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Keratin/polyethylene oxide bio-nanocomposites reinforced with ultrasonically functionalized graphene


Polyethylene oxide (PEO) functionalized graphene (f-G) was prepared by ultrasonication of pristine graphene in PEO aqueous solution. Feasible sonication protocol of PEO degradation and graphene functionalization enabled fabrication of solvent casted nanocomposites. Additionally, the steps to form new bio-nanocomposite films have been described, taking the advantage of the combination of graphene, PEO and keratin fibers from poultry feather waste, to design the aforementioned bio-nanocomposite films, which allow extraordinary properties that would have promising applications as eventual packaging materials and enable bio-waste keratin to be converted to added-value material. Compared to neat PEO, addition of only 0.3 wt.% f-G provided increase of 92 % of storage modulus. These findings are similar to the nanoindentation results, which yielded the increases for reduced modulus of same composition for about 92 %. Nanoindentation testing shows that the incorporation of 0.3 wt.% f-G increased reduces modulus and hardness of keratin-PEO blend for about 155 and 99 %, respectively.

1. Introduction

The green chemistry became a new perspective for prevention of pollution in an economically feasible way, through the use of chemicals and processes that are environmentally friendly. Chicken feathers from poultry production are natural renewable source of fibrous protein, keratin. In green chemistry, keratin found implementation use in films, packaging, building materials and bioplastics. Keratin also found applications in membrane devices for separation and adsorption and biomedical applications in different forms, such as films, hydrogels, sponges, fibers and scaffolds. Functional groups within keratin structure enable strong intermolecular interactions, so the poor mechanical properties, especially fragile nature of pure keratin, and low solubility in most used solvents, indicated functionalization and mixing keratin with other polymers, natural or synthetic, like chitosan, cellulose, gelatin, fibroin, nylon, PEO, PLLA and similar, as model for overcoming this problem. New perspective is grafting carbon nanotubes and graphene structures with keratin, or generally speaking, opportunity to modify carbon structures with natural polymers, which provide a possibility of producing bio-nanocomposites.

Polyethylene oxide is a low cost, commercially available biodegradable material, with favorable mechanical features and processability. Due to high polarity, this material is compatible with a large number of chemical substances, solvents and polar nanofillers with applications in biomedicine and in electrolytes within lithium batteries and solar cells. Changing the structure of PEO with irradiation, producing macromolecules with radical end and recombination with cleavage of polymer chains, opens new perspective in application of this polymer.

Due its outstanding thermal, electrical and mechanical properties graphene sheets are used in a wide range of applications such as electric devices, solar cells and, as in our study, as reinforcement in composite films. New perspective of graphene structure could be incorporation in polymer blend systems. Advantage and the benefits of graphene’s application are in very low contents. Use of organic polymers, with both low and high molecular weight, mixed with graphene provide manufacturing materials with superior characteristics. Most researchers based their work on transformation of inert graphene surface, improving it with various chemical, radical-type or oxidation reactions. These methods usually involve multi-phase organic synthesis, which are often very time consuming and sometimes economically inefficient. The use of ultrasound for graphene was limited on mechanical and physical effects, but recently, high intensity ultrasound through the possibility of chemical treatment provides activation of inert graphene surface in feasible manner. Incorporation of graphene structures in multicomponent systems is not sufficiently investigated, and as such opens many possibilities for the production of composite films, especially if one of the components
represents a renewable resource. Currently, to the best of our knowledge, there have been no reports on graphene functionalization with PEO with ultrasonic irradiation and no reports on incorporation of functionalized graphene (f-G) within PEO. Furthermore, in this research application of feather keratin as an additional linking polymer in keratin-PEO nanocomposite was thoroughly investigated. Keratin is naturally derived polymer, and due to its biocompatibility and biodegradability, it facilitates biomedical applications of composites with keratin phase. Due to similarity with host tissue, amino acid sequences of keratin interact and support cellular attachment and proliferation.149 This would promote such composites as good candidate for wound dressing, scaffolds, drug delivery or water filtration. This would contribute in increase of need for renewable waste materials and production of biodegradable composites, which could be used as good substitute for traditional nanocomposites.

