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# Effect of emulsion polymerization and magnetic field on the adsorption of albumin on poly(methyl methacrylate)-based biomaterial surfaces

**Loredana E. Nita · Aurica P. Chiriac**

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**Abstract** The adsorption of bovine serum albumin (BSA) onto the surfaces of poly(methyl methacrylate) (PMMA) and of methyl methacrylate copolymer with 2,3-epoxypropyl methacrylate, it was investigated. The polymeric matrices were obtained through radical emulsion polymerization with and without the presence of a continuous external magnetic field (MF) of 1,500 Gs intensity. Two types of surfactant agents were used for polymers' synthesis: a classic one sodium lauryl sulphate (SLS) and $\beta$-cyclodextrin (CD). The protein adsorption was conducted in the presence as well as in the absence of MF, by varying the coupling conditions, respectively, the temperature, pH and albumin/polymer ratio. The study underlines the assistance of MF during the adsorption process, materialized into growth of the BSA adsorbed quantity. Thus, MF presence during adsorption determines the doubling of the BSA adsorbed quantity onto the surface of polymers prepared in the MF. The adsorption process was also related to the tensioactive used for the synthesis of polymeric matrices. The higher content of the adsorbed BSA corresponds to the polymers with CD instead of SLS. The fact was attributed to the catalytic activity of the MF, which determines the molecules distortions, the growth of distance interactions and the modifications of the angles between bonds, with benefit effect upon adsorption.

L. E. Nita · A. P. Chiriac (✉)
"Petru Poni" Institute of Macromolecular Chemistry,
Grigore Ghica Voda Alley No. 41A, 700487 Iasi, Romania
e-mail: achiriac1@yahoo.com

## 1 Introduction

Polymers have been the first choice as biomaterials due to their good mechanical properties and chemical passivity at the contact with biological fluid. PMMA is one of the frequently used polymers as substrate [1]. It is well known the use of PMMA or methyl methacrylate (MMA) copolymers for tissue engineering, microarrays, biosensors, immobilized enzymes or biomolecules, MEMS (*Micro-Electro-Mechanical Systems*) Enzyme reactors, DNA hybridization, etc. [2–4]. There have been critical obstacles for the application of PMMA owing to properties as hydrophobicity, poor biocompatibility and fouling, that induce surface functionalization prior to the attachment of a bioactive compound. The surface modification is an effective way to improve the biocompatibility by maintaining the bulk properties of the polymer. Various modifications of the PMMA substrates have been attempted by using different techniques, like polyatomic ion deposition, physical adsorption, biomolecule adsorption, chemical modification, and so on [5, 6]. Several surface modification techniques have been developed to improve wetting, adhesion, and printing of polymer surfaces by introducing a variety of polar groups, with little attention to functional group specificity. However, when surface modification by attaching a bioactive compound is a precursor phase, this technique must be tailored to introduce a specific functional group. The techniques that modify surface properties by introducing random and non-specific groups are less useful in bioconjugation to polymer surfaces. Even so, the polymer coating is a facile and versatile method for surface modification, because polymers have unique advantages over other materials owing to their physical and chemical properties which can be easily achieved through a properly molecular design [7].

By immobilization of extracellular matrix (ECM) proteins such as collagen, gelatin, elastin, or fibrin to polymers, the enhancement of cell adhesion and proliferation has been reported [8–10]. The protein adsorption process was intensively studied due to the importance of the phenomenon for biomedical field as well as in industrial applications [11]. 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 [12].

The major interactions involved in the protein adsorption onto different surfaces are considered as being: hydrophobic interaction, electrostatic interaction, and hydrogen bonding [13].Some researchers concluded that hydrophobic interaction is the most important phenomenon during protein adsorption, and the increase of the hydrophilicity will result in a low level of adsorption [14]. Other authors claimed that hydrogen bonding or electrostatic interaction is more important than hydrophobic interaction [15]. Finally it was concluded that hydrophobic interactions are the major driving force of adsorption, but the participation of electrostatic interactions play an important role, too. In addition, by analyzing the adsorption phenomena with only one interaction it cannot be justified, because every interaction force involved in the protein adsorption affects the process [16]. It can be concluded that the ability to organize and manipulate protein nonspecific adsorption requires a detailed understanding of the mechanism that controls these processes. Although the adsorption isotherms for protein are given in the literature, the equilibrium is difficult to obtain because the adsorption of protein on solid surface determined conformational and orientation changes of adsorbed layer. This denaturation is often associated with losses of the secondary and tertiary structure of protein that determines the irreversible adsorption. In addition, there are not well understood the interaction and relaxing processes, such us denaturation and orientation, as well as the scale of time for them [17].

