Immobilization of Some Biomolecules onto Radiation-Grafted Polyethylene Beads for Possible Use in Immunoassay Applications

S. Lotfy,[1] K. A. Moustafa[2]

[1]*National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City 11731, Cairo, Egypt*
[2]*Hot Labs. Center, Atomic Energy Authority, P.C. 13759, Cairo, Egypt*

Received 6 July 2010; accepted 3 January 2011
DOI 10.1002/app.34076
Published online 22 September 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Functionalization of polyethylene (PE) beads was accomplished via radiation induced graft copolymerization of acrylic acid/acrylamide (AAc/AAm) binary comonomer of different compositions onto such beads. Factors affecting the grafting yield were optimized and the occurrence of the grafted chains was confirmed by following the FTIR spectra of the grafted beads. SEM analyses were used to follow the variation of the morphology of the grafting and immobilization onto PE beads. Some bio-active molecules such as Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Thyroid-stimulating hormone (TSH), and Prolactin were immobilized to the radiation functionalized PE beads. The parameters may affect the immobilization process such as degree of grafting, temperature, and pH of the coupling buffer and the coupling period were investigated. The obtained results show that the grafting of AAc offers a better immobilization environment than those of AAm and their copolymer. It is found that the highest immobilization degree would be achieved at pH 7 and 37°C for 24 h. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 3725–3733, 2012

Key words: radiation; grafting; immobilization and immunoassay

INTRODUCTION

The development of immunoassay technology is a success story especially for the clinical laboratory and still continues to be a vibrant area of research.[1] The development of a wide variety of solid phase separation methods has contributed significantly to the reliability and simplification of immunoassays. Antibody-coated beads or tubes are very popular and most convenient but are usually expensive and require great skill and control at all stages of manufacture.[2] An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result. An antibody–antigen complex is also known as an immuno-complex. "Immuno" refers to an immune response that causes the body to generate antibodies, and "assay" refers to a test. Thus, an immunoassay is a test that utilizes immunocomplexing when antibodies and antigens are brought together. Immunoassays are different from other types of laboratory tests, such as colorimetric tests, because they use antibody–antigen complexes to generate a signal that can be measured. In contrast, most routine clinical chemistry tests utilize chemical reactions between the reagents (a solution of chemicals or other agents) and patient sample to generate a test result.

Radioimmunoassay is the most widely used technique for measuring relatively low concentrations of hormones in plasma. In many immunoassay techniques, the analyte is quantified by removing the unreacted (free) label in the system from the reacted (bound) label. This entails either physically absorbing the unreacted labeled compound or precipitating the bound label using another antibody. Modern procedures use solid phase techniques where the immune reaction takes place on a solid surface, allowing the unreacted components to be easily washed away, thereby leaving only bound label to be detected. Many different types of solid phases are employed including microtitre plates, plastic test tubes, plastic beads, latex micro particles, and plastic films.[3]

The immobilized biomolecules FSH (Follicle-stimulating hormone), LH (Luteinizing hormone), TSH (Thyroid-stimulating hormone), and Prolactin are often used in conjunction with each other in the workup of infertility in both men and women. Their levels are used to help determine the reason a man has a low sperm count, also useful in the investigation of menstrual irregularities, and to aid in the diagnosis of pituitary disorders or diseases involving the ovaries or testes. In children, FSH and LH are

Correspondence to: S. Lotfy (samaz711@yahoo.com).

used to diagnose delayed or precocious (early) puberty. LH is sometimes measured in relation to gonadotropin releasing hormone (GnRH) to distinguish between primary or secondary disorders involving the hypothalamus, pituitary gland, or the gonads. Subsequent blood samples are drawn at specified times and the level of LH is measured.

FSH, LH, TSH, and prolactin testing is used to: diagnose a thyroid disorder in a person with symptoms, screen newborns for an underactive thyroid, monitor thyroid replacement therapy in people with hypothyroidism diagnose and monitor female infertility problems, help evaluate the function of the pituitary gland (occasionally), and screen adults for thyroid disorders, although expert opinions vary on who can benefit from screening and at what age to begin. Prolactin levels are used, along with other tests, to help determine the cause of galactorrhea, determine the cause of headaches and visual disturbances, diagnose infertility in females, diagnose prolactinomas, evaluate anterior pituitary function (along with other hormones) and monitor treatment of prolactinomas and detect recurrences. So the immobilization of such macromolecules will be economically useful for testing their levels with locally-prepared kits.

Graft polymerization is a well-known method for modification of the chemical and physical properties of polymeric materials, and is of particular interest for achieving specifically desired properties as well as excellent polymer quality, since various commercial polymers can be used as the grafting substrate. Graft polymerization can be achieved by ionizing radiations, UV, or chemical initiators. Radiation grafting is one of the most promising methods because of its large penetration into polymer matrix, rapid and uniform formation of active sites for initiating grafting throughout the matrix.[4,5]

The radiation-induced grafting of acrylamide (AAm) and acrylic acid (AAc) onto polyethylene (PE) is of great importance as modified PE is of interest for various applications (membranes, biomaterials, coatings, and so on). The grafting technique and properties of modified surface of PE has been described in detail.[6] There is considerable practical interest in PE modified by the grafting of acrylic acid especially for the synthesis of biocompatible materials. PE, containing complexes formed between polyacrylic acid (PAAc) and polybasic on its surface, which make it a very promising material from the point of view of use in medicine. In the last case, the hydrophilicity of PE modified by PAAc is very important.[4,7]

In this study, acrylic acid (AAc), acrylamide (AAm) individually and copolymer were selected as the hydrophilic monomers to provide two different types of functional groups, to the surface of PE beads to use it as a solid support for immobilization of some biological molecules.

