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Protein adsorption of radiation functionalized LDPE sheets

Amr El-Hag Ali · Naeem M. El-Sawy ·
El-Sayed A. Hegazy · Ahmed Awadallah-F

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Abstract Low density polyethylene (LDPE) sheets were modified by radiation-induced graft copolymerization of N-vinyl pyrrolidone (NVP) and acrylonitrile (AN) binary comonomer of different molar ratios. The factors affecting the radiation modification process were optimized. The radiation-modified LDPE sheets were further modified via the chemical treatment of the grafted nitrile groups with thiourea. The modification processes were confirmed by FTIR and the modified LDPE sheets were characterized by studying their swelling behavior. The protein adsorption ability of the modified LDPE sheets was examined towards creatinine, as protein derivative and γ-globulin fraction (II). Adsorption kinetics were calculated and possibility of selective adsorption was evaluated.

Keywords LDPE · Grafting · Radiation · Protein adsorption

Introduction

The attachment of proteins to surfaces has many applications in the food, chemical, biotechnology, and medical diagnostic industries [1]. New applications in these industries using nanotechnology have been developed, including biosensors and micro-arrays for testing blood serum for the presence of proteins associated with diseases [2]. Procedures that can result in increased protein attachment to surfaces and achieve longer times over which the attached proteins remain functional are therefore valuable. Polymer plastics are suitable as surfaces for attaching proteins because of their lightness, strength, ease of forming into shapes [3], stability in air, and resistance to corrosion or breakdown in many harsh chemical environments. Protein adsorption

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A. El-Hag Ali (✉) · N. M. El-Sawy · E.-S. A. Hegazy · A. Awadallah-F
Department of Polymer Chemistry, National Center for Radiation Research and Technology,
Atomic Energy Authority, P.O. Box 29, Nasr City 11731, Cairo, Egypt
e-mail: elhagali_a@hotmail.com

onto polymeric materials is a very complex process which is the overall result of various types of interaction between the polymeric material, the solvent (water) and any other solutes such as ions present in buffer [4]. The sum of attractive and repulsive forces between a protein and material will determine the extent of deposition [5].

The incorporation of hydrophilic groups onto the polymeric surfaces is an effective pathway to increase the density of cellular attachment by reducing the contact angle of the treated surface [6]. An effect of chemical treatment is the introduction of oxygen-containing groups onto the surface, which may be important in the attachment process [7]. There are two main mechanisms that explain the attachment of proteins to the surfaces; covalent bonding via a suitable chemical linker and physical sorption to the surfaces. In the time, the attachment of proteins to surfaces via covalent bonding offers more permanent attachment, it suffer from serious disadvantages such as high cost of chemical linker and the possibility of the bonding via inappropriate binding site in the protein which may negatively affect its activity. The process of protein adsorption from an aqueous solution onto a solid surface is typically described in three steps. Firstly, transportation of the protein from the solution towards the solid surface occurs. This is followed by attachment of the protein to the surface, and finally the protein structure undergoes a conformational change after adsorption [8].

The surface modification methods can be grouped into general categories: physical adsorption, grafting coupling, and grafting copolymerization. Grafting copolymerization of hydrophilic polymer chains to polymeric surfaces has been considered as a promising technique for improving their physical properties. Radiation-induced graft copolymerization is a well-established technique for producing polymeric materials that combine the chemical and physical properties of both the base polymer and the grafted monomer [9]. The use of γ-radiation gives a uniform distribution of radical initiating sites and graft copolymer properties can be varied in a predicted and controlled manner.

Polyacrylonitrile (PAN) and acrylonitrile-based copolymers have been successfully applied as membrane materials for hemodialysis [10], ultrafiltration [11], enzyme-immobilization [12], and pervaporation [13]. However, due to the relatively poor hydrophilicity and/or biocompatibility for this type of membrane, some additional processes, such as chemical modification, must be achieved to enhance the ability for protein adsorption. On the other hand, polyvinyl pyrrolidone (PVP) possesses excellent biocompatibility with living tissues and extremely low cytotoxicity.

