JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 11, November 2007, p. 3431 - 3434

Magnetic field effect upon albumin immobilization

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Copolymers of methyl methacrylate (MMA) with 2, 3 epoxypropyl methacrylate (GMA) were tested as matrices for the coupling of the bioactive products. The preparation of polymeric matrices was realized by classic emulsion polymerization (CW) and also comparatively obtained through unconventional procedure in the presence of a magnetic field (MF) of 1500 Gs intensity. As tensioactive substance β-cyclodextrin was used owing to the future biomedical application of the polymeric matrices. In the paper, the albumin (BSA) adsorption onto the synthesised polymeric matrices, it was study. Thus, it was compared the coupling process performed with or without the magnetic field presence. It was also studied the influence of the temperature upon the albumin adsorption yield. The yield of BSA adsorption is higher in case of the copolymers matrices synthesised in the MF presence. At the same time the BSA adsorption performed in the MF presence evidences decreased yields of coupling. For the polymeric system taken into study, the optimum temperature that allows the albumin adsorption at the most higher amount it was determined as being 30 ^{0}C.

(Received March 23, 2007; accepted November 1, 2007)

Keywords: Albumin adsorption, Magnetic field effect, Methyl methacrylate

1. Introduction

Protein adsorption has been studied actively for decades because of its importance in the wide range of biomedical and industrial applications [1].

Protein adsorption on solid surfaces is a complex phenomenon and involves many dynamic steps, between them the bond formation between surfaces and proteins. Therefore, the kinetics of the adsorption of proteins on various surfaces has been studied by many researchers using various methods such as: ellipsometry, Fourier transform infrared spectroscopy (FTIR), radiolabeling techniques, reflectometry, and total internal reflection fluorescence (TIRF) [1, 2].

The ability to control and manipulate nonspecific adsorption of proteins requires a detailed understanding of the mechanisms that controls the adsorption. Although conventional adsorption isotherms for proteins can be found in the literature, equilibrium is rarely achieved because the adsorption of the protein molecules onto solid surfaces frequently results in conformational and/or orientational changes within the adsorbed layer. Because protein structures are relatively unstable, protein tends to unfold, allowing internal regions to form additional contacts with a particular solid surface. This denaturation is often associated with loss of secondary or tertiary structure and results in irreversible adsorption [3].

More than that, the response of a particular protein to an interface is essentially determined by the surface chemistry of the substrate. Surface properties have a tremendous effect on the mechanism of adsorption, its rate and extent [4, 5].

The adsorption of proteins onto polymeric microspheres having diameters in the range of 0.1 to several micrometers is important in the field of biomedical applications, and hence it has been studied from all viewpoints for the last two decades [6]. However, the results were not consistent due to the complexity of protein adsorption. Major interactions involved in the protein adsorption are classified as follows [7]: hydrophobic interaction, electrostatic interaction, and hydrogen bonding. Some researchers concluded that hydrophobic interaction is the most important aspect of protein adsorption, and increasing of the hydrophilicity will result in a low level of adsorption [8]. Others claimed that hydrogen bonding or electrostatic interaction is more important than hydrophobic interaction [9]. Recent has been statute that hydrophobic interactions are the major driving force of adsorption, but the participation of electrostatic interactions play an important role [10]. When the adsorption is controlled by the surface charge, not the net charge, the electrostatic contributions are the most important. These different results are mainly due to the narrow range of experiments, that is, using only low carboxylated microspheres, or using only high sulfonated ones, and so forth. Therefore, the experiments should be performed in the full range, from not modified to fully modified surfaces. In addition, analyzing the adsorption phenomena with only one interaction cannot be justified, because the interaction forces involved in the protein adsorption affect each other [11].

The theory of perturbation applied to static interactions in a magnetic system (colloidal suspension) has been developed in [12, 13] in order to explain the experimental results obtained in ferrofluids and in polymeric magnetic samples.

In the paper, copolymers of methyl methacrylate (MMA) with 2, 3 epoxypropyl methacrylate (GMA) were tested as matrices for coupling of albumin (BSA) as bioactive product. The preparation of polymeric matrices was realized by classic emulsion polymerization (CW) and also comparatively obtained through unconventional procedure in the presence of a magnetic field (MF) of 1500 Gs intensity. As tensioactive substance

β-cyclodextrin was used owing to the future biomedical application of the polymeric matrices. The albumin adsorption onto the synthesised polymeric matrices it was correlated with the coupling conditions, respectively the presence or absence of the continuous magnetic field and the temperature.

2. Experimental part

2.1. Materials

Methyl methacrylate (MMA) (c > 99 wt %, Merck) and 2, 3 – epoxypropyl methacrylate (GMA) (c > 97 wt %, Fluka) were freshly distilled before use.

β- Cyclodexrin (CD) from Fluka vacuum dried at 80 °C for 12 h were used as tensioactive agent.

Potassium persulphate ($K_2S_2O_8$) – KPS was twice recrystallized from twice distilled water.

In all experiments twice distilled water was used.

The bovine albumin-BSA (fraction V, c > wt 98 %) from Sigma was used as a bioactive substance.