EXPERIMENTAL

Materials and methods

Polyethylene beads are produced by (El-Nasr for Medical Supplies, Egypt). Acrylamide (AAm) and Acrylic acid (AAc) of purity, 99.9%, was supplied by (Merck, Germany). FSH, LH, prolactin and TSH antigen or tracer were obtained from Siemens medical solutions diagnostics company USA. Water was double distilled shortly before use. The other chemicals, such as, solvents, inorganic salts, and other reagents were reagent grade and used without further purification.

Graft copolymerization

Polyethylene beads were washed with acetone, dried in a vacuum oven at 50°C, weighed, and then immersed in aqueous monomer solutions of AAc, AAm, and their binary comonomer of different concentrations and compositions in a Pyrex bottle. The reactant aqueous solution mixtures in the glass bottle were exposed to gamma radiation from a 60Co source at a dose rate about 6 kGy h^{-1} using a gamma cell available in NCRRT, AEA, Cairo, Egypt.

The grafted polyethylene beads thus obtained, were removed and washed thoroughly with hot distilled water, and then soaked overnight in distilled water to eliminate the residual monomer and homopolymer. The polyethylene beads were then dried in vacuum oven for 24 h at 50–60°C and then weighed. The degree of grafting was calculated as follows:

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

where W_o and W_g represent the weights of the initial and grafted fibers, respectively.

(FTIR) and spectrophotometric measurements

Analysis by IR and UV spectrophotometer were carried out using Jasco Fourier Transform Infrared spectrophotometer and spectrophotometer, Japan. The measurement was carried out in the region of wave number 4000–400 cm^{-1} keeping air as a reference. Analysis by UV spectrophotometer was carried out in the range from 190 to 900 Å.

Scanning electron microscope (SEM)

The surface topography of the polyethylene beads were studied using JEOL SEM-25 scanning electron

microscope. Prior to examination, the samples were dried under sputter-coated gold.

Immobilization of some biomolecules onto the surface of prepared beads

The immobilization of some antigens namely; FSH, LH, Prolctin, and TSH was performed using variable concentrations of antigens. A number of 100 beads were incubated with various antigens of variable concentrations in 10 mL of 0.01*M* phosphate buffer at variable pH values. The beads were then washed three times with 0.01*M* phosphate buffer saline. The blocking step was performed by addition of 10 mL of 0.01*M* phosphate buffer saline containing 0.5 g % BSA, then the beads were incubated for 1 h, then washed with 10 mL of 0.1*M* phosphate buffer saline and kept in such phosphate buffer saline at refrigerator.

Assay performance

All reagents were raised at room temperature and into a corresponding plastic test tubes the following were added: one coated bead with a specific antigen and 200 μL ^{125}I anti-FSH, LH, Prolactin, or TSH according to the type of the bead used then the tubes were mixed gently and incubated for 24 h at room temperature, then each tube was decanted and washed two times with 2 mL D.W. then counted for 60 s using gamma counter to detect the bound fractions.

Determination of bound protein concentrations using Ponceau S stain

The bound protein was determined by staining with Ponceau S.[8] PE-beads (blank, grafted and bounded) were immersed for 1 h into a solution of Ponceau S (0.1% (w/v), in 5% acetic acid) then washed three times with water, immersed for 1 h into 5% acetic acid and again washed three times with water. Then, the protein/dye complex was quantitatively eluted with 3.0 mL 100 m*M* NaOH solution (1 h). Equilibration of the beads with each solution was enforced by using a Vortex mixer. The beads were removed, the solutions were neutralized by addition of 50 μL 6*M* HCl, and the absorbance of the solution was measured at 515 nm. Protein amounts were calculated based on a calibration which was performed by applying known BSA amounts ($m = 0.001$–1 mg).

RESULTS AND DISCUSSION

Radiation modification of inert polymeric materials was a subject of interest for many research groups to import and mix different chemical characteristics to such substrates. Although radiation-induced grafting of either AAc, AAm, or their binary comonomer onto LDPE was studied by number of researchers,[9–11] it was important to optimize the grafting conditions to produce homogeneously-grafted PE pellets with degree of grafting appropriate for the proposed application. Following the literature, according to literature Mohr's salt (ammonium ferrous sulfate) of concentration 0.1*M* and sulfuric acid of concentration 0.2*M*[12] were added to the reaction medium to suppress homopolymer formation, and facilitating the isolation of the resulting copolymer.[13]

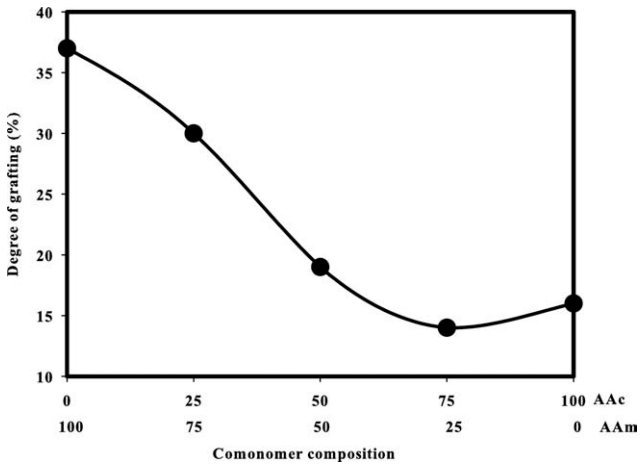

Figure 1 Effect of the comonomer composition on the degree of grafting, monomer concentration; 20%, H_2SO_4 concentration; 0.2*M*, Mohr's salt concentration; 0.1*M*, irradiation dose; 20 kGy.

