mixture of single and double unfolded chains of hydrophilic character, whose hydrated states form a 3D network.^[34] These random spiral molecules have a high degree of freedom to reorganise their hydrophilic moieties, which could have preferred orientations (spatial arrangement of atoms and ligands at the top surface) resulting in a lower contact angle.^[35] This result qualitatively agrees with those reported by Białopiłotrowicz and Janczuk,^[36] who concluded that the chains’ side orientations were responsible for the wettability changes caused by the addition of gelatin in aqueous solution.

Regarding the effect of plasma preactivation of the PET support, all gas-cold plasma improved the wettability of the gels, as contact angles decreased. Analysing the capability of the plasma to change the surface properties of the samples formed on the activated supports, it is clear that the most efficient treatment was obtained using oxygen-plasma, particularly at the 3% gelatin concentration, where the contact angle decreased from $121 \pm 8^\circ$ to $16 \pm 8^\circ$. This significant decrease denotes an improvement in wettability of the surface. The gelation on cold plasma-treated support strongly altered the surface polarity, and hydrophilic bonds/groups appeared, creating intensive hydrophilic interactions at the activated surface/water interface, manifested by the decrease in the contact angles.

In the case of gels with the highest gelatin concentration, a smaller effect of the plasma was found. The difference between the “untreated” and “treated” samples gradually decreased, being the lowest at 6% gelatin concentration, where the contact angle slightly decreased from $62 \pm 8^\circ$ to $48 \pm 8^\circ$. This loss of efficiency could be related with the

strength of the biopolymer chains, which was explained in the previous section (Figure 1a,b). Higher gelatin concentrations led to a stronger gel, a larger number of bonds between the gel and the protein molecules appeared, which probably hindered the introduction and reorganisation of the polar groups (not readily inserted between biopolymer chains) and, in turn, their interactions.

The effect of argon- and air-plasma treatments of the PET plates on the contact angles of mixed gels at 3% gelatin concentration was also noticeable, as it dropped from $121 \pm 8^\circ$ (“untreated” samples) to $61 \pm 4^\circ$ in the case of argon-plasma pretreatment; and to $52 \pm 2^\circ$ in the case of air plasma. As discussed above, these results show that both pretreatments are less effective than that of the oxygen-plasma, which could be explained by the lower intensity of the interactions of the functional groups between the support surface and the deposited gel. Pérez-Huertas et al.^[22] have studied the surface chemistry of PET after air- and argon-plasma activation. It was found that, after the activation, water contact angles dropped from $83.5 \pm 3.3^\circ$ (untreated PET) to $26.0 \pm 1.7^\circ$ and to $13 \pm 4^\circ$, respectively. This was reported to be due to the appearance of functional groups (originated by the treatment) involving the C, O and N atoms, measured by the X-Ray Photoelectron Spectroscopy (XPS) technique. Probably these new functional groups resulted from the interaction between the groups in the activated PET surface and the gel surface in contact with it. Moreover, the changes in the contact angles for all gels were similar for both argon and air pretreatments (within $\pm 5.0^\circ$). This suggests that both plasma-acting mechanisms should be based on similar contributions of functional groups, originating from the treated PET surface. Pérez-Huertas et al.^[22] found that the contributions of the polar groups on PET surface were hardly 2.5% different when comparing air- and argon-treatments by XPS spectra, which may explain such a similar behaviour in the gels.

It is worth mentioning that analogous behaviour was observed for the receding contact angles used for the calculation of SFE but not discussed here. In the case of the nonpolar diiodomethane, the advancing and receding contact angles did not exhibit significant changes. As diiodomethane interacts with the surface by only London-dispersion forces, the changes in the contact angle after the plasma treatment were not significant.

3.3 | Surface free energy

Figure 4 represents the SFE as a function of the gelatin concentration and the type of plasma pretreatment.

It is shown that the largest change of SFE was obtained with oxygen-plasma at the lowest concentration of gelatin, from 20.4 ± 3.2 to 71.8 ± 0.3 mJ/m^2 . The decrease of contact angles induced by the plasma treatment of the support