As it is well known through very weak perturbations of the magnetic field (MF) one can control chemical kinetics and thus, the course and the rate of reactions that normally require much higher chemical energies [18–25]. The intervened magnetokinetic effects were attributed to the changes in the multiplicity of the radical pairs owing to the MF influence. Beside the modifications brought to the evolution of the reactions, the MF influences the properties of the resulted products, such as: molecular weight, thermal stability and interval of glass transition temperature, dielectric and mechanical properties as well as conductibility, of the polymers obtained in the presence of a continuous electromagnetic field [23, 24].

Adsorption at interfaces of polymers is employed as a powerful technique for modifying the surface properties, such as surface energy, electric charge, lubricity, optical properties, biocompatibility, etc. A large number of parameters affect the adsorption. These factors include, the structure of the polymer, polymer chain length and the extent of polydispersity, nature of interaction of the polymer with the solvent and with the surface, concentration of the polymer in the solution, temperature, etc. Magnetic effects can be in this order of technological interest because they offer a new way to perform the adsorption which can be sustained by the catalytic activity of the MF that can re-shape the molecules by growing of distance interactions and by modifying of angles between bonds [26–30].

The correlation between different surfactants—sodium lauryl sulfate (SLS) and $\beta$-CD—and the presence of a continuous electromagnetic field was put into evidence upon the emulsion polymerization of MMA and emulsion copolymerization of MMA with GMA. The research underlined the coupling possibilities of the influence of the MF—growth of the reaction rate and conversion explained through radical pairs mechanism—with a combination of the cage effect and conformational control afforded by CD. The presence of MF and CD during the syntheses induced the increase of $T_g$ as well as of thermal stability of PMMA and P(MMA-*co*-GMA) copolymer [31].

In the present paper the adsorption of bovine serum albumin (BSA) onto the surfaces of PMMA and of MMA copolymers with 2,3-epoxypropyl methacrylate (GMA), it was evaluated. The polymeric matrices were obtained through radical emulsion polymerization with (MF) and without (CW) the presence of a MF of 1,500 Gs intensity. The BSA adsorption onto the surfaces of synthesized polymeric matrices was also realized in the presence or absence of the continuous MF, by varying the coupling conditions, respectively, the temperature, pH and albumin/polymer ratio.

## 2 Materials and methods

### 2.1 Materials

The monomers MMA (c >99 wt%, Merck) and 2,3-epoxypropyl methacrylate (GMA) (c >97 wt%, Fluka) were freshly distilled before use. The tensioactive products sodium lauryl sulphate (SLS—from Sigma, purity >95 wt%), was used without further purification, and $\beta$-CD (CD—from Fluka) was dried 12 h in vacuum at 80°C. The initiator potassium persulphate (KPS) was twice recrystallized from twice distilled water. The BSA (fraction V, c >98 wt%, from Sigma) was used as tested protein for

adsorption. In all experiments twice distilled water was used.

## 2.2 The polymer matrix preparation

The polymers—PMMA and P(MMA-*co*-GMA)—were prepared through radical emulsion polymerization process classical performed (CW) and in the presence of a continuous external MF of 1,500 Gs, procedures that were previously described [31, 32]. The reactions were made in the same conditions for both variants of synthesis with or without the MF presence, respectively, the same reaction composition with 0.8 wt% initiator (KPS) and 3 wt% surfactant—SLS or CD—, the comonomers/water ratio was ¼. The similar shape and geometry of vessel, the same reaction temperature—70°C—as well as the type and the rate of stirring there were used during the syntheses. The prepared macromolecular 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. Table 1 gives the samples type and their correspondent code used into the present study.

The polymeric particles were swelled in chloroform vapor at 20°C and 2 Torr to obtain a relaxed polymeric structure with a lattice able for the future adsorption of protein (the kinetic data were previously presented) [25]. After swelling the particles were washed in twice distilled water until the chloroform was completely remove (inspection by FT-IR spectra).

## 2.3 Biocompatibilization procedure

*The adsorption procedure*: The adsorption of albumin (BSA) onto polymeric surfaces it was carefully achieved. BSA was dissolved in phosphate buffer solution at a concentration of 0.37% and at different pH values described in the following sections. In view of coupling, the polymer sample and BSA solutions were stirred together for 120 min with or without the MF presence of 1,500 Gs intensity. From time to time the residual protein amount from solution was taken and determined with a Jenway 6305 UV/V spectrophotometer.

### *2.3.1 Kinetic of albumin adsorption*

For the kinetic study BSA was dissolved under stirring in a phosphate buffer solution with pH = 7, at 25°C and for 120 min. The coupling process was performed onto the polymeric samples mentioned in Table 1, also with or without the presence of the MF. The samples from the solution of the mixture between polymeric matrices with protein were analyzed gravimetrically at intervals to determine the residual protein amount. The steady state of adsorption was considered the value registered for three equal consecutive measurements of the residual protein amount.

### *2.3.2 Influence of temperature*

In a previous study it was presented the temperature influence upon BSA adsorption—the process was realized at 25, 30, 35 and 40°C—and the solution pH was 7 [32].