Optimizing the factors affecting the radiation functionalization process

Enriching the functionality of the inert polymeric substrate would be achieved via the introduction of two functional groups of different chemical nature and behavior. Therefore, it is important to study the effect of the comonomer composition on the degree of grafting that the presence of more than one monomer may lead to the retarding or the enhancement of the grafting process. The grafting of acrylamide and acrylic acid in a binary monomer mixture at various compositions onto PE beads were investigated at an overall comonomer concentration of 20 wt %. The results obtained are illustrated in Figure 1. It is observed that the grafting yield increases with the decrease in the acrylic acid content in the system. Its significant increases start at AAc/AAm composition (75/25).

The influence of comonomer concentration on the grafting process may affect its kinetic parameters. Solvent which may favor the diffusion of the comonomer into the trunk polymer may also dilute the comonomer and result in the reduction of the

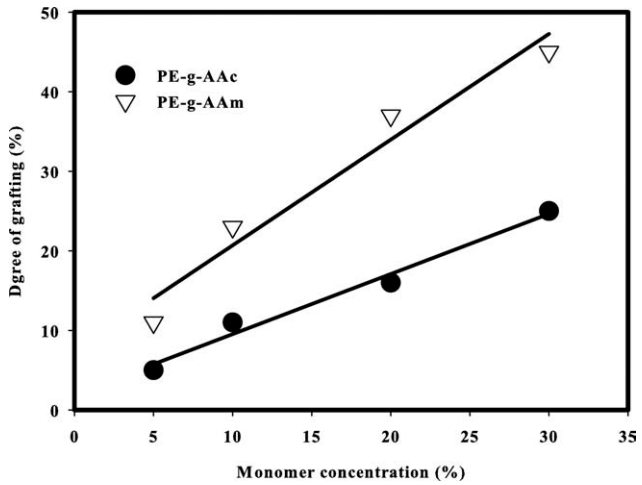

Figure 2 Effect of the monomer concentration on the degree of grafting, H_2SO_4 concentration; 0.2*M*, Mohr's salt concentration; 0.1*M*, Irradiation dose; 20 kGy.

propagation rate. The influence of monomer concentration on grafting reaction was studied at a constant dose of 20 kGy. The grafting percentage of AAc and AAm onto PE beads as a function of monomers concentration under the conditions of our experiment is shown in Figure 2. As the concentration of monomers increases, the grafting degree also increases. The initial increase in the grafting yield is due to the higher probability of association between the monomers and polymer macroradicals when higher amounts of monomers are present. Thus, more concentrated solutions lead to an increase in the propagation reaction. There is an optimum composition of the diluents and monomer at which the effect of chain transfer is more pronounced and also in the initiation of new grafting sites with longer chain higher degree of grafting is obtained. The selective monomers concentrations not more than 30% as the planed work need low graft percent. Generally, the initial rate should be largely dependent on the diffusibility of monomer into the polymer matrix. The same behavior has been observed by other researchers in the grafting of vinyl monomers on different polymers.[14]

Infrared analysis was made for the grafted PE beads to confirm the formation of the graft copolymer and the biomolecules coupling is shown in Figure 3. The surfaces of the ungrafted and the grafted PE beads were analyzed by FTIR spectroscopy. The peaks between 2942 and 2866 cm^{-1} correspond to the various aliphatic CH stretching modes. The peaks near 1450 and 1380 cm^{-1} are the CH_2 and CH_3 deformation bands, respectively. The grafted PAAc layer is characterized by the carbonyl C=O stretching band at 1732 cm^{-1}, and C—O band at 1100–1250 cm^{-1} and carboxylateion peak at 1500 cm^{-1}[15] and finally stretching broad band of OH peak in the range of 2900–3300 cm^{-1}. The presence of polyacrylamide chains is indicated by the spectrum of the modified copolymer that shows a strong absorption near 1650 cm^{-1} involving C=O stretching and NH_2 deformation (amide I and amide II bands) and a broad absorption band around 3400 cm^{-1}, which is a doublet, attributed to asymmetric and symmetric NH_2 stretching.[16]

Immobilization of the bioactive molecules

Rapid and reversible

The protein (hormone) staining and the reversible staining of hormone on the surface of the PE-grafted beads and also the release of the protein/dye complex (biological molecule/Ponceau S stain) during the elution process of biological molecule from the grafted beads within 1 h are confirmed using the Ponceau S stain,[17] which is a rapid and reversible staining method for locating protein that were used to confirm the bound of the hormones on the surface of the grafted PE beads.

Influence of copolymer composition on the bound of biomolecules

Figure 4 illustrated that the bound percent of biological molecules occurred as function of the copolymer composition. From the figure, it is obvious that the use of PAAc as grafted chains possess the