To improve the protein adsorption properties of the low density polyethylene (LDPE) sheets, AN and NVP was grafted onto the LDPE sheets by means of ionizing radiation-induced grafting and copolymerization. The grafting conditions such as comonomer composition and concentration and irradiation dose were optimized to achieve the optimum grafting degree. The physical and chemical properties of the grafted sheets were characterized using FTIR, and studying their swelling behavior and thermal stability. Chemical treatment was carried out to the nitrile group of acrylonitrile to improve their protein adsorption properties. The effect of preparation conditions and chemical treatment on the adsorptivity of the grafted sheets was investigated. The competitive and cumulative adsorption of creatinine as a protein derivative and γ-globulin was studied.

Experimental

Materials

Low-density polyethylene (LDPE) produced by (Shooman Co. for plastic, Cairo, Egypt). Acrylonitrile (AN) and N-vinyl-2-pyrrolidone (NVP), of purity (99% Merck, Germany) were used without further purification. Other chemicals such as solvents and inorganic salts were reagent grade and used as received.

Radiation functionalization of LDPE sheets

LDPE sheets were washed with acetone, dried at 50 °C in oven until constant weight is reached and then immersed in the monomer-solvent mixture in glass tubes. The direct radiation-induced grafting was used as a technique in nitrogen atmosphere. The glass tubes containing the monomer solution and the polymer substrates were subjected to ^{60}Co-gamma irradiation at dose rate 4.6 kGy/h. The grafted polymers were removed and washed thoroughly with excess dist. water in order to remove the homopolymer that may be formed, and then soaked overnight in dist. water to extract the residual monomer and homopolymer, which may included in the grafted polymer. These grafted polymers were then dried in oven at 40 °C for 24 h and weighed. The degree of grafting was calculated by using the following equation:

$$\text{Degree of grafting } (\%) = \frac{\left(W_g - W_o\right)}{W_o} \times 100$$

where W_o and W_g represent the weights of original and grafted LDPE sheets, respectively.

Fourier-transform infrared (FTIR) measurements

Mattson 1000, Unicam, England in the range from 400 to 4,000 cm^{-1} was used to confirm the formation of graft copolymer.

Treatment of grafted LDPE by thiourea

The grafted LDPE-g-(PAN/PNVP) sheets were reacted with Thiourea in alkaline ethyl alcohol solution at 70 °C for 24 h. The complete conversion of $\mathrm{C{\equiv}N}$ was determined by using FTIR spectra.

Protein adsorption experiments

Batch adsorption experiments were carried out by shaking a sample (LDPE-g-(PAN/PNVP) treated with thiourea of apparent surface area of 40 cm^2 with 10 mL aqueous Creatinine or γ-Globulin fraction (II) buffer solution of pH 8 at 30 °C for 24 h. The amount of Creatinine or γ-Globulin fraction (II) adsorbed at various conditions such as soaking time and initial solution concentration was calculated by the difference of the concentrations in mg/g before and after adsorption using Jasco UV/V is spectrophotometer model V-S60 at 236 and 202 nm for Creatinine or γ-Globulin fraction (II), respectively.

Scanning electron microscopy (SEM)

Surface morphology of the specimens was investigated by JEOL JEM-5400 (Japan) scanning electron microscope (SEM) after gold deposition in vacuum for 3 min.