### *2.3.3 Influence of pH*

Seven pH values were tested 2, 3, 5, 7, 8, 10, 12, respectively and during adsorption the solution temperature was maintained constantly at 30°C.

**Table 1** The samples structure and code

| Sample | Cod | Syntheses performed | | Tensioactiv product | |
|---|---|---|---|---|---|
| | | Classic | In MF | SLS | $\beta$-cyclodextrin |
| PMMA | 1 | X | – | X | – |
| PMMA | 2 | – | X | X | – |
| P(MMA-*co*-GMA) = 97:3 | 3 | X | – | X | |
| P(MMA-*co*-GMA) = 97:3 | 4 | – | X | X | – |
| P(MMA-*co*-GMA) = 75:25 | 5 | X | – | X | – |
| P(MMA-*co*-GMA) = 75:25 | 6 | – | X | X | – |
| PMMA | 1′ | X | – | – | X |
| PMMA | 2′ | – | X | – | X |
| P(MMA-*co*-GMA) = 97:3 | 3′ | X | – | | X |
| P(MMA-*co*-GMA) = 97:3 | 4′ | – | X | – | X |
| P(MMA-*co*-GMA) = 75:25 | 5′ | X | – | – | X |
| P(MMA-*co*-GMA) = 75:25 | 6′ | – | X | - | X |

#### 2.3.4 Influence of BSA/polymer ratio

Three polymer/albumin ratios were taking in consideration 2/1, 1/1, 1/2, respectively. During the adsorption process the solution temperature of 30°C as well as pH of 7 was maintained constantly.

Every experiment was made three times and the average values (mg BSA/g polymer) were graphic represented.

### 2.4 Structure characterization

#### 2.4.1 The ζ potential

The ζ potential was determined on a Malvern Zetasizer Nano ZS instrument at two different temperatures 22 and 37°C, respectively (Table 2). The ζ potential was appreciated from the electrophoretic mobility ($\mu$) using the Smoluchowski relationship,

$$\xi = \eta\mu/\varepsilon, \text{ where } k\alpha \gg 1$$

where $\eta$ is the viscosity, $\varepsilon$ is the dielectric constant of the medium, $k$ and $\alpha$ are Debye–Hűckel parameter and the particle radius, respectively.

It is also presented: (1) the average diameter (MMD) as statistic parameter of the particle distribution, representing 50% of the particles that have the diameter higher than that mentioned in Table 2; and (2) the specific surface of adsorption (SSA) as being the entire surface of the particles divided to their load.

Each determination was made three times and their average value it is presented.

#### 2.4.2 FT-IR spectra

The structure of the studied compounds was confirmed through FT-IR spectra (figure not shown) (on a DIGILAB, Scimitar Series, USA, spectrophotometer, the resolution recording was 4 $cm^{-1}$) (concentration used: 3 mg sample in 500 mg KCl tablet). Thus FT-IR characteristic bands were put into evidence at 1,080 $cm^{-1}$ and 1,300 $cm^{-1}$ for C–O absorption; at 1,380 $cm^{-1}$ C–H absorption corresponding to the methyl group; 1,690–1,760 $cm^{-1}$ absorption of the carbonyl (C=O); and at 2,800 and 3,100 $cm^{-1}$ CH stretching. BSA film spectrum indicates for amide region, the peaks around of ~1680, 1,657, 1,637 and 1,622 $cm^{-1}$. The vibration of the protein carboxylic groups of side-chains corresponds at 1,715 $cm^{-1}$.

## 3 Results and discussion

### 3.1 Influence of the comonomer content upon the BSA adsorption

Figures 1 and 2 illustrate the GMA concentration effect upon the BSA adsorbed amount in direct dependence with the adsorption conditions when the process takes place by stirring with or without the MF presence.

The analysis of Figs. 1 and 2 evidences the increase of the BSA adsorbed amount proportional with the comonomer (GMA) percent, the fact being attributed to the supplementary possibilities to produce intermolecular forces between the matrix and the attached bioactive substance, as well as owing to the network structure type which corresponds to the copolymer composition more adequate to generate a bioactive composite. The BSA adsorbed amount depends on the method performed during the polymer synthesis, being higher when the polymeric structures were synthesized in the MF (samples 2, 4, 6, 2′, 4′, 6′).