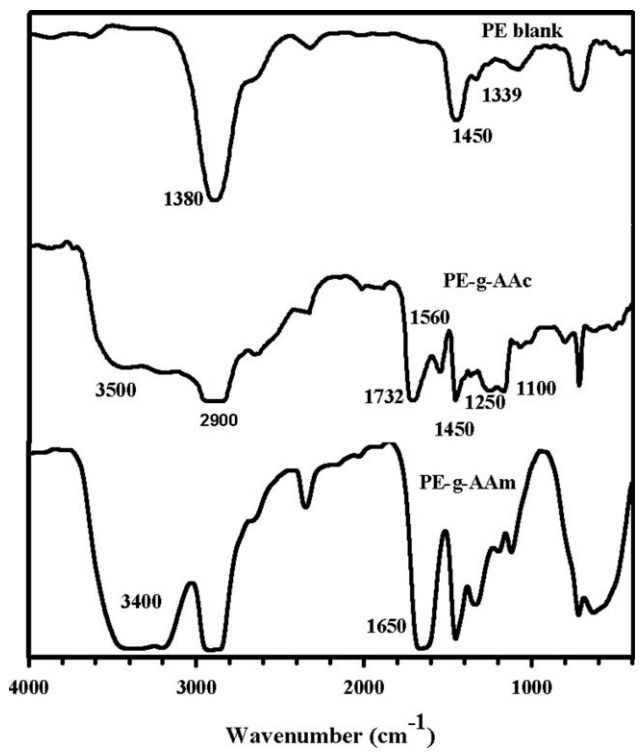

Figure 3 FTIR for the ungrafted PE bead, PE-*g*-AAc and PE-*g*-AAm.

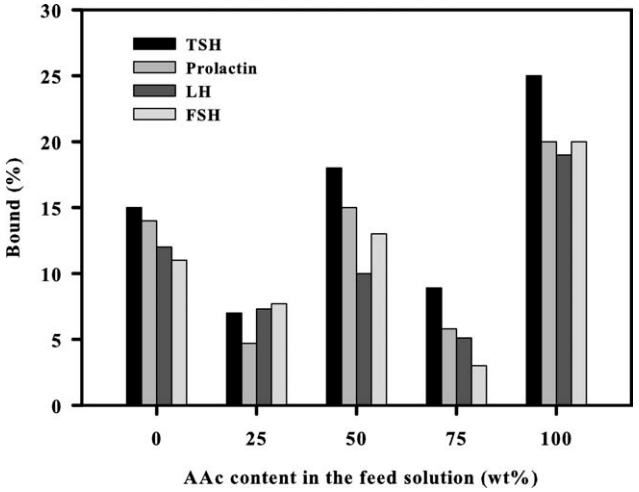

Figure 4 Coupling of PE-*g*-AAc/AAm copolymer at different compositions with some biological molecules, biological molecules concentration (5 mIU mL^{-1}).

bounding percent. The effect of the polymer composition on the bounding of biological molecules was examined by the different hydrophilicity between acrylic acid and acrylamide monomers. Both parameters affect the chemical composition of the surface and consequently, the electrostatic surface charge of the grafted beads effect the bound percent of the biological molecules.

Influence of PE-*g*-AAc degree grafting on bound of biomolecules

Figure 5 indicated the effect of degree of grafting percent of PE-*g*-AAc on the bound of biomolecules, (which determined using gamma counter to detect the bound fractions) on the surface of the beads, where by increasing the degree of grafting the bound percent were increased for FSH, LH, prolactin, and TSH. The pore size of the polymer increased with increase in the degree of grafting which enhance the degree of hydration.[18] Therefore, substrate can penetrate into the pores and the interior of the matrix and consequently the overall rate of Ag/Ab reactions will be enhanced. There are two advantages to employing covalently bound antibodies to an insoluble carrier such as our PE-*g*-AAc, AAm, or AAc/AAm as opposed to covalently crosslinked antibodies which are simply linked to one another rather than to a heavy polymer carrier. These advantages are the ability to separate the bound and unbound fractions with low-speed centrifugation and the high utilization of antibody.

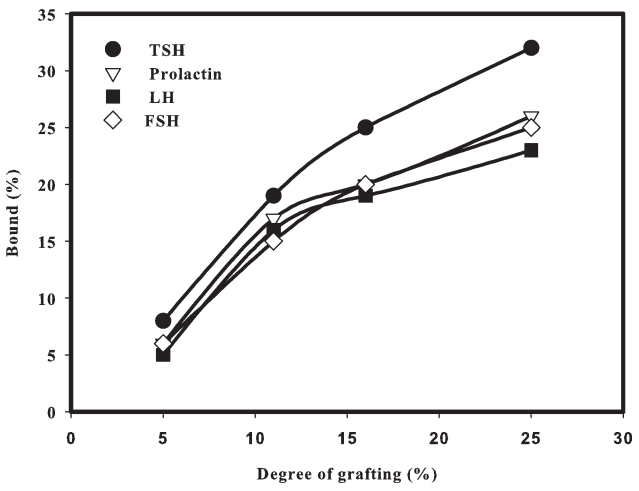

Figure 5 Coupling of PE-*g*-AAc with some biological molecules, biological molecules concentration (5 mIU mL^{-1}).

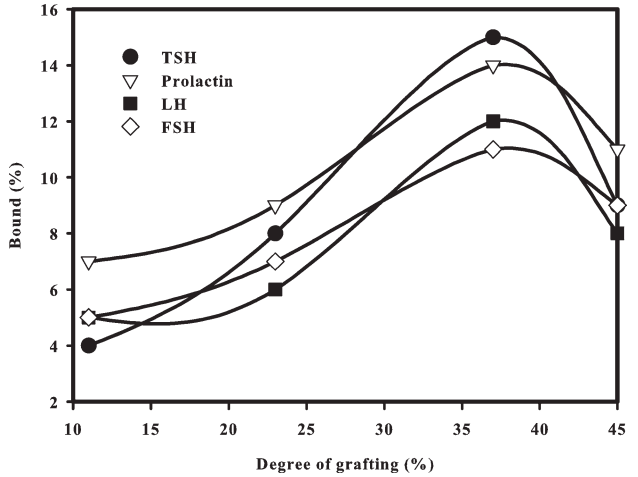

Figure 6 Coupling of PE-*g*-AAm with some biological molecules at different degree of grafting, biological molecules concentration (5 mIU mL^{-1}).