In this study, we report ultrasound functionalization of graphene with polymer with high molecular weight and degree of disintegration (PEO), which resulted in a shorter period of ultrasonic treatment. The functionalization with PEO showed to be an effective method for grafting on graphene surface. The f-G was successfully incorporated in PEO and keratin-PEO blend yielding composites with exceptionally high increase of mechanical properties.

2. Experimental
2.1. Materials

In experimental part, polyethylene oxide (PEO), molecular weight Mw = 600 000 g/mol (ACROS Organics) and graphene nano platelets (Cheap Tubes, plazma-Argon, 1-2 µm diameter, > 99 wt% of purity) were used as purchased. Deionized water (DI) (resistance of 18 MQ cm) was used for the preparation of solutions. Keratin obtained from chicken feathers (waste from poultry plant – Perutnina Ptuj, Slovenia), in aqueous solution 23.15 g/L, was used for preparation of composite films.

2.2. Extraction of keratin from chicken feathers

Keratin was obtained from chicken feathers through the process of extraction. Waste feathers were subjected to pre-treatment, which involved washing, drying and fine milling to a size of 0.5 mm. For removing fatty acids, feathers were soaked in a Soxhlet extractor in petrol ether for 12 h at 40-60 °C. To complete evaporation of petrol ether, feathers were dried in a vacuum oven for 24 h. Thus obtained feathers were treated with an aqueous solution of urea, mercaptoethanol and sodium dodecyl sulfate (SDS). Derived extract was filtered and dialyzed with cellulose membranes (MWCO 6000-8000 Da) for 72 h, yielding the aqueous solution of keratin. Keratin concentration of dialysate was determined using protein assay kit, based on Hartree-Lowry assay.50 Keratin solution was stored at 5 °C until application.

2.3. Grafting graphene with ultrasound

0.5 g of polymer (PEO) was dissolved in 100 ml of deionized water (DI) for 2 h until the polymer was completely dissolved, and then the solution was treated with ultrasound (Sonics Vibra Cell VCX 750 W, 19 mm Ti horn) on 22 kHz and 300 W for 2 h with maintaining temperature at 30 °C.44 At specified time interval a part of the solution was taken for monitoring the degree of degradation through changes of viscosity. Within the process of polymer degradation the optimal condition of irradiation treatment was determined. Considering the optimal conditions, 0.5 g of PEO was dissolved in DI water. After 2 h, solution with addition of 50 mg pristine graphene (p-G) was vigorously stirred for 40 min and then ultrasonically treated at 22 kHz and 300 W for 70 min with constant temperature of 30 °C. The resulting mixture was centrifuged at 2000 rpm for 20 min, decanted and centrifuged again at 2000 rpm for 30 min, in order to remove p-G in the form of black sediment. Obtained black solution with dissolved f-G was filtered (200 nm) under vacuum, and washed 3 times with DI water to remove ungrafted PEO. Figure 1 presents schematic illustration of functionalization.

2.4. Preparation of nanocomposite films

Nanocomposite films were obtained using a simple solution casting method on glass surface. Firstly, neat PEO (0.5 g) was dissolved in DI water for at least 2 h on magnetic stirrer at 40 °C. When the solution became transparent, pristine graphene (p-G) was added until the graphene reached the content of 0.3 wt.% The mixture was stirred for 12 h before solvent casting. The composite films with f-G were prepared in the same manner as p-G. The keratin-PEO blend was obtained by dissolving PEO for 2 h, followed by
mixing with a keratin solution with the content 90/10 ratio (keratin/PEO) for 6-12 h at 40 °C. Other content ratios keratin-PEO is also prepared (95/5, 40/60, 60/40, 5/95; see Supplement Material) as confirmation of benefits proposal blend with 90/10 ratio (in further text marked as Keratin-PEO). The bio-nanocomposite film keratin-PEO/f-G was prepared firstly by forming the polymer blend and then subsequently adding 0.3 wt.% of f-G into polymer blend. The mixture was stirred for 12 h before solvent casting. Formed blends were poured in Petri dishes and dried in oven on 40 °C for 48 h, and then in vacuum oven on 50 °C for another 12 h.