Differences for the BSA adsorbed amount intervene related also to the adsorption conditions, respectively, slowly stirring achieved with or without the MF presence. Thus, for the adsorption performed in the absence of the field the BSA adsorbed amount increases from 25 mg BSA/g polymer (sample 5 synthesized without the MF) to about 30 mg BSA/g polymer (sample 6 synthesized in the

**Table 2** ζ potential, average diameter and specific surface of adsorption of the studied polymeric sample

| Sample | MMD (μm) | | SSA ($m^2$/g) | | Zeta potential, ξ (mV) | | | |
|---|---|---|---|---|---|---|---|---|
| | C | MF | C | MF | C | | CM | |
| | | | | | 22°C | 37°C | 22°C | 37°C |
| PMMA (SLS) | 0.401 | 5.042 | 0.3 | 0.28 | –23.9 | –21.2 | –26.6 | –23 |
| PMMA (CD) | 0.423 | 7.53 | 0.68 | 0.75 | –20.5 | –19 | –22 | –21 |
| P(MMA-*co*-GMA) (SLS) (97/3) | 6.942 | 9.046 | 1.07 | 1.16 | –25.2 | –22.1 | –28.9 | –25 |
| P(MMA-*co*-GMA) (CD) (97/3) | 11.14 | 13 | 1.13 | 1.19 | –15.2 | –12.1 | –18.9 | –15 |
| P(MMA-*co*-GMA) (SLS) (75/25) | 11.6 | 13.1 | 16.1 | 1.75 | –13.9 | –11.2 | –16.6 | –13 |
| P(MMA-*co*-GMA) (CD) (75/25) | 18 | 20 | 16.4 | 2.16 | –10.5 | –9 | –12 | –11 |

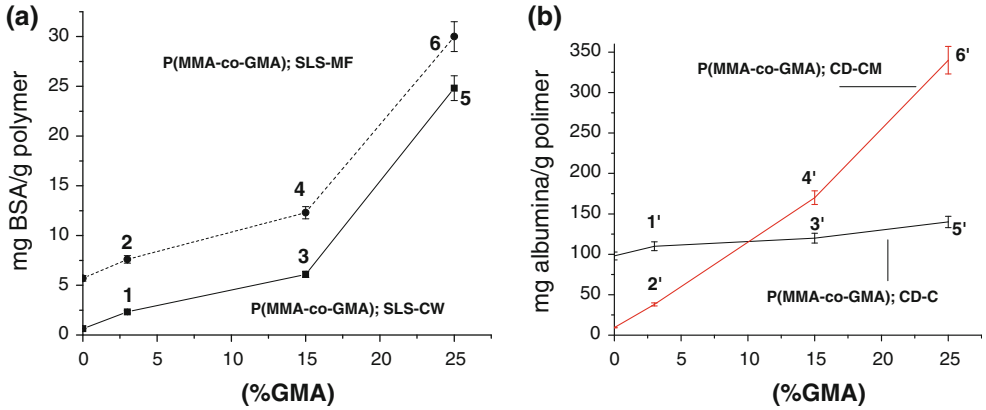


**Fig. 1** The comonomer presence influence on the BSA adsorbed amount (coupling in the absence of MF); copolymers synthesized with SLS (**a**) and CD (**b**) as tensioactive substances, respectively syntheses performed with or without the MF presence

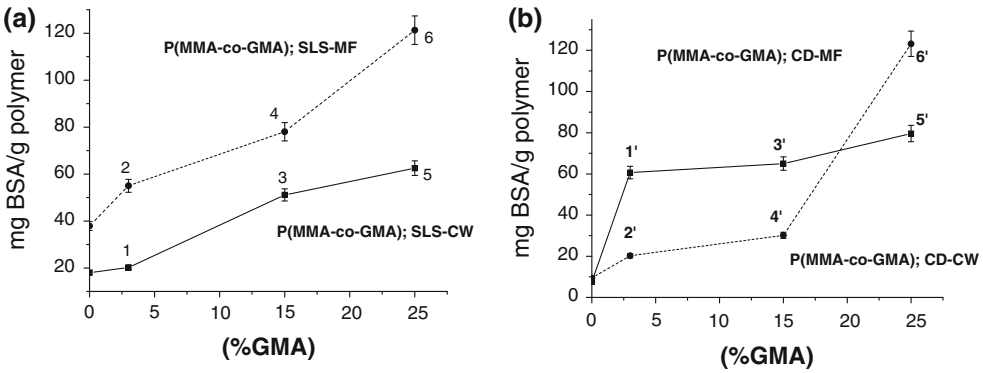


**Fig. 2** The influence of GMA concentration on BSA adsorbed amount when adsorption was performed in the MF presence; copolymers synthesized with SLS (**a**) and CD (**b**) as tensioactive substances, respectively syntheses performed with or without the MF presence

MF presence). The same behavior is registered in the case of polymers prepared with $\beta$-CD as tensioactive. The maximum BSA adsorbed amount of about 150 mg BSA/g polymer in case of sample 5′ (classic synthesized) becomes greater of about 330 mg BSA/g polymer for sample 6′ (the same copolymer composition as 5′, excepted the synthesis which was performed in the MF presence—Table 1).

The favorable effect of MF for the BSA adsorption process is sustained by the macromolecular chains' structure which benefited by the MF presence during synthesis, as well as by the adsorption process itself. Thus, for the adsorption performed in the MF presence, the maximum adsorbed amount of BSA is—for the copolymers synthesized with SLS—of about 60 mg BSA/g polymer in the case of polymer 5, and it growths at about 120 mg BSA/g polymer for its correspondent polymer synthesized in the MF presence (sample 6). Also, the replacement of SLS with CD, during the synthesis process, determines the increase of the BSA adsorbed amount from about 80 mg BSA/g polymer—sample 5′—to about 130 mg BSA/g polymer—sample 6′.