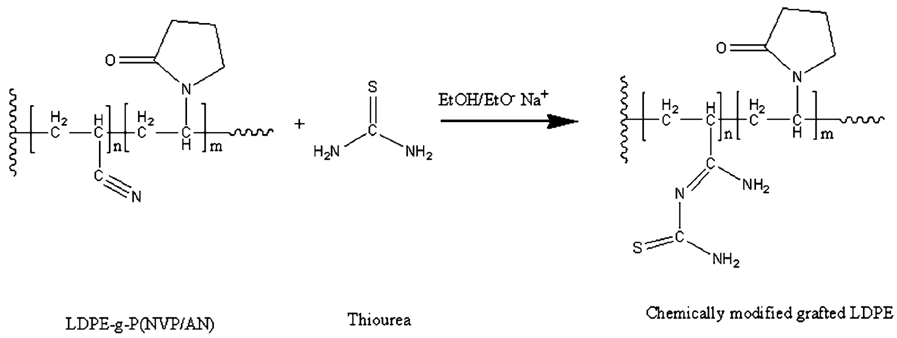
Results and discussion

The physical and chemical properties of LDPE sheets were improved via radiation-induced graft copolymerization of AN/NVP binary comonomer in our previous article. In such article, the parameters controlling the radiation grafting process were optimized to produce suitable and homogeneous-grafted LDPE sheets with considerable grafting yield [14].

Chemical treatment of the cyano groups

Polyacrylonitrile and AN-based copolymers are particularly appropriate for chemical improvement due to the reactive nitrile groups present within the polymer chains. The chemical modification of AN-based copolymer has been the object of numerous studies since these modification lead either to the improvement of the physico-chemical properties or to the obtaining polymers with new properties. The mechanism of such treatment is extensively studied [15]. Scheme 1 shows a schematic diagram for the chemical modification of LDPE-g-(PNVP/PAN) sheets with thiourea.

Figure 1 shows the FTIR spectra of the untreated grafted LDPE, LDPE-g-(PAN/PNVP) treated with thiourea in comparison to the blank LDPE. The FTIR spectrum



Scheme 1 Schematic diagram represents the chemical modification of LDPE-g-P(NVP/AN) sheets with thiourea

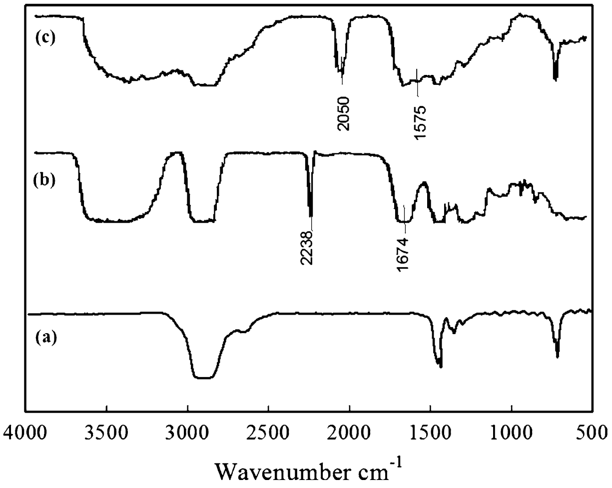


Fig. 1 FTIR spectra for (*a*) blank LDPE, (*b*) LDPE–g-(PAN/PNVP) and (*c*)Treated LDPE-g–(PAN/PNVP) films by thiourea, respectively, at degree of grafting (350 wt%) containing comonomer solution composition 50:50 mol%

of LDPE-g-(PAN/PNVP) shows the characteristic sharp band at 2,238 cm^{-1} that belong to C≡N groups and absorption bands appeared at 1,350 cm^{-1} and 1,674 cm^{-1} that belong to C–N and stretching vibration of the C=O groups of pyrrole rings of PVP. In addition, a new band appeared at the range of 3,200–3,600 cm^{-1} which may due to some water associated with the carbonyl groups of pyrrole rings.

The chemically treated grafted films shows the disappearance of the absorption band at 2,238 cm^{-1} corresponds to cyano- groups and instead new bands appears at 1,575 and 2,050 cm^{-1} which assigned to C=N and N=C=S groups, respectively. Such new bands confirm that the reaction of the cyano groups with thiourea as a result of thiourea treatment and the formation of $H_2N–C=N–CH_2NS$ instead of C≡N.