Influence of PE-*g*-AAm degree grafting on bound of biomolecules

Figure 6 indicated the effect of PE-*g*-AAm on the bound of biomolecules on the surface of the beads, where by increasing the degree of grafting the bound percent were increased and reached to maximum at degree of grafting around 40% and after which the bound percent started to decrease for FSH, LH, prolactin, and TSH. The results indicate that there are effect for the stereo structure of the bounded biomolecules and the grafted monomers.

Influence of the degree grafting on the bounded protein concentration

Figure 7 shows the bounded protein concentration, (which determined using the Ponceau S Stain) against degree of grafting for PP-*g*-AAc and PP-*g*-AAm. It can be seen that as the percent graft increases the bounded protein percent increases for both systems, also the bounded protein percent for PP-*g*-AAc is higher than that for PP-*g*-AAm. The increase in protein concentration with grafting may

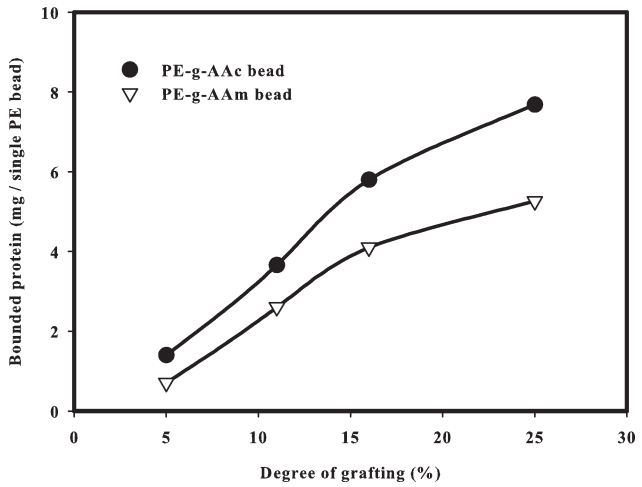

Figure 7 Effect of the degree of grafting percent on the bonding of biological molecule. Biological molecules concentration (5 mIU mL^{-1}).

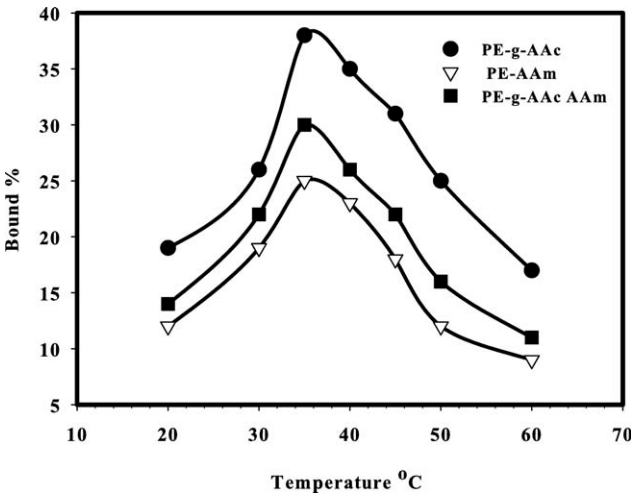

Figure 9 Coupling of PE-*g*-AAc, AAm, and AAc/AAm copolymer with Anti-TSH molecules at variable coupling temperatures.

be due to the increase in the chain length of the grafted polymer. The bounding protein is a function of chain length, i.e., the longer the chain of the polymer the less effective the protein adsorption.[19]

Effect of pH on the immobilization of Anti-TSH

It is generally accepted that the bound percent is affected by buffer conditions such as a kind, pH, and concentration (ionic strength) in bounding procedure.[20,21] Thus, in this work, the effects of the buffer solution on the bounding of Anti-TSH were investigated. Bounding was carried out in a different buffer solution to determine a suitable buffer for the bounding of Anti-TSH. Figure 8 indicated that by increasing pH of coupling buffer the bound percent increased till it reached the optimum at pH 7.5, where at this pH the maximum bound percent was obtained, while after this pH the bound percent started to decrease and this agree with that of Ref. [22]. The protein (Anti-TSH) has a quaternary structure and is stabilized at a suitable pH. If the pH is changed the protein conformation is changed and becomes unstable, thus play down the bounding percent. Therefore, in this work, the influence of pH in coupling reaction was investigated. These results indicate that the pH of buffer solution in the coupling reaction affected the yield of protein.

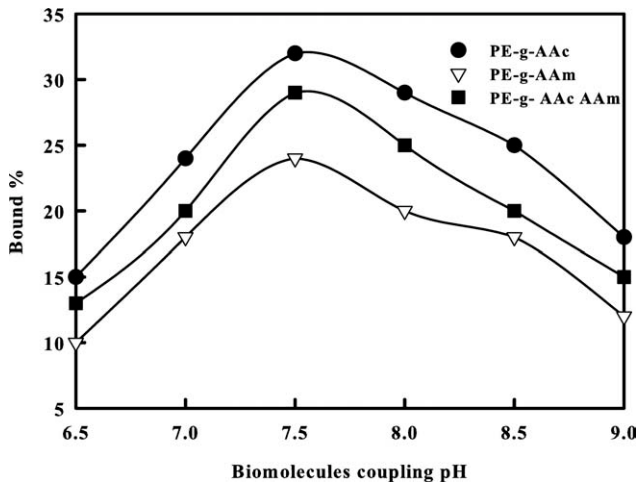

Figure 8 Coupling of PE-*g*-AAc, AAm, and AAc/AAm copolymer with Anti-TSH molecules at variable pH.