3. Characterization

Elemental analyses were performed using a VARIO EL III Elemental analyzer.
Fourier transformed infrared (FTIR) spectra were recorded in the 4000–400 cm\(^{-1}\) range on a BOMEM spectrophotometer (Hartmann & Braun, MB-series), using the KBr wafer technique. Structural changes of polymer with ultrasonic treatment and structure of composites were identified with optical microscopy (Olympus CX41).
Thermal properties of PEO films were examined in a nitrogen atmosphere from room temperature to 80 °C at a heating rate of 10 °C/min using a differential scanning calorimeter (DSC, Q10 TA Instruments, USA). To determine the melting temperature (T\(_m\)), melting enthalpy (\(\Delta H_m\)) and the degree of crystallinity (X\(_c\)) samples were heated to 80 °C and kept for 10 min. Then, they were reheated at a heating rate of 10 °C/min. Melting temperatures (T\(_m\)) were measured from the second cycle as the temperature at the top of the endothermic peak, T\(_{m\,\text{max}}\). The area under the endothermic peak determined the melting enthalpy, \(\Delta H_m\). The crystallinity degree of films (X\(_c\)) was determined from DSC analysis based on following Eq. (1):

\[
X_{c,\text{PEO}} = \frac{\Delta H_{m,\text{PEO}}}{\Delta H_{m,\text{PEO}}^0 \cdot \omega}
\]

where \(\Delta H_{m,\text{PEO}}\) is the melting enthalpy of PEO and \(\Delta H_{m,\text{PEO}}^0\) is the melting enthalpy of 100 % crystalline PEO (213.7 J/g) for the PEO molecular weight of 600 000 g/mol \(30\), \(\omega\) is mass fraction of PEO.

Thermogravimetric analysis (TGA) was performed using a SDT Q600 simultaneous DSC-TGA instrument (TA Instruments). Samples were heated from the room temperature to 600 °C at a heating rate of 10 °C min\(^{-1}\) under nitrogen atmosphere.

The morphology of the fracture surface of composite films obtained with liquid nitrogen were observed by a field emission scanning electron microscope (FESEM), (JSM 5800, Tescan Mira 3), operated at 2 kV.

The nanoindentation experiments on neat polymer and composite films were performed using a Triboscope T950 Nanomechanical Testing System (Hysitron, Minneapolis, MN) equipped with a Berkovich indenter type in situ imaging mode. A peak load of 2 mN was applied for all samples with the load-hold-unload of 10-20-10 s for each segment. Nine indentation measurements were performed for each sample and the average values and standard deviations are reported.

Dynamic mechanical analysis (DMA, Q800 TA Instruments, USA) was performed successively through two different procedures, both in the film tension clamp mode. First procedure was performed in the multi frequency mode, with frequency range from 0.1-10 Hz, in an isothermal condition of 30 °C and initial amplitude of 1 µm, in order to determine frequency effects on storage modulus. After first, second procedure was consequently performed, so the storage modulus from the end of procedure one was initial storage modulus of the second procedure, and then another sample was measured. A second procedure was performed in order to determine temperature effects on storage modulus and tan δ. The temperature ranged from 30 °C to 120 °C with a heating rate of 3 °C/min, with constant frequency of 1 Hz and amplitude of 15 µm. The samples size was approximately 40 mm × 5 mm × 0.2 mm.

4. Results and discussion

4.1. Elemental analysis of keratin

Elemental analysis of neat keratin determined the contents of: 12.78 % of N and 3.72 % of S. This confirmed a “hard keratin” content of sulfur, which is >3 % according to Tonin et.al. \(^{31}\) Keratin is known to have an amphoteric character owing to the presence of amino and carboxylic functional groups in the molecular structure. The point of zero charge of feather keratin determined by titration method was at the pH 5. The pH value of keratin solution and blend solutions prepared with it were \(\approx 8\), because at this pH most of functional groups were deprotonated, which enabled favorable linking with other components in the blends.