Effect of chemical treatment on the hydrophilicity of the grafted sheets

The process of protein adsorption to hydrophilic or hydrophobic surfaces is related to the capability of the protein molecule to displace water molecules bounded to such surface. Hence, the surface hydrating water molecules play mediating or moderating agent in protein–surface interaction process [16].

The improvement in the hydrophilicity of the LDPE sheets as a result of grafting with (PAN/PNVP) and chemical treatment with thiourea are shown in Fig. 2. The figure shows that the hydrophilicity of LDPE sheets remarkably increased by increasing the degree of grafting. Further increment in the hydrophilicity of the grafted sheets as a result of chemical modification because the conversion of the hydrophobic nitrile groups into the hydrophilic $H_2N–C=N–C(NH_2)=S$ groups.

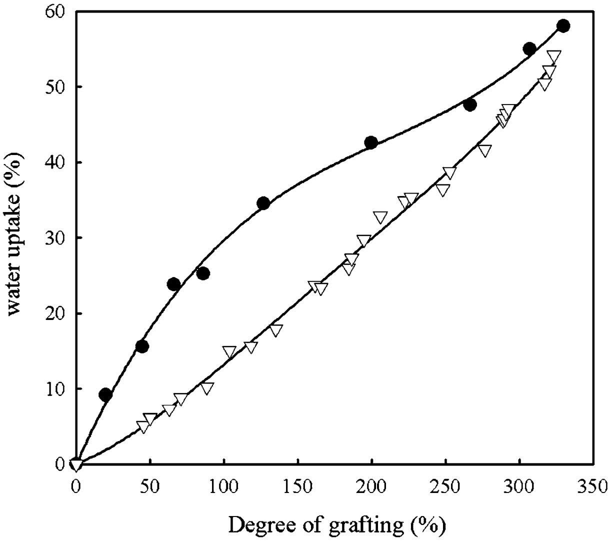


Fig. 2 The effect of degree of grafting and chemical treatment on their water uptake versus degree of grafting LDPE-g-(PAN/PNVP) (*open inverted triangle*) and treated grafted LDPE with thiourea (*filled circle*) at grafted comonomer composition 50:50 mol%

Adsorption of proteins and their derivatives on the radiation functionalized sheets

Recently, wide applications of protein-based polymers have been growing fast in many applications such as separation, adsorption and immobilization of proteins to make scaffold for biotechnology applications [10–13]. These scaffolds were designed for a specific function using the biologically available 20 amino acids and subsequent chemical modification of side chains with functional groups to mimick the natural protein [17, 18]. In addition to the production and disposable of protein-based polymers which could be used in an environmentally friendly manners. In fact, there are critical parameters governing the proteins adsorption process onto a solid surface are enthalpic and entropic forces related to the free energy of interaction between protein molecules and the substrate surface, which, in turn, depends on the surface free energy parameters of the interacting systems [5].

Effect of creatinine initial solution concentration

Figure 3 shows the relationship between the amounts of creatinine adsorbed (mg/g) and its solution concentration (mg/L). It was observed that by increasing the initial solution concentration of creatinine, the amount of creatinine molecules adsorbed on treated LDPE-g-(PAN/PNVP) films increases. This increment of adsorption of creatinine reaches 53 mg/g then level off. Such increment in adsorption by increasing the creatinine concentration might be attributed to the increment of the