2.2 The microspheres preparation

The polymeric particle, respectively poly(methyl methacrylate – co – 2, 3 epoxypropyl methacrylate) was realized through emulsion polymerisation reaction: classic (CW) and in the presence of a continuous external magnetic field of 1500 Gs, procedure which was previously described [12-14].

The reactions were made in the same conditions for both variants of synthesis with or without the MF presence: the same reaction composition (25 wt % GMA and 75wt % MMA, 0.8% wt potassium persulfate – the initiator (KPS) and 3% wt β- Cyclodexrin the surfactant, ratio comonomers / water being 1/4), similar vessel shape and geometry, same reaction parameters – 70 °C – and type and rate of stirring.

The synthesised compounds were precipitated in methanol, purified by reprecipitation in methanol from acetone solution, dried under vacuum at room temperature for 48 h and stored in desiccators.

In Fig. 1 are depicted SEM pictures of the synthesised copolymers in CW (a) and in MF presence (b). Samples present a narrow distribution with submicronic size. At the same time, the polymeric matrices synthesised in MF present smallest size comparatively with the samples synthesised in CW.

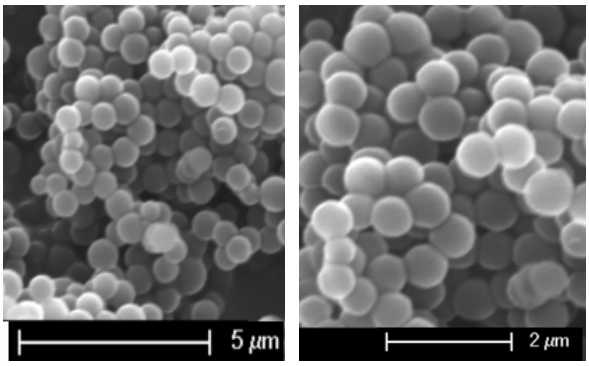
2.2.1 Swelling study

The swelling of dried polymer particles was performed in chloroform vapour at 20 °C at 2 Torr. The organic solvent absorbed was determined by weighing the samples, at various intervals of time, with a precision electronic balance (A&D Co. Ltd. HR 200).

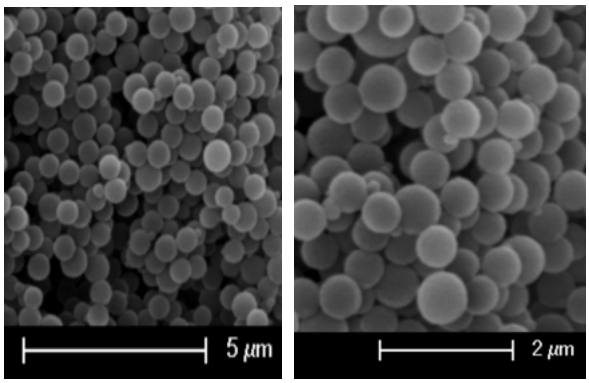
The swelling percentage ratio (%S) was calculated from the following equation:

$$\%S = \frac{M_t - M_o}{M_o} \times 100 \quad (1)$$

where M_t is the weight of the swollen polymer at time t and M_o being the weight of the dried polymer at time 0. The solvent in taken by the initially dried polymers was pursued approximately for 30 hours, until equilibrium.



a)



b)

Fig. 1. The SEM pictures of the copolymer synthesised in CW and in MF presence.

2.3 Biocompatibilization procedure

The adsorption of albumin (BSA) onto microspheres was carefully achieved. BSA was dissolved in phosphate buffer solution (pH 7) at (25 °C). The sample solutions were stirred for 120 min with or without the MF presence. The residual protein concentrations were determined by spectrophotometric method with a Jenway 6305 UV/V spectrophotometer. The influence of the temperature at 25 °C, 30 °C, 35 °C, and respectively 40 °C upon the BSA adsorption was also studied, using the adsorption procedure with or without the MF presence.

3. Results and discussion

3.1. Swelling study

Swelling curves are plotted in Fig. 2. It is evidenced the decrease of the swelling capacities of the copolymers synthesized in MF comparing with the sample classic

synthesised. This behaviour is attributed to the ordered structure of the polymeric matrix resulted owing to the MF presence during synthesis. These data allow establishing the interval of time to achieve the maximum relaxation structure for the polymeric network for their future use in coupling of the bioactive substance. Thus, it is remarkable an increase of the swelling degree until 800 – 1000 min, the swelling equilibrium being reached after that. The swelling rate decreases in the MF presence from two to ten times, as well as α_{max} with few exceptions, as can be observed from Table 1.

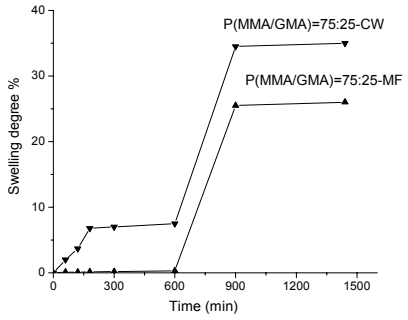


Fig. 2. Swelling degree of the samples obtained by CW and in MF presence.