Effect of temperature on immobilization of Anti-TSH

Figure 9 illustrated that the bound percent was increased by increasing temperature till it reached maximum bound percent at 37°C and after this temperature the bound percent started to decrease and this is due to the denaturation of the biological

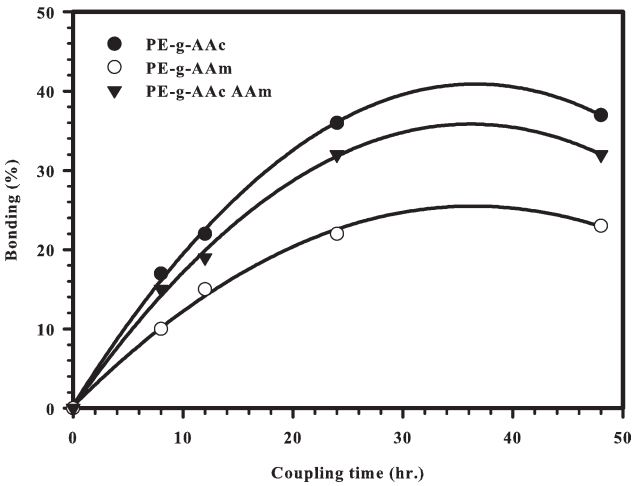

Figure 10 Coupling of PE-*g*-AAc, AAm, and AAc/AAm copolymer with anti-TSH molecules at variable coupling time.

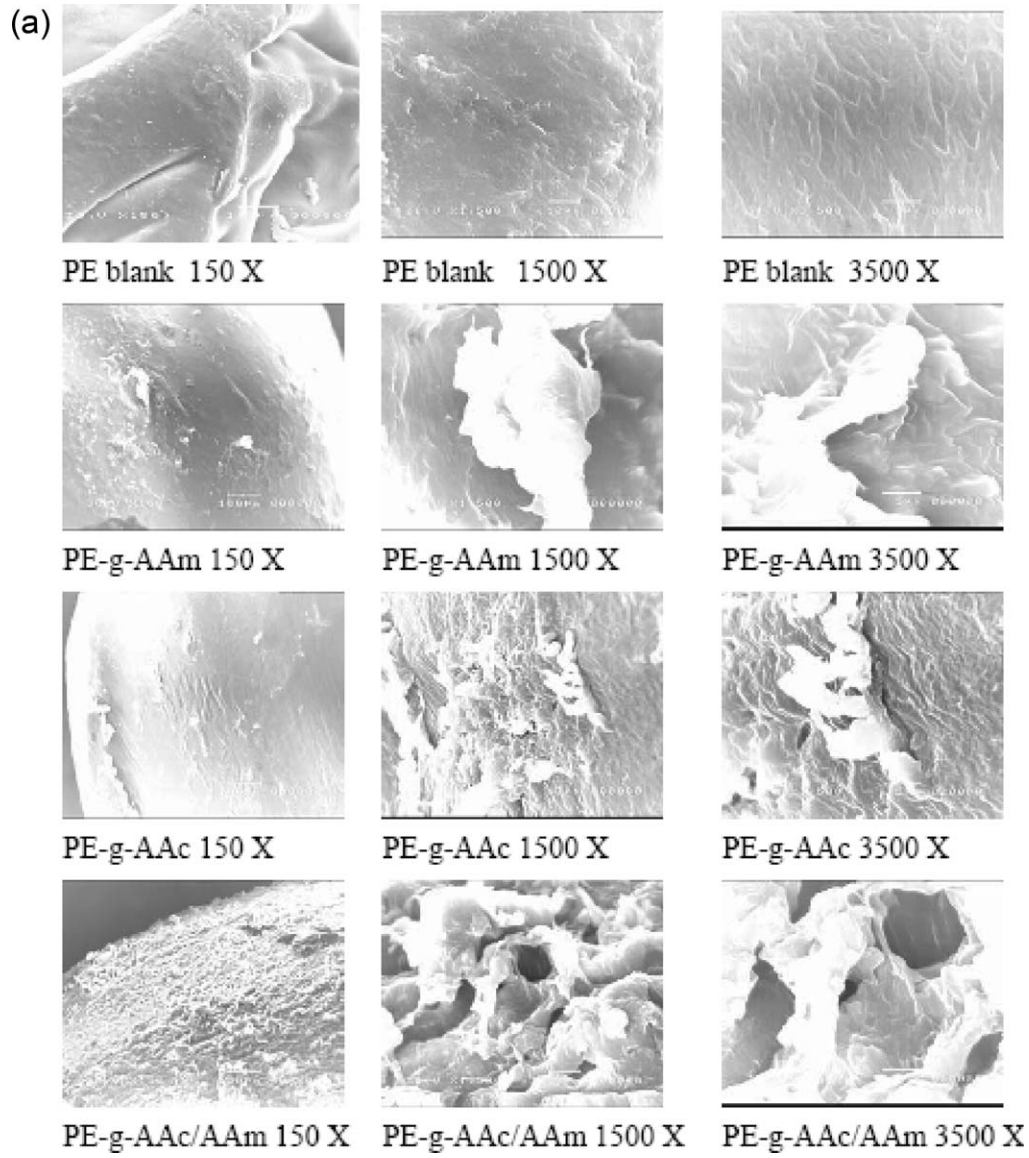

Figure 11 (a) Scanning electron patterns of external surfaces of ungrafted PE beads and PE grafted with AAm, AAc, and copolymer AAm/AAc. (b) Scanning electron patterns of external surfaces of ungrafted PE beads and immobilized PRL hormone as a model example of PE grafted with AAm, AAc, and copolymer (AAm/AAc).

molecules which are protein in nature, so the optimum temperature of coupling buffer is 37°C. These results confirm the important role of temperature in the protein binding. The changes bounding percent according to temperature optima could be due to the fact that the actual temperature in the microenvironment of the matrix was lower than that in bulk solution.[23] It is well-known that the activity of immobilized proteins, especially in a covalently bound system, is more resistant against heat and denaturing agents than the soluble form.