These data allow the following conclusions: (1) the presence of the functional comonomer—GMA—has a positive influence upon the BSA adsorption process; (2) the polymers synthesized in the MF presence have a structure more disposed for the BSA adsorption, proportionally with the comonomer content (GMA); (3) the presence of CD instead of SLS as tensioactive substance during the synthesis process also determines the increase of the BSA adsorbed amount.

These conclusions are in good agreement with the average diameter and the SSA of the studied polymeric samples (Table 2) with normal proportional dependence between the average diameter of polymeric particles or their SSA and the adsorbed amount of BSA. As it is well known, the particles acquire a charge for their adsorption onto a surface, which, in our case, it is proven by $\zeta$ potential values of the polymeric matrices [33]. At the same time, the adsorption process goes better for smallest values of $\zeta$ potential when the repulsion forces between BSA and the polymeric surface are also smaller.

With these conclusions the selected samples for the kinetic analysis of the BSA adsorption process are the following: P(MMA-*co*-GMA)(5)(75/25–SLS–CW), P(MMA-*co*-GMA)(6)(75/25–SLS–MF), P(MMA-*co*-GMA)(5′)(75/25–CD–CW) and P(MMA-*co*-GMA)(6′)(75/25–CD–MF), respectively.

### 3.2 The influence of different factors on BSA adsorption process

#### *3.2.1 Kinetic study of the BSA adsorption*

According with equilibrium data, the curves that illustrate the BSA adsorbed amount onto the polymeric surfaces related on time, are presented in Figs. 3, 4, 5, and 6. As it is well known the protein adsorption from aqueous solution to a solid surface follows, generally, three steps, respectively, the protein molecules diffusion from the solution to the interface, the attachment of protein molecules to the active sites of the surface, and the rearrangement of the molecules' structure after adsorption [34]. The last step is considered as being the most important in controlling the protein adsorption kinetic, as well as for the future characteristics and properties of the substrate surface.

From Figs. 3a, 4, 5, and 6a, which illustrate the kinetic evolution of the BSA adsorption onto the selected polymeric samples, it is observed a faster process at the beginning of coupling, when the surface of the matrix is

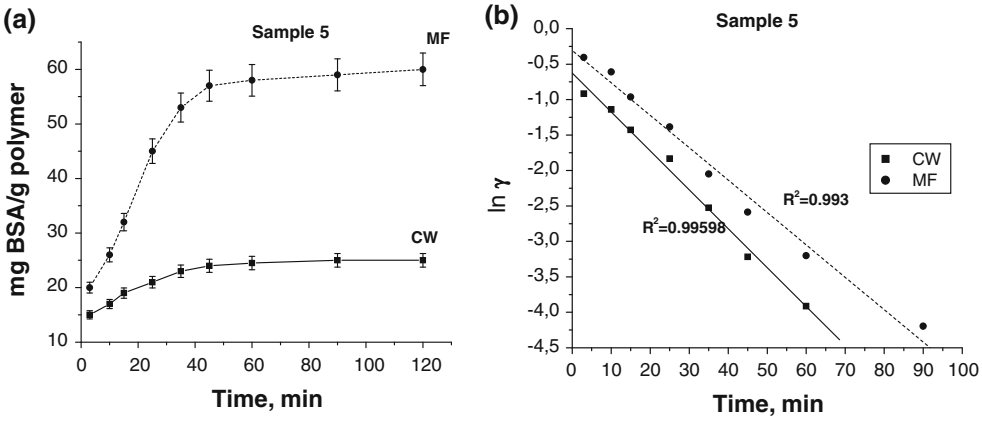


**Fig. 3** Shape (**a**) and kinetic (**b**) of the BSA adsorption on sample 5 (Table 1) performed in (MF) and without (CW) the magnetic field presence

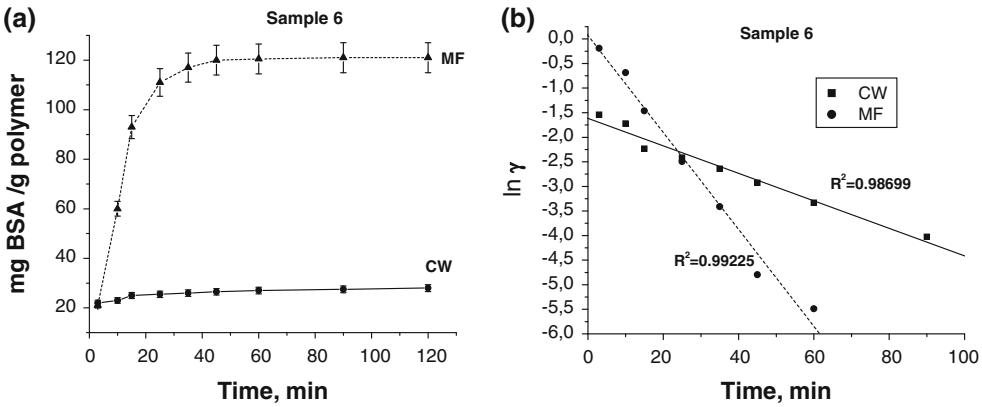


**Fig. 4** Shape (**a**) and kinetic (**b**) of the BSA adsorption in case of sample 6 (Table 1) achieved in (MF) and without (CW) magnetic field