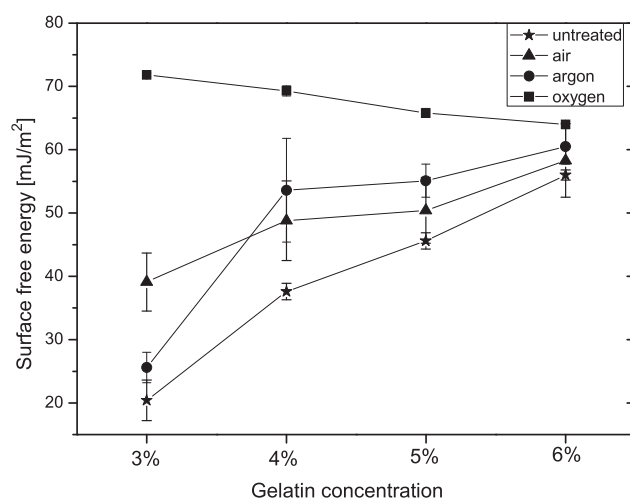


FIGURE 4 Surface free energy for the mixed gels surface calculated by contact angle hysteresis approach

confirmed an increase of the SFE of the gel. In the case of the air-plasma treatment, the change was up to 39.1 ± 2.6 and 25.6 ± 2.4 mJ/m^2 for the argon-plasma treatment. The effect of increasing gelatin concentration exhibited opposite trends when comparing air- and argon-plasma treatment with oxygen plasma treatment. The downward trend showed by oxygen-treated gels is likely because SFE reached its maximal value (at 3%) and further oxidation could only reduce it. As shown in our previous paper,^[22] the functional polar groups appearing in the PET plates after cold plasma treatment, could be the responsibility of the SFE changes of the gels deposited on these plates. These findings support a new methodology for material activation, in those cases where they cannot be exposed to the plasma working conditions, as it occurs in the case of biopolymers-gel type materials.

Summarising, the effect of increasing gelatin concentration in the gel is to reduce the differences between the SFE of “untreated” and “treated” samples. In this way, the sample with the highest gelatin concentration (6%) shows an SFE difference of only approximately 7 mJ/m^2 , regardless of the type of plasma used. Comparing the SFE values for the samples of 3% and 6% gelatin concentration, a loss of efficiency of the plasma treatment is found. As discussed in the previous section, these small differences may be accounted for by the different chemical composition of the samples, obtaining stronger gels by the addition of more gelatin (Figure 1a,b). For a better understanding of the above behaviour, the samples with 6% gelatin concentration were subjected to a topography study.

3.4 | Surface topography

The samples with 6% gelatin composition were most suitable for this analysis because of the small differences between the

TABLE 1 Topography parameters of the mixed gels formed on PET plates, before and after plasma modification

Parameters	Untreated	Air-plasma	Argon-plasma	Oxygen-plasma
Ra (nm)	481 ± 80	1,148 ± 233	1,320 ± 290	1,655 ± 163
RRMS (nm)	618 ± 103	1,493 ± 304	2,100 ± 357	2,155 ± 375
Rt (μm)	10.6 ± 1.7	22.6 ± 8.7	30.6 ± 5.4	35.3 ± 8.3

Note: Surface = 0.9 × 1.3 mm.

Abbreviations: PET, polyethylene terephthalate; Ra, average roughness; RRMS, quadratic mean of the surface roughness; Rt, maximum roughness height.

“untreated” and “treated” ones. Table 1 shows the surface topography parameters, Ra, RRMS and Rt, of the gels produced on PET plates previously activated by cold plasma.

As a general trend, all the parameters increased after the plasma treatment, indicating that the surfaces became rougher. The value of Ra for the “untreated” gel was 481 ± 80 nm. Then, after the oxygen-, argon- and air-plasma treatments, Ra rose up to 1,655 ± 163 nm, 1,320 ± 290 nm and 1,148 ± 233 nm, respectively. The increase of Ra has been attributed to the etching effect induced by the bombardment of the energetic particles produced during the plasma treatment, which excited the support where the gels were formed.^[37] As found in most experiments reported in this study, oxygen-plasma pretreatment of the support was the most effective in altering the surface and bulk properties of the gels. It is interesting to compare these results with those obtained by our group using single PET plates.^[22] There, the average Ra parameter of the untreated PET plates was 828 ± 61 nm; after the air-plasma activation, 856 ± 123 nm and the argon-plasma activation, 1,000 ± 119 nm. These values are considerably lower than those reported in the present work for the gels built on PET support. Taking all these findings into account, we could say that the technique of plasma “indirect activation” seems to be quite efficient. One simple explanation could be that most polar groups of the surface do not usually remain on the top surface, which is exposed to air but are buried into the inner part of the surface region, according to our working methodology. In addition, the soft nature of gels could allow the polar groups originated after PET activation to be readily inserted into the biopolymer chains. Another observation is that the untreated gel surface is relatively smoother than the treated ones, with randomly dispersed roughness over the surface (Figure 5).