4.2. Structural and thermal analysis of grafted graphene

FTIR spectroscopy was used to reveal interactions in f-G between graphene and PEO attached to graphene surface (Fig. 2.a). FTIR analysis of p-G showed stretching at 2921 and 2815 cm\(^{-1}\) due to C–H methylene group and peak at \(\approx 3426\) cm\(^{-1}\) derived from hydroxyl group. Peak at 1634 cm\(^{-1}\) also derives from vibration of deformation of a hydroxyl group which suits skeletal vibration of stretching C=C bonds within graphene structure. Due to terminal hydrogen groups, FTIR spectra of neat PEO showed stretching at 3429 cm\(^{-1}\). Spectra also revealed symmetric and asymmetric stretching vibrations from methylene group. Strong peak at 1670 cm\(^{-1}\) belongs to deformation of terminal hydrogen group of PEO macromolecule. Peaks at 1460, 1143, 984 and 840 cm\(^{-1}\) derived from CH\(_2\) group (scissoring) and its deformation (twisting and wagging). Asymmetric and symmetric stretching of –C=O–C= ether chain appears at 1389 and 1111 cm\(^{-1}\). FTIR spectra of f-G displayed weak interactions between PEO and graphene, but electrostatic and hydrogen bonding indicated changes without destroying shape or characteristics of composed materials. Changes at 1630 cm\(^{-1}\) refer to stretching interaction
between aromatic C=C graphene skeleton and aliphatic PEO structure.

In Fig. 2b weight loss of p-G is about 3%, due to adsorbed moisture on graphene surface. Comparative TGA analysis of p-G, PEO and f-G determine that the content of PEO decorating graphene surface within f-G is about 7.7%.

![Fig. 2. a) FTIR spectra and b) TGA analysis of pristine graphene, neat PEO and functionalized graphene](image)

DSC analysis of degree of crystallinity indicated 60 minutes of ultrasonic irradiation for functionalization. With further increase of ultrasonic time values of DSC parameters decreased (see Fig. 3a and Table 1. Supplement Material), due to cleavage of C–C bonds under polymer chains which suggest partial or complete polymer degradation. This observation of crystallinity increase was followed by optical microscopy (Fig. 3(b-f)), which revealed growth of spherulites with the increase of ultrasound treatment period. Therefore, use of ultrasonic irradiation provides PEO chain radicals, through polymer degradation, and produces reactive places on graphene surface, induced by ultrasonic cavitation. Also, high intensity ultrasound by shear forces enables exfoliation of graphene into single- or few-layer sheets. So, this one step functionalization of ultrasonic irradiation provides soluble f-G compared to insoluble p-G in water, suggesting covalent grafting of PEO chains onto graphene surface, rather than plain physisorption. Also, Figure S1. (see Figure S1. Supplement Material) reveals a favorable dispersion of f-G, without agglomerates identified with the addition of p-G into PEO. Shen and coworkers, in their study used polymer with low molecular weight and degree of disintegration for functionalization of graphene. Within our research we demonstrated possibility of using polymer with higher molecular weight and degree of disintegration, which resulted in the shorter time of ultrasonic irradiation than one found in literature. This functionalization proved to be effective, because it is easy for processing, less time consuming and resulted in good dispersion of f-G in polar solvents.

![Fig. 3. a) DSC analysis and (b-f) optical microscopy images of crystalline changes of PEO after 0, 10, 30, 60 and 120 min of ultrasound treatment](image)

4.3. Structural and morphological analysis of composite films

Figure 4. shows FTIR spectra of composite films of: neat PEO, PEO with functionalized graphene, keratin-PEO blend composite film and hybrid composite film keratin-PEO/f-G (FTIR spectra of all composite films of neat PEO, PEO/p-G, PEO/f-G, keratin, keratin-PEO and keratin-PEO/f-G are given in Figure S2 in Supplement Material).

![Fig. 4. FTIR spectra of: a) neat PEO, b) composite film PEO/f-G, c) keratin and d) composite film keratin-PEO/f-G](image)
Addition of f-G in keratin-PEO blend system revealed some changes and new signals between 1400 and 800 cm\(^{-1}\) wavenumbers. Appearance of signals at 1400, 1346 and 847 cm\(^{-1}\) detected in PEO/f-G composite and new signals at 990 and 920 cm\(^{-1}\) suggested asymmetric and symmetric vibrations of ether C–O–C bonds and CH\(_2\) group deformation (twisting and wagging) of polymer toward amid III and carbonyl groups on protein. These signals confirmed bonding within constituents, beside enhanced intermolecular hydrogen bonding between nanofiller (f-G) and polymer blend.