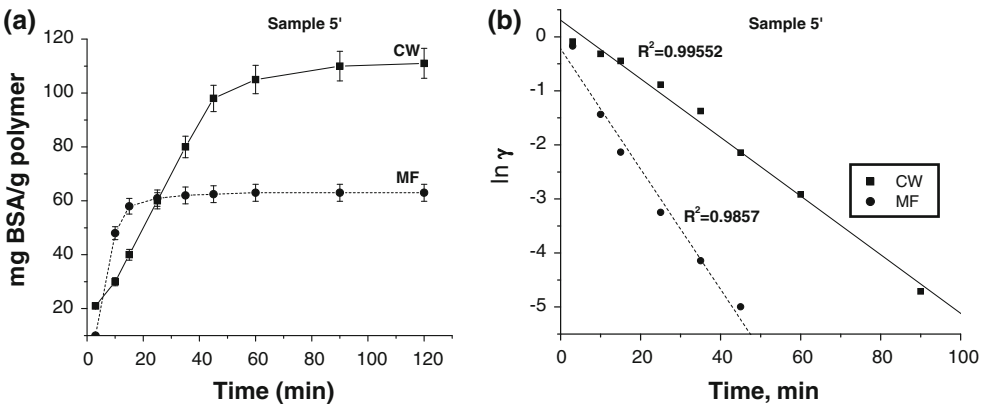


**Fig. 5** Shape (**a**) and kinetic (**b**) of BSA adsorption on sample 5′ realized in (MF) and without (CW) magnetic field

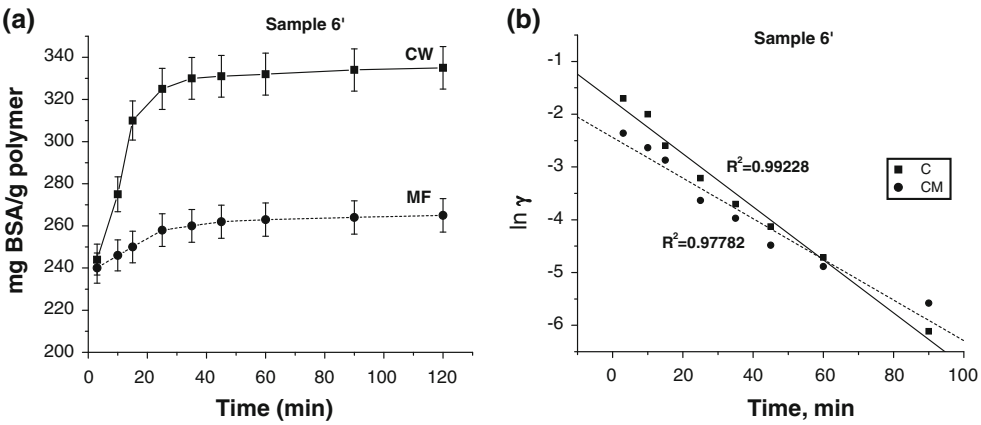


**Fig. 6** Shape (**a**) and kinetic (**b**) of BSA adsorption on sample 6′ (Table 1) performed in (MF) and without (CW) magnetic field

free and the adsorption kinetic is lead by the diffusion of molecules from the solution to the solid surface. The type of transport in this case corresponds to Fickian diffusion. The reaching of the coupling equilibrium it was considered as being attained after 120 min of stirring when the BSA adsorbed amount is almost the same with that registered after 60 min process of physically adsorbed protein. After that a barrier from BSA adsorbed molecules is formed. The molecules of BSA which are still present in solution must overtake this barrier to interact with the solid substrate and this process is slower. This behavior indicates a first order kinetic process which can be described by the equation:

$$-\frac{dC}{dt} = kC, \quad C = C_0 \exp(-kt)$$

where $C_0$ and $C$ represents the concentration of BSA at time 0 and $t$, respectively; $k$ is rate constant of BSA adsorption process. The BSA adsorbed amount in time will be:

$$Q = Q_m[1 - \exp(-kt)] \text{ or } \ln\gamma = -kt, \quad \gamma = \frac{Q_m - Q}{Q_m}$$

were $Q_m$ represents the maximum amount of adsorbed BSA and $Q$ represents the amount of adsorbed BSA at the $t$ moment.