The largest differences are obtained when activating the support with oxygen plasma. This is also evident in the side profile of the roughness (Figure 6). The gels obtained on the nonactivated surface and those subjected to air- and argon plasma have a comparable profile of roughness, while in the case of gelling on an oxygen-plasma activated surface, the profile has periodically repeated surface unevenness. Their distance (mm) and height (μm) testify to the fact that they can be completely

wetted by water, which is what the drop-in wetting of water does.

3.5 | Confocal microscopy

As a part of broader surface characterisation, an alternative noncontact profilometry technique is presented by means of confocal images of the gels (6% gelatin), before and after oxygen-cold-plasma treatment. As shown in Figure 7, the areas marked red are gelatin while those in black are albumin. As can be seen from Figure 7 (left), albumin forms quite small clusters and the surface is dominated by gelatin, whereas in the case of the surface obtained on the activated plate by oxygen plasma, the albumin forms large clusters (Figure 7, right). Under some conditions, disulphide covalent bonds from albumin molecules could play an important role in cross-linking and stabilising the structure of the gel.^[38] Cold-plasma activation caused probably deprotonation of the sulfhydryl groups of albumin and formation of a larger number of disulphide bonds, which results in a higher aggregation. Such a structure leads to a reduction in the value of water wetting contact angles (see Figures 3 and 4) because albumin is more hydrophilic than gelatin.

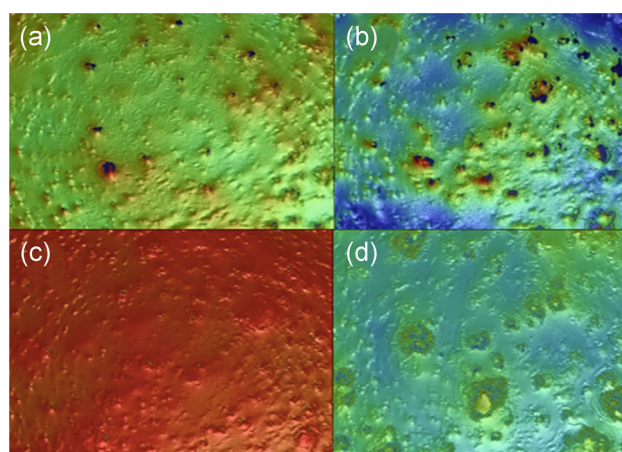


FIGURE 5 Surface topography (top images) of mixed gels produced on polyethylene terephthalate (a) untreated, (b) air-plasma, (c) argon-plasma and (d) oxygen-plasma treated supports; scanned area 1.3 mm × 0.95 mm

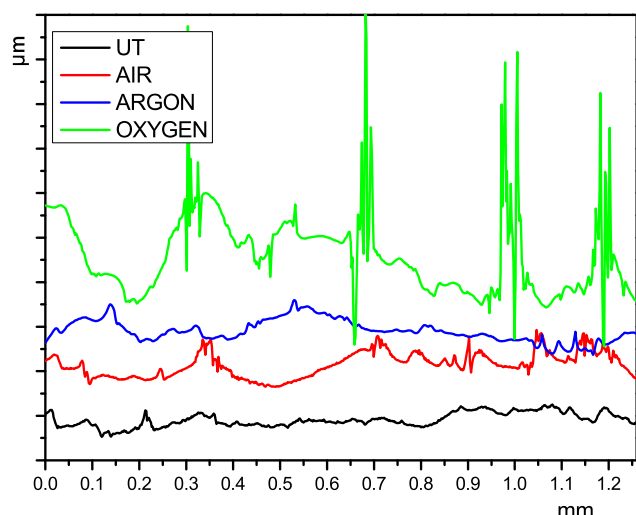


FIGURE 6 Side roughness profiles. Surface unevenness distribution: separation (mm) and height (μm)

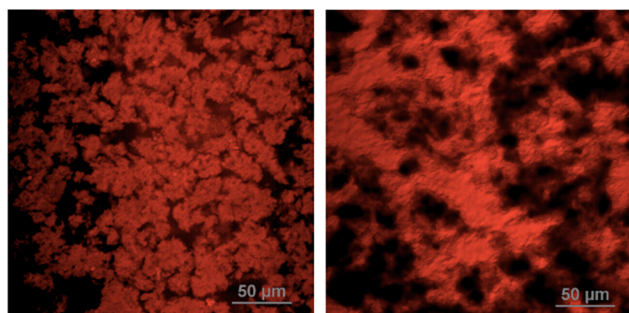


FIGURE 7 Confocal microscopy images of mixed gel (6% gelatin) produced on untreated (left) and oxygen-plasma treated support (right)