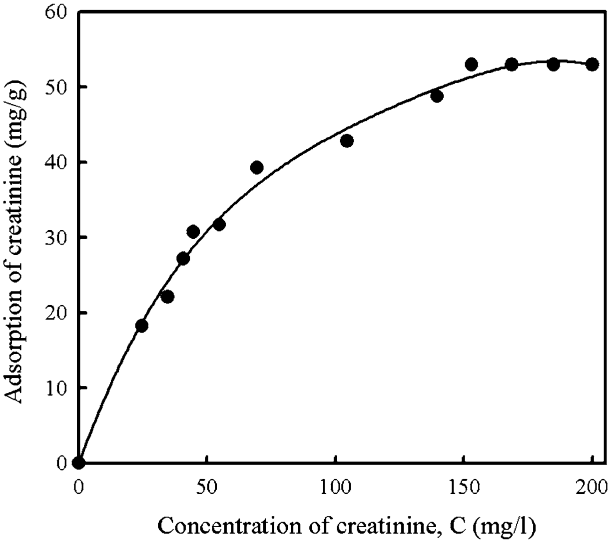


Fig. 3 Effect of the initial solution concentration of creatinine on the adsorption capacity of LDPE-g-(PAN/PNVP) film by thiourea

probability number of creatinine molecules into solution, consequently, it is increasing the chances to react with active functional groups of treated grafted films.

Adsorption isotherm of creatinine

Freundlich equation is considered one of the most widely used mathematical model to show the relationship between adsorbate concentrations and adsorbents. Figure 4 displays an empirical expression encompassing the surface heterogeneity and exponential distribution of active sites and their energies. Freundlich equation was tested in the following form [19]:

$$\log C_{\mathrm{ads}} = \log A + 1/n \log \mathrm{Ce}$$

where C_{ads} is the amount of adsorbate, C_{e} is the amount of adsorbate in liquid phase at equilibrium, A and $1/n$ are characteristic Freundlich constants. The results give the calculated value of $A = 4.36$ which indicates to surface heterogeneity and $1/n = 0.49$ indicating to intensity of sorption and sorption capacity.

It is possible to calculate and know the features of heterogeneity of energies over the surface by using the Dubinin-Radushkevich model as shown in Fig. 5, which expresses the linear form of Dubinin-Radushkevich isotherm as below [20]:

$$\ln C_{\mathrm{ads}} = \ln X_{\mathrm{m}} - \beta\varepsilon^2$$

$$\varepsilon = \mathrm{RT} \ln\left[1 + \frac{1}{C_{\mathrm{e}}}\right]$$

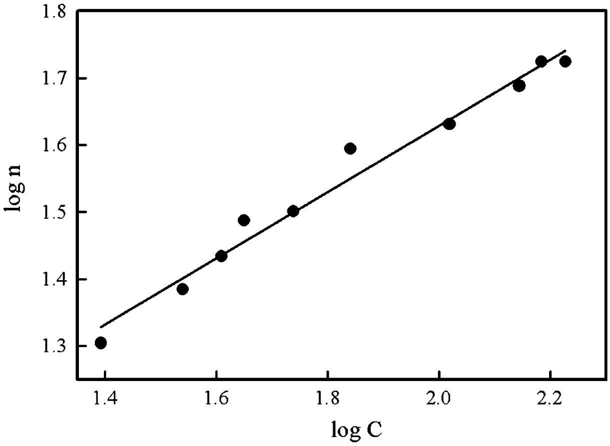


Fig. 4 Achievement of Freundlish equation on adsorption of creatinine molecules

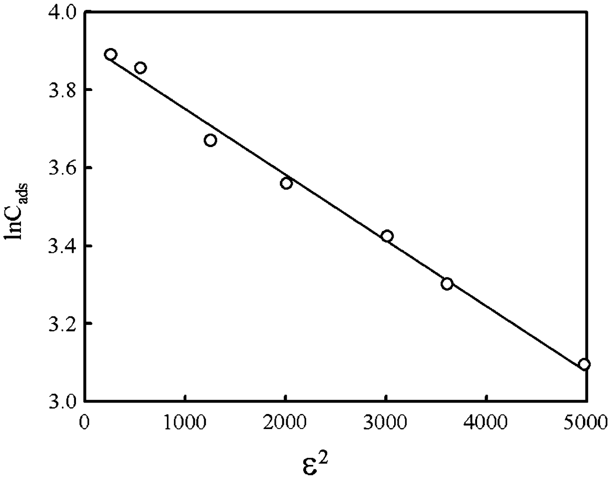


Fig. 5 Achievement of Dubinin-Radushkevich model for adsorption of creatinine molecules