Comparative analysis of SEM and optical microscopy of composite films (Fig. 5. and Figure S3 Supplement Material) revealed the influence of crystallinity on composite films, which resulted in improvement of mechanical features. Optical images of composite films with both, pristine and f-G, show slight changes of spherulites, but the SEM images revealed maintained smooth surface of PEO/f-G compared to neat PEO. Morphology of blend with addition of PEO in keratin remains unchanged, because keratin inhibits crystallization process of PEO, from changing morphology of spherulites to total absence of crystallinity with increase of keratin content in keratin-PEO blend (see Figure S4 Supplement Material). Optical images of keratin-PEO and keratin-PEO/f-G show composite films with uniform structure and SEM images of these films show good adhesion of PEO on the keratin fiber surfaces. Addition of f-G supported morphology structure of polymer and polymer blend matrix. In FTIR spectra we observed intermolecular hydrogen bonding between constituents of both composite films, keratin-PEO and keratin-PEO/f-G, which anticipated thermal stability and increase of mechanical properties of these materials.

4.4. Thermal and mechanical characterization

Thermal analysis of keratin indicates weight loss in water and moisture vaporization below 150 °C, followed by decomposition and destruction of keratin structure up to 400-420 °C. Comparison of residual mass at 600 °C addition of f-G in PEO and polymer blend system indicated improvement in thermal stability (Table 1.). DSC analysis showed that addition of graphene structures in polymer led to decrease of crystallinity of nanocomposite films, which is in accordance with work of Shen et al. (Fig. 6.).

<table>
<thead>
<tr>
<th>Sample</th>
<th>ΔH(_m), (J/g)</th>
<th>T(_m), (°C)</th>
<th>X(_m)</th>
<th>Residual mass at 600 °C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO</td>
<td>149.0</td>
<td>64.44</td>
<td>0.70</td>
<td>2.28</td>
</tr>
<tr>
<td>PEO/p-G</td>
<td>117.3</td>
<td>64.83</td>
<td>0.55</td>
<td>1.11</td>
</tr>
<tr>
<td>PEO/f-G</td>
<td>114.8</td>
<td>66.75</td>
<td>0.53</td>
<td>3.07</td>
</tr>
<tr>
<td>Keratin-PEO/f-G</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Fig. 5. Optical images of a) neat PEO, b) PEO/p-G, c) PEO/f-G, d) keratin-PEO and e) keratin-PEO/f-G

Fig. 6. DSC analysis of a) PEO, b) PEO/p-G, c) PEO/f-G, d) keratin-PEO/f-G

Mechanical properties of composite films were studied using nanoindentation and DMA methods. Measured results of reduced elastic modulus and hardness are shown in Fig. 7. (see Table 2. Supplement Material). Addition of small content (0.3 wt.%) of p-G increased the modulus and hardness of PEO nanocomposite for about 5 and 33 %, respectively. Functionalization of graphene significantly increased the composite modulus and hardness for about 92 % and 190 %, respectively, proving in that way the success of proposed ultrasonic graphene functionalization. Incorporation of same content (0.3 wt.%) of f-G in blend of PEO and keratin polymers yielded significant increase of mechanical properties, i.e. modulus and hardness were increased for 155 % and 99 %, respectively. Namely, 10 wt.% of PEO enable changes in keratin to restrain high mechanical properties and provide enough elasticity of blend films with high keratin content.
The practical application of keratin-based products was limited due to their poor strength and flexibility. Neat keratin films are shown to be too fragile for practical use, but the addition of plasticizers resulted in a relatively strong, flexible, and biodegradable films and their potential use as biomaterials in medical applications. We also investigated alternative preparation techniques for creating keratin films with more suitable mechanical properties in addition to creating blended keratin systems with PEO polymers. Because of all the above, material properties of neat keratin and blends with high content of keratin are measured only by nanoindentation since the fragile nature of keratin does not enable the DMA measurement proper boundary conditions. Addition of reinforcements in the form of graphene enables the DMA testing in proper manner. Incorporation of small content of PEO (10 wt.%) and graphene (0.3 wt.%) increased the keratin modulus and hardness for about 64 and 49%, respectively. It is worth noting that the use of 90 wt.% keratin, as waste abundant materials leads to remarkable enhancement of mechanical properties. Benefits of such approach are twofold, namely, the use of bio-waste component contributes to more advanced material and on the other hand, this could lead to the use of significant amount of waste material for fabrication of materials with added value. The improvement of basic polymer mechanical properties is achieved by introducing materials of increased stiffness (keratin and especially graphene), change of crystallinity of polymer systems, more effective distribution and exfoliation of graphene flakes in polymer matrices and achieved bonding of particle and matrix phases.