Table 1. The swelling rate of the copolymers.

Time for swelling (min)	Swelling rate $\times 10^{-3}$ % min^{-1}		BSA Adsorption (mg/g) at 60 min performed			
			Classic		In MF	
	Classic	In MF	Polymeric matrix synthesised		Polymeric matrix synthesised	
0-120	31	1	Classic	In MF	Classic	In MF
120-600	5.6	0.3	230	330	80	115
600-1440	29	27				
α_{max}	35	28				

3.2. The BSA adsorption

The plotting of BSA adsorption – amount onto microspheres surface against time is presented in Fig. 3. It is observed an initial period which corresponds to a rapid adsorption followed by a slower process of adsorption. The adsorption – amount of BSA at about 60 min is almost the same with that registered at 120 min. It can be concluded that the adsorption reaches the equilibrium within 60 min. From the profiles, the increase in the adsorption-amount is evident in case of classic adsorption for both samples of polymers synthesised with or without the MF presence. At the same time the copolymers synthesised in the MF presence determined an increase of the amount of albumin adsorbed as it is show in Table 1.

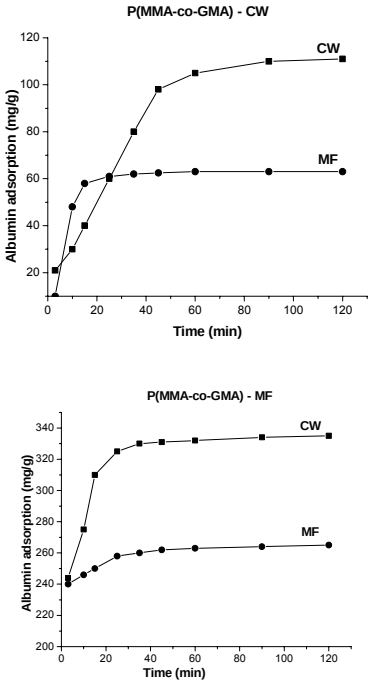


Fig. 3. Time profile of BSA adsorption.

However it is obviously that the copolymers synthesised in MF presence have a better capacities for BSA adsorption. This was the reason for choosing this sample in order to study the influence of the temperature (Fig. 4).

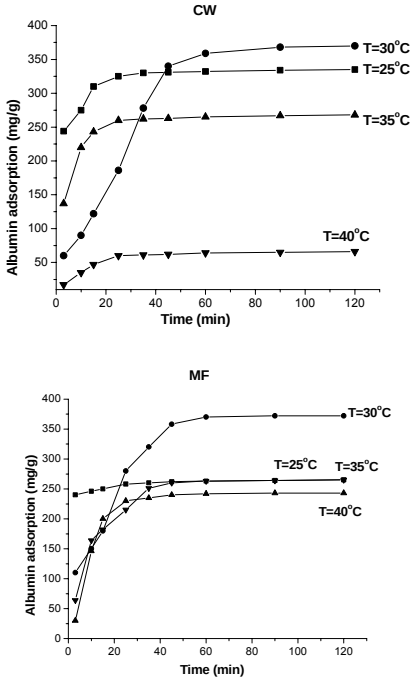


Fig. 4. The influence of the temperature upon of BSA adsorption.

The temperature effect upon BSA adsorption was investigated by varying the temperature in the range of 25 ^{o}C to 40 ^{o}C. The results, depicted in Fig. 4, evidence a direct dependence between the BSA adsorption and the increase of the temperature up to 30 ^{o}C. Further increase of the temperature are not the properly conditions for the adsorption of BSA owing to the growth of the system entropy that induces the diminution of the possibilities to create intermolecular forces as well make the forces between the BSA molecules and polymer surface weaker, and adsorption decreases. At the same time at higher temperatures, the escaping tendency of the BSA molecules from the surface to the bulk solution increases, this also results in decreases adsorption. It was concluded that the optimum temperature for adsorption is 30 ^{o}C, temperature which also allow for the protein molecules to acquire a structure properly for coupling, which result in a greater adsorption. However it must be mentioned that the MF presence near to temperature increase determines the decrease of the BSA adsorption. This is also the result of the thermal agitation that disturbs the structure adequate to coupling.

4. Conclusions

Methyl methacrylate copolymers with 2, 3 – epoxypropyl methacrylate – monomer bearing functional groups, with controlled dimension and size of particles, synthesised by emulsion polymerization classic and in the MF presence, by means of β-cyclodextrin as surfactant, were used as matrices to be coupled with bioactive substances the albumin (BSA). The adsorption of the albumin was performed also in the magnetic field presence.

The adsorption of BSA is higher in case of the copolymers synthesised in the presence of the magnetic field. At the same time, the capacity of BSA adsorption is decreased when the coupling process is performed in magnetic field.

The temperature influences the BSA adsorption process, the optimum of temperature which allows the adsorption of the higher amount of albumin is 30 ^{o}C for the polymeric system examined in this study.

Acknowledgements

The study was realised with the financial support of a Grant included into 2005-2007 CNCSIS and CEEX 10/2005 program.

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