4 | CONCLUSION

The studies reported in this paper show that there is a significant modification of the surface as well as of the structural bulk conformation of gel films formed in situ on PET plates pretreated by cold plasma. As a general trend, the effect of plasma pretreatment to the gels is to provide a more hydrophilic character to their surfaces. The increase in the wettability of the surface of the gels is manifested by a significant decrease of the contact angles after the activation of the support. The most effective pretreatment was obtained by the oxygen-cold plasma, which induced the higher hydrophilicity of the samples. The SFE of the gels was shown to be a function of the gelatin concentration in the albumin/gelatin mixed gels and the type of plasma pretreatment. Wetting of the gel samples was affected by the formation of micropores on the surface of the mixed

gels and characterised by a huge increment of the roughness parameters of the gel. It was found that the stiffness of gel samples produced using the methodology proposed in this paper can also be tuned, as manifested by the oscillatory rheological technique. The findings could be exploited for the development of active or intelligent ingredients, scaffolds or tissue engineering films, where the active compound release can be controlled depending on the product quality and expected shelf-life.

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How to cite this article: Pérez-Huertas S, Terpiłowski K, Tomczyńska-Mleko M, Mleko S. Surface modification of albumin/gelatin films gelled on low-temperature plasma-treated polyethylene terephthalate plates. *Plasma Process Polym.* 2019;e1900171. <https://doi.org/10.1002/ppap.201900171>

Appendix 5

Solid surface energy data (mJ/m²) for common polymers

Name	SFE at 20 °C	Dispersive contrib. SFE	Polar contrib. SFE
Polyethylene-linear PE	35.7	35.7	0
Polyethylene-branched PE	35.3	35.3	0
Polypropylene-isotactic PP	30.1	30.1	0
Polyisobutylene PIB	33.6	33.6	0
Polystyrene PS	40.7	34.5	6.1
Poly- <i>p</i> -methyl styrene PMS	39.0	35	4
Polyvinyl fluoride PVF	36.7	31.2	5.5
Polyvinylidene fluoride PVDF	30.3	23.3	7
Polytrifluoroethylene P3FEt/PTrFE	23.9	19.8	4.1
Polytetrafluoroethylene PTFE	20	18.4	1.6
Polyvinylchloride PVC	41.5	39.5	2
Polyvinylidene chloride PVDC	45.0	40.5	4.5
Polychlorotrifluoroethylene PCTrFE	30.9	22.3	8.6
Polyvinylacetate PVA	36.5	25.1	11.4
Polymethylacrylate PMAA	41.0	29.7	10.3
Polyethylacrylate PEA	37.0	30.7	6.3
Polymethylmethacrylate PMMA	41.1	29.6	11.5
Polyethylmethacrylate PEMA	35.9	26.9	9.0
Polybutylmethacrylate PBMA	31.2	26.2	5.0
Polyisobutylmethacrylate PIBMA	30.9	26.6	4.3
Poly(<i>tert</i> -butylmethacrylate) PtBMA	30.4	26.7	3.7
Polyhexylmethacrylate PHMA	30.0	27.0	3
Polyethyleneoxide PEO	42.9	30.9	12.0
Polytetramethylene oxide PTME	31.9	27.4	4.5
Polyethyleneterephthalate PET	44.6	35.6	9
Polyamide-6,6 PA-66	46.5	32.5	14
Polyamide-12 PA-12	40.7	35.9	4.9
Polydimethylsiloxane PDMS	19.8	19.0	0.8
Polycarbonate PC	34.2	27.7	6.5
Polyetheretherketone PEEK	42.1	36.2	5.9

For surface energy components (e.g. dispersive and polar, hydrogen bonding, acid-base contributions etc.), corresponding (single) references and data at room temperatures. 15 Jun 2018 - www.surface-tension.de

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Scientific activities

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1. **S. Pérez-Huertas**, K. Terpiłowski, M. Wisniewska, V. Zarko, Influence of Polyvinylpyrrolidone adsorption on stability of silica aqueous suspensions- effects of polymer concentration and solid content, *Physicochem. Probl. Miner. Process.* 53 (2016) 121–135.