Effect of coupling time on immobilization of Anti TSH

The effect of incubation time on the local solid phase was studied throughout 48 h, ranging from 8 to 48 h at room temperature. The results obtained in Figure 10 illustrated that the bound percent reached to maximum after 24 h of incubation with the biological molecules. It was observed that the sensitivity increases with increasing incubation time. Incubating the standard or sample for overnight with stirring was adequate for getting the optimum sensitivity. Steady state was reached and accordingly 24 h was selected as immunoreaction's time. So the optimum coupling time was 24 h and this as reported by Ref. 24.

In a solid-phase system, the antibodies are physically adsorbed to polymer, such factors as the pH, temperature, and protein concentration of the incubating solution affect the amount of antibody bound. These factors cause disadvantages which are not present when the covalently bonded insoluble polymer

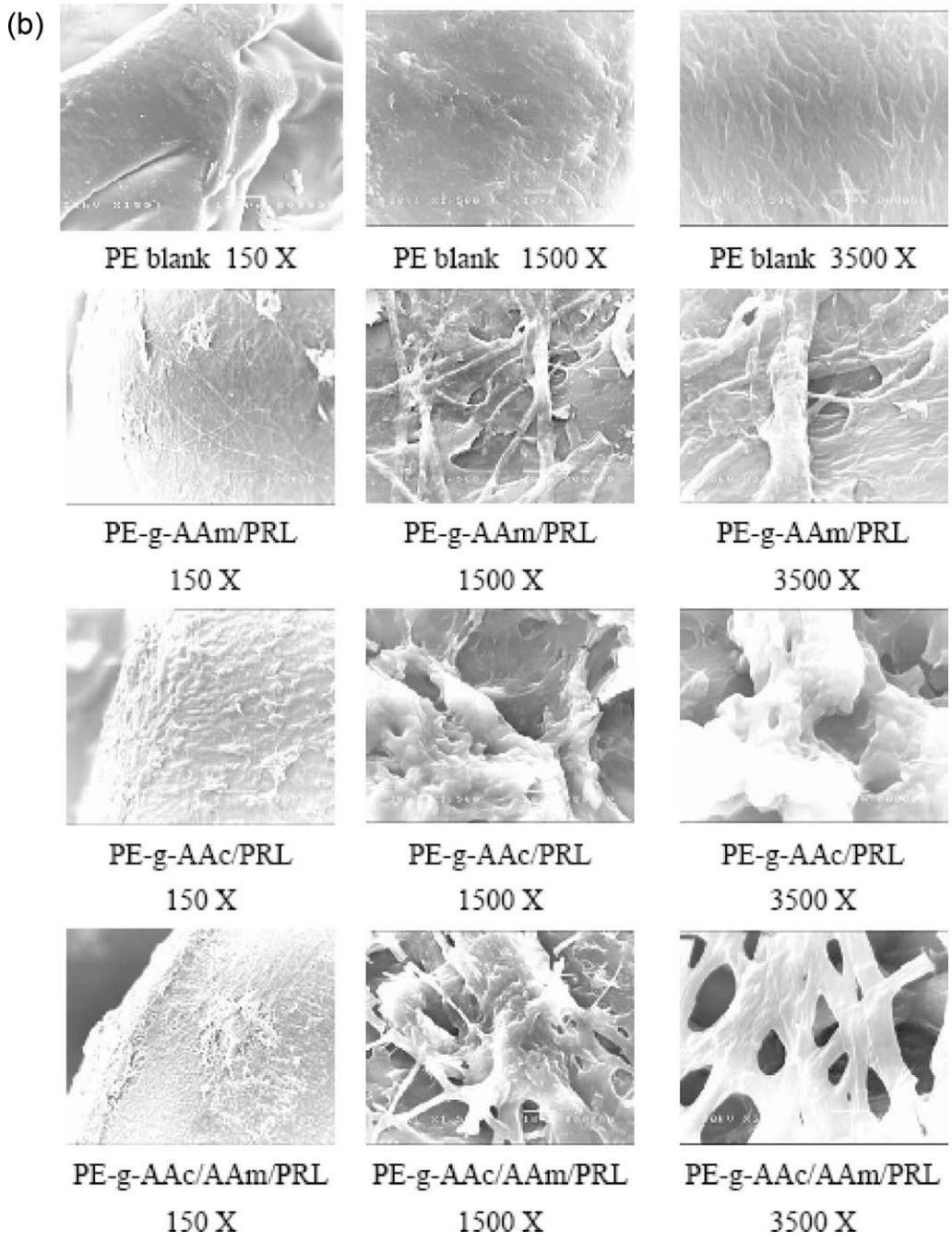

Figure 11 (*Continued*).

method is used. A solid-phase system which uses antibodies, which are covalently bonded to a water insoluble carrier, offers the most advantages in that all the necessary prerequisites of an assay are found in one unit. These three prerequisites are having the antibody, the tracer activity, and the means for separating the bound and unbound fractions.