Storage modulus of neat PEO and blend composite films was determined as a function in multi frequency and temperature mode (Fig. 8.). Due to polymer crystallinity, PEO and keratin show affinity for polymorphic modifications, which represent ability of material to change crystal orientation and crystallinity phases with applied tension force. Slight increase of storage modulus through multi frequency mode is result of orienting crystalline phases of PEO and nanocomposite films. Several studies of overcoming a fragile nature of keratin indicated compatibility for producing films and fibers with high content of keratin with PEO. Nanoindentation measurements demonstrated changes in mechanical properties of keratin-PEO blend (see Figure S5. Supplement Material) and in accordance with nanoindentation results and the inability of measuring films with high keratin content, DMA is used to emphasize the influence of graphene. Addition of graphene increase storage modulus for 23%. Also addition of only 0.3 wt.% graphene provided increase of 92% of storage modulus compared to neat PEO, while the keratin-PEO/graphene show increase for about 151% due to crystallinity changes. These findings are similar and follow the same trend observed in nanoindentation results, which yielded the increases for reduced modulus of same composition for about 5, 92 and 155%.
presented as a function of temperature (Fig. 8b). After procedure one, E’ of PEO film at 30 °C was 453.4 MPa, which significantly decreased at about 85 °C through second procedure. The same behavior showed composite film with graphene structure (p-G and f-G), with rapid decrease at about 90 °C. E’ of keratin-PEO/f-G film showed decrease trend, but it reached a storage modulus value of about 591.7 MPa till the end of investigated temperature range, due to presence of keratin and its polymorphic modification ability. For the sake of the real term applications, scope of testing was due to presence of keratin and its polymorphic modification ability. About 591.7 MPa till the end of investigated temperature range, observed of rubbery plateau above melting temperature sharply due to the melting of the crystalline region in the PEO. Observation of rubbery plateau above melting temperature suggests physical crosslinking of nanocomposite structure with addition of both, p-G and f-G (see Figure S5a. Supplement Material). The tan δ curve of the neat PEO and nanocomposites as a function of temperature is presented in the Supplement Material. (see Fig. S6b.) The tan δ peak magnitudes of nanocomposites compared to neat polymer magnitudes decreases after the loading of graphene and keratin, in the range of 0.49 to 0.13 (at 90 °C). There were very small changes of the storage modulus and tan δ in the rubbery region of the keratin-PEO/f-G sample. This probably happens because keratin inhibits PEO crystallization process and PEO interferes with the keratin self-assembly at the appropriate level by inducing protein structure with high thermal stability. Results of this study demonstrated the possibility of fabrication of composite blend films with high content of keratin from chicken feather waste. To overcome the fragile nature of neat keratin, blending keratin with small content of PEO and incorporation of ultrasonically treated graphene produces films with remarkable mechanical properties, revealing the possibility of using widely available bio-waste material, such as keratin for fabrication of functional materials with added value.

**5. Conclusion**

This study confirmed ultrasonic irradiation as an effective method for grafting on graphene surface with PEO. Keratin-PEO/f-G bio-nanocomposite films have been successfully fabricated by solvent casting method. Compared to neat PEO addition of f-G show increase for 92 % of storage and reduced moduli observed in DMA and nanoindentation results. Nanoindentation testing shows that the incorporation of 0.3 wt.% f-G increased reduced modulus and hardness of keratin-PEO blend for about 155 and 99 %, respectively. Reinforcement is generated due to crystallinility changes and effective load transfer between reinforcing and matrix phases. These findings open possibilities for utilization of abundant waste keratin for fabrication of new materials with significantly improved mechanical properties.

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**References**


Graphical Abstract
85x73mm (300 x 300 DPI)