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Also, the mean sorption energy is deduced as follows:

$$E = \frac{1}{\sqrt{-2\beta}}.$$

Figure 5 shows the linear relationship between ln C_{ads} and ε^2 the slop of such relation represents activity coefficient (β) and intercept represents maximum amount of adsorbate on the adsorbent surface (X_{m}). From the results, it was found that the activity coefficient (β) = -1.76×10^{-4} and maximum amount of adsorption (X_{m}) required to cover all surface is 51 mg/g. Consequently, the transfer of one mole of creatinine molecules to the surface of treated LDPE-g-(PAN/PNVP) film requires a value of free energy (*E*) of 51.90 J/mol.

Selectivity between creatinine and globulin molecules in mixture

Figure 6 shows the selective affinity of the prepared functional polymer films towards creatinine and gamma globulin fraction (II) molecules during adsorption process at initial concentration 29 mg/L. It was observed from the results that the creatinine molecules were adsorbed about eight times more than gamma globulin fraction (II) molecules.

The results suggest that the selectivity of treated LDPE-g-(PAN/PNVP) films towards creatinine molecules is higher than that of gamma globulin fraction (II). Such affinity might due to the lower molecular weight and smaller stereo dimensions of creatinine molecules which allows more opportunities to creatinine molecules to easily penetrate the matrix of polymer substrate and adsorb almost individually on the surface.

SEM investigation of creatinine and globulin molecules adsorption on treated LDPE-g-(PAN/PNVP) films by thiourea

Scanning electron micrographs of the grafted LDPE films, treated grafted LDPE films with thiourea, creatinine and gamma globulin fraction (II) molecules adsorbed separately on treated LDPE-g-(PAN/PNVP) films by thiourea at initial concentration 29 mg/L were shown in Fig. 7a–d, which depicts the morphological structures of different films surfaces.

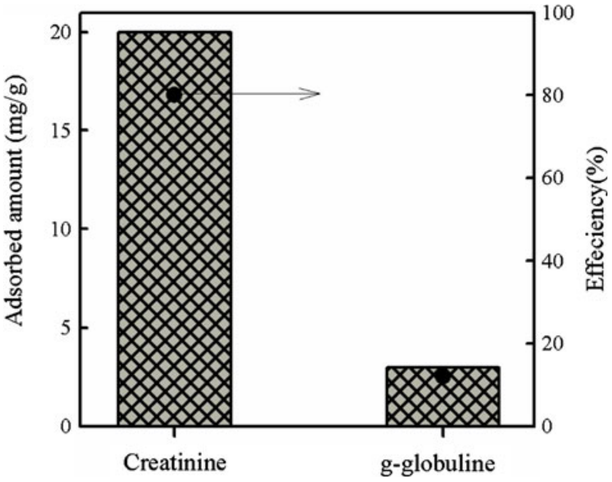

Fig. 6 Selectivity capacity of treated LDPE-g-(PAN/PNVP) films by thiourea dependable upon adsorbate-type