The representation of ln $\gamma$ versus time (Figs. 3b, 4, 5, and 6b) is a straight line with slope $k$. The values of the rate constant, $k$, are presented in Table 3.

The adsorption rate constant depends on polymeric surface—respectively, the polymer composition—, on the variant of used tensioactive, as well as the presence or absence of the MF during the synthesis or during the adsorption. The greater rate of the adsorption corresponds to sample 5′ but maximum of the adsorbed BSA is recorded on sample 6′. With this new information, the next step in the study was the evidencing of parameters influence—temperature and pH—upon BSA adsorption onto sample 6′.

### 3.2.2 *Temperature influence onto BSA adsorption*

The influence of temperature on BSA adsorption was previously investigated by varying the temperature in the field of 25–40°C [32]. The results evidenced a direct dependence between BSA adsorption and the increase of temperature up to 30°C. Further increase of temperature is not properly for BSA adsorption, since the growing of the system entropy induces the diminution of the possibilities to create intermolecular forces. At the same time, with growing of the temperature, the tendency of BSA molecules tend to escape from the polymer surface into the solution. For the studied polymeric structures it was concluded the optimum temperature for adsorption as being 30°C, when the protein molecules acquire the properly structure for coupling [34]. As a consequence the temperature used for BSA adsorption in the future investigation was about 30°C.

**Table 3** The rate constant of BSA adsorption

| Sample | $k$ ($\times 10^{-2}$ mg g$^{-1}$ min$^{-1}$) | |
|---|---|---|
| | BSA adsorption through stirring in absence of MF | BSA adsorption through stirring in the MF presence |
| 5—P(MMA-*co*-GMA)(75:25)(SLS)(CW) | 5.488 ± 0.221 | 4.57 ± 0.222 |
| 6—P(MMA-*co*-GMA)(75:25)(SLS)(MF) | 2.797 ± 0.186 | 9.864 ± 0.552 |
| 5′—P(MMA-*co*-GMA)(75:25)(CD)(CW) | 5.426 ± 0.21 | 11.135 ± 0.81 |
| 6′—P(MMA-*co*-GMA)(75:25)(CD)(MF) | 5.025 ± 0.256 | 3.853 ± 0.337 |

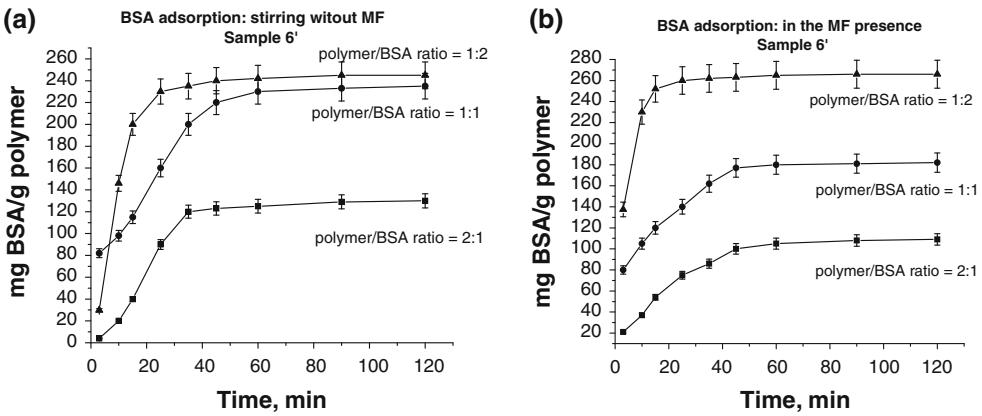


**Fig. 7** The BSA adsorbed amount related on polymer: BSA ratio—1:2; 1:1; 2:1—process performed by stirring without the MF (**a**) and in the MF presence (**b**)

### *3.2.3 Influence of polymer/BSA ratio on BSA adsorption*

The influence of polymer/BSA ratio on the BSA adsorbed amount onto the polymeric surface is illustrated in Fig. 7.

The study of the influence of ratio between polymer and BSA is important from two points of view. Firstly, it can be established the ratio at which BSA adsorbed content is maximum. Secondly, the final structure of protein layer often depends on the rate by which it is formed: at low concentration of protein the adsorption is slowly and there is longer time available for conformational changes to be occurred. By contrast, at high concentration of protein and high adsorption rates, the neighboring molecules may sterically prevent conformational changes [34, 35]. To have the best amount of coupled BSA it was found that optimum ratio between polymeric matrix (P(MMA-*co*-GMA: 75/25) synthesized in MF with $\beta$-CD as tensioactive) and protein is 1–2 and the adsorption is performed in MF. In these conditions the amount of the adsorbed albumin was 240 mg BSA/g polymer in the absence of the field (Fig. 7a) and 260 mg BSA/g polymer for the adsorption in the MF presence (Fig. 7b).