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<https://doi.org/10.1002/ppap.201900171>

Conference participations

1. L. Holysz, K. Terpilowski, **S. Pérez-Huertas**, M. Tomczyńska-Mleko, S. Mleko, Advances materials and technologies, 27-31 August 2017, Palanga (Lithuania) “Investigation of the effect of low temperature plasma and k-carrageenan on wettability of gelatine layers”

2. M. Mielniczek, K. Terpilowski, L. Holysz, **S. Pérez-Huertas**, M. Chodkowski, Advances materials and technologies, 27-31 August 2017, Palanga (Lithuania) “Surface properties of gelatine gels deposited on the plasma activated glass support”

3. **S. Pérez-Huertas**, K. Terpilowski, M. Tomczyńska-Mleko, S. Mleko, Advances materials and technologies, 27-31 August 2017, Palanga (Lithuania) “Physicochemical properties of gelatine/egg white albumin mixed gels”

4. K. Terpilowski, M. Chodkowski, **S. Pérez-Huertas**, Sixteenth Young researchers conference-Materials science and engineering, 6-8 December 2017, Belgrade (Serbia) “Graphene and graphene/diamonds metal composites used in military industries”

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6. **S. Pérez-Huertas**, K. Terpilowski, L. Holysz, M. Chodkowski, XVI Polish-Ukrainian Symposium, Theoretical and experimental studies of interfacial phenomena and their technological applications, 28-31 August 2018 Lublin, “Surface properties of gelatine gels deposited on solid supports”

7. K. Terpilowski, **S. Pérez-Huertas**, Y. Bolbukh, 22nd International symposium on surfactants in solution, 3-8 June 2018, Oklahoma, “Surface properties of plasma activated chitosan foils”

8. K. Terpilowski, O. Goncharuk, **S. Pérez-Huertas**, M. Nastaj, Seventeenth Young researchers conference-Materials science and engineering, 5-7 December 2018, Belgrade (Serbia) “Macro and micro wettability of hydrophobic rough surface”

9. D. Rymuszka, K. Terpilowski, **S. Pérez-Huertas**, M. Tomczyńska-Mleko, 6th European young engineers conference, 24-26 April 2017 Warsaw, “Stability of water silica suspensions with polymer addition”

10. **S. Pérez-Huertas**, K. Terpilowski, M. Chodkowski, XVI Polish-Ukrainian Symposium, Theoretical and experimental studies of interfacial phenomena and their technological applications, 28-31 August 2018 Lublin, “Surface properties of egg white isolate/gelatine mixed gels deposited on PET support activated by cold plasma”

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- 1.- **S. Pérez-Huertas**, K. Terpiłowski, M. Tomczyńska-Mleko, M. Wesołowska-Trojanowska, M. Kawecka-Radomska, M. Nastaj, S. Mleko, Surface Properties of Whey Protein Gels, **Journal of the Chemical Society of Pakistan**, 41 (2019), **(5%)**
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<https://doi.org/10.1002/ppap.201900171>, (5%)

Wyrażam zgodę na dołączenie wyżej wymienionych publikacji do rozprawy doktorskiej mgr. inż. Salvadora Pérez-Huertasa.

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Oświadczenie

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<https://doi.org/10.1002/ppap.201900171>, (5%)

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June 02-2020

STATEMENT

Herby I confirm that my contribution to the below publication consisted mainly in discussion of obtained data. My contribution to below papers can be estimated at about:

S. Pérez-Huertas, K. Terpiłowski, M. Tomczyńska-Mleko, K. Nishinari, S. Mleko, Surface and rheological properties of egg white albumin/gelatin dispersions gelled on cold plasma-activated glass, **Food Hydrocolloids**, 96 (2019) 224–230 (5%)

I agree this paper to be included in the PhD thesis by Salvador Pérez-Huertas.



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Oświadczenie

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S. Pérez Huertas, K. Terpiłowski, M. Tomczyńska–Mleko, S. Mleko, Ł. Szajnecki, Time-based changes in surface properties of poly (ethylene terephthalate) activated with air and argon-plasma treatments, **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, 558 (2018) 322-329, (5%)

Wyrażam zgodę na dołączenie wyżej wymienionych publikacji do rozprawy doktorskiej dr Salvadora Pérez-Huertasa.

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Oświadczenie

Niniejszym oświadczam, że mój wkład w powstanie poniższych publikacji polegał na udziale w opracowaniu wyników badań.

1.- **S. Perez-Huertas**, K. Terpiłowski, M. Tomczyńska-Mleko, M. Wesołowska-Trojanowska, M. Kawecka-Radomska, M. Nastaj, S. Mleko, Surface Properties of Whey Protein Gels, **Journal of the Chemical Society of Pakistan**, 41 (2019), (5%)

Wyrażam zgodę na dołączenie wyżej wymienionych publikacji do rozprawy doktorskiej dr Salvadora Pérez-Huertasa.