Scanning electron microscope (SEM)

SEM analyses were used to follow the variation of the morphology of the grafted and immobilized PE beads. The presence of polyacrylic acid and/or polyacrylamide is observed by electron micrography of the modified copolymer surface shown in Figure 11(a). The surface morphology of the grafted beads is different from that of virgin (blank) PE beads. The scientific impact of exploring more than one magnification of each sample is to clarify the fine details found inside the grafted agglomeration, as the protein bounded on the surface of the grafted PE beads is clearly noticed making as a fiber around all over the grafted PE beads surface which could not be clear with the lower magnification. While the lower

magnification need to clarify just the several holes distributed all over the surface of the beads.

One notices a clear change on the PE beads surface with the emergence of several holes distributed all over the surface of the beads. By comparing SEM images of grafted with AAm, AAc, and copolymer of (AAm/AAc) and virgin PE beads, at different magnifications factor of 150, 1500, and 3500× the scientific impact of exploring more than one magnification of each sample to clarify the fine details found inside the grafted agglomeration. The surface of the grafted PE beads exhibited granular structure, and the height of the granules increased linearly with their diameters confirming the grafting of monomers.[25] In Figure 11(b) the protein bounded on the surface of the grafted PE beads is clearly noticed making as a fiber around all over the grafted PE beads surface.

CONCLUSIONS

In this study, we developed an improved solid phase form of some biological molecules such as FSH, LH, prolactin, and TSH antigens using radiation-induced graft polymerization of acrylic acid and acrylamide onto polyethylene beads. The optimum condition of immobilization process on the surface polymeric beads were found to be pH 7.5, temperature 37°C, biological material concentration 400 μg mL^{-1}, grafting percent of PE-*g*-AAc 25%, PE-g-AAm 40%, copolymer composition PE-*g*-AAc/AAm (50/50), and coupling time 24 h; this solid phase form can be used for biological application in the field of bioaffinity assays as well as environmental monitoring.

The authors thank Assistant Prof. Dr. Amr El-Hag Ali for his fruitful guidance through the discussion of the results.

References

1. Peter, B. L.; Lori, J. S.; Daniel, W. C. Clin Chim Acta 2001, 314, 1.
2. Mehany, N. L.; Kolaly, M. T. E.; Ayyoub, S. M.; Hassan, S. E. M. J Radioanal Nucl Chem 2005, 265, 71.
3. Ghosh, R. Rapid antibody screening by membrane chromatographic immunoassay technique. J Chromatography B, 2006, 844 1, 21, 163.
4. Mitra, D; Varshney, L.; Francis, S.; Dhanawade, B. R; Sabharwal, S. Radiat Phys Chem 2008, 78, 42.
5. Carreón-Castro, M. P.; Rivera, E.; Cruz, J. J.; Zavaleta, G.; Gutiérrez-Nava, M. Thin Solid Films 2010, 518, 4136.
6. Nasef, M.; Hegazy, E. A. Prog Polym Sci 2004, 29, 499.
7. Dondi, D.; Buttafava, A.; Faucitano, A.; Arimondi, M.; Ballabio, O.; Caracino, P. Radiat Phys Chem 2009, 78, 521.
8. Mathias, U.; Heike, M.; Annett, O.; Hans-Georg, H. J Membr Sci 1996, 115, 31.
9. Jingxin, L.; Xia, L. Eur Polym Mater 2001, 37, 771.
10. Roberto, S.; Francesco, P.; Mantia, L.; Loredana, C.; Giovanni, P.; Sara, F.; Pierluigi, M. Polymer 2003, 44, 6951.
11. Yang, W.; Ranby, B. Eur Polym Mater 1999, 35, 1557.
12. Young, C. N.; Joon-Ha, J. J Appl Polym Sci 1997, 63, 1101.
13. Hui, B.; Chen, J.; Yang, L.; Li, J.; Pei, Y.; Shi, L. J Radioanal Nucl Chem 2004, 260, 673.
14. Hegazy, E.-S. A.; AbdEl-Rehim, H. A.; Kamal, H.; Kandeel, K. A. Nucl Instrum Methods A 2001, 185, 235.
15. Huang, C.-Y.; Lu, W.-L.; Feng, Y.-C. Surf Coat Technol 2003, 167, 1.
16. Colthup, N. B. L.; Daly, L. H.; Wiberley, S. E. Introduction to Infrared and Raman Spectroscopy; Academic Press: New York, 1964.
17. Borcherding, H.; Hicke, H. G.; Jorcke, D.; Ulbricht, M. Ann N Y Acad Sci 2003, 984, 470.
18. Kumakura, M.; Kaetsu, I. J Appl Polym Sci 1984, 29, 2713.
19. Geoffrey, O.; Esben, T.; Imre, V.; Ricardas, M.; Per, M. C. J Colloid Interface Sci 2010, 349, 265.
20. Shen, G.; Cai, C.; Wang, K.; Lu, J. Anal Biochem 2011, 409, 22.
21. Yin, Y.; Bax, D.; David, R. M.; Marcela, M. M. B. Appl Surf Sci 2010, 256, 4984.
22. El-Batal, A. I.; Atiab, K. S.; Eid, M. Radiat Phys Chem 2005, 74, 96.
23. Trelles, J. A.; Quiroga, F.; Britos, C.; Eduardo, E.; Smolko, M. G. Radiat Phys Chem 2010, 79, 241.
24. Paradkar, S.; Vrinda, C.; Jyotsna, N.; Sivaprasad, N. J Radioanal Nucl Chem 1999, 241, 561.
25. Zouahri, A.; Elmidaoui, A.; Ameduri, B.; Hervaud, Y.; Boutevin, B. Eur Polym Mater 2002, 38, 2247.