### *3.2.4 pH influence on BSA adsorption*

The BSA adsorption is a process dependent on pH, especially when molecules are adsorbed on the charged surfaces. The pH effect on BSA adsorption onto polymeric particles surface was investigated in the range of 3–10 (Fig. 8).

BSA molecules present a maximum of structural adaptability at acid pH that corresponds to the unpackaged protein structure. It is observed an increasing of the adsorbed BSA amount with pH up to small alkaline pH values. The maximum of adsorbed BSA is recorded at pH = 7 (about 240 mg/g) for adsorption by stirring in the absence of the MF (Fig. 8a), respectively pH = 8 (about 430 mg/g) for the adsorption in the MF presence (Fig. 8b). The continuous increase of pH determines the negative

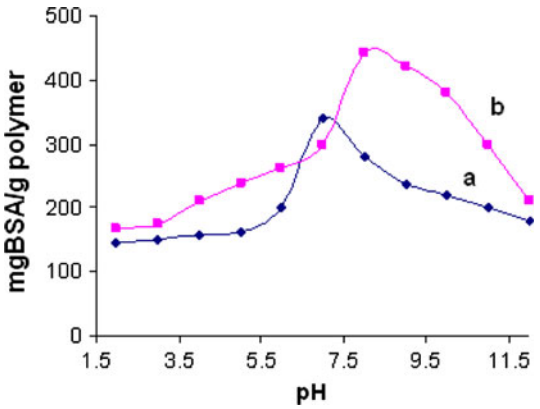


**Fig. 8** The BSA adsorbed amount related on pH values; adsorption performed by stirring without the field (*a*) and in the MF presence (*b*)

charging of the BSA molecules and due to the repulsive forces between BSA and substrate the adsorption decreases constantly. There are papers that mention the maximum adsorption of BSA at its isoelectric pH, respectively pH = 4.7 [36, 37]. Onto the surface of the studied polymeric structure the maximum of adsorbed BSA was registered at pH slow alkaline [33, 34]. Interactions of ionizable groups vary with pH, and the stability of a protein depends on the number of bound or released protons. Many proteins are maximally stable near pH 6, regardless of their isoelectric points. Stability varies smoothly with pH, suggesting the presence of compensating interactions in the constellation of ionizable groups. Consistent with this behavior, the maximum of the adsorbed BSA onto polymeric matrix (P(MMA-*co*-GMA)(75/25) synthesized in MF with $\beta$-CD as tensioactive) registered at pH slow alkaline, was attributed to the interfacial pH and conformational hypotheses [34].

At the same time the best results from the determining of the protein adsorption process correspond to the adsorption variant performed in the presence of the MF (Fig. 8, except the point at pH 7). This aspect was justified by the catalytic effect of the MF which may induce

orientation of the molecular structure. Thus, owing to the modification of the strength constant introduced by the inductive and electromeric effects supplementary appeared into the structures found in the MF conditions, they are re-shaped through growing of distance interactions and modification of the angles between bonds as well as by growing interactions between molecules.

Further investigations in order to evidence the interdependence between the polymer synthesis and the coupling method as well as the BSA content with or without the presence of the MF—are in progress.

## 4 Conclusions

The BSA adsorption onto the surfaces of PMMA and of MMA copolymer with 2,3-epoxypropyl methacrylate, it was investigated. The polymeric matrices were obtained through radical emulsion polymerization with and without the presence of a continuous external MF of 1,500 Gs intensity. Two types of surfactant agents were used for polymers' synthesis: a classic one (sodium lauryl sulphate—SLS) and $\beta$-CD. The coupling process was realized by physical adsorption performed by stirring with or without the presence of the MF and by varying the coupling conditions, respectively, the temperature, pH and albumin/polymer ratio.

The study underlines the assistance of the MF upon the adsorption process which it is concretized into growth of the BSA adsorbed amount onto polymeric matrices.

Also, the study evidences the increase of adsorbed protein content proportional with the amount of functional comonomer (2,3-epoxypropyl methacrylate).

Thus, the maximum of the adsorbed albumin content was recorded onto the poly(methyl methacrylate-*co*-2,3-epoxy propylmethacrylate) (75/25) synthesized in the MF presence and using $\beta$-CD as surfactant, when the adsorption was performed in the optimum conditions, respectively in the MF presence, at pH = 8 and the temperature of 30°C with a ratio between polymer and albumin of about 1–2.

Practically, the MF presence during adsorption determines the doubling of the BSA adsorbed quantity onto the surface of polymers prepared in the MF. The adsorbing process is also related to the nature of the tensioactive used for the synthesis of polymeric matrices. Higher content of the adsorbed BSA corresponds to the polymers prepared with CD instead of SLS.

This behaviour during adsorption was attributed to the catalytic activity of the MF, which determines the molecules distortions, the growth of distance interactions and the modifications of the angles between bonds with benefit effect upon adsorption.

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