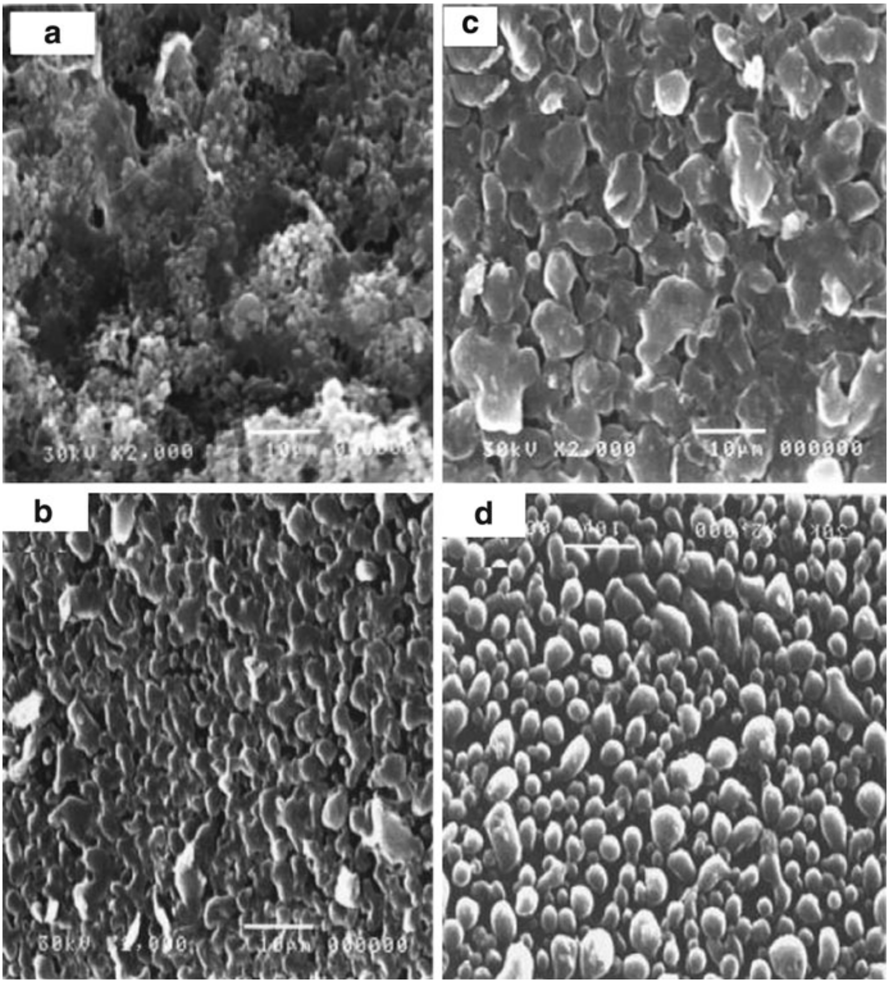

Fig. 7 SEM micrographs of **a** LDPE-g-(PAN/PNVP) film, **b** treated LDPE-g-(PAN/PNVP) film by thiourea, **c** creatinine molecules adsorbed on treated LDPE-g-(PAN/PNVP) film by thiourea, and **d** gamma globulin fraction (II) molecules adsorbed on treated LDPE-g-(PAN/PNVP) film by thiourea, respectively

It can be seen that the grafted LDPE films were found their morphological structure to be as shallow forest Fig. 7a. Meanwhile, Fig. 7b depicts the morphological structure of treated LDPE-g-(PAN/PNVP) films by thiourea as cracks in thirsty earth. Figure 7c describes the morphological structure of adsorption of creatinine molecules on the treated LDPE-g-(PAN/PNVP) film by thiourea in which was shown as nutshells of fish. While, Fig. 7d illustrates the morphological structure of immobilized gamma globulin fraction (II) molecules on treated LDPE-g-(PAN/PNVP) film surface by thiourea and it was depicted as caviar.

Conclusion

Low density polyethylene films obtained hydrophilic character via importing hydrophilic groups to its structure by means of radiation-induced grafting of NVP/AN binary comonomer followed by chemical modification of the nitrile groups with thiourea. Parameters that may affect the radiation grafting process were optimized and the prepared modified films were characterized. The results clearly show that the improvement in the hydrophilic character acquired the modified films the ability to absorb globular proteins such as γ-globulin fraction II and protein derivatives such as creatinine. The protein adsorption isotherms were estimated and possibility for selective adsorption was examined.

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