The possibilities to obtain some bioactive composites based on macromolecular structures and a protein, there are presented. The biocompatible materials have as macromolecular matrices a (homo)-polymer of methyl methacrylate and three (co)-polymers of methyl methacrylate with different percentage of 2,3-epoxypropyl methacrylate, aspects concerning their syntheses and several of their characteristics being also mentioned. The used bioactive product was albumin. The procedure for protein immobilization as well as differences between macromolecular matrices composition are correlated with the coupling capacity of the bioactive product.

Key words: emulsion polymerization, crosslinked, functionalized copolymers, albumin, bioactive substance, biocompatibilization.

INTRODUCTION

The application of polymeric materials for medical purpose is growing very fast. Polymers have found applications in biomedical fields as tissue engineering, implantation of medical devices and artificial organs, prostheses, ophthalmology, dentistry, bone repair and many other applications [1]. Polymer based delivery systems enable controlled slow release of drug into the body [2]. Polymeric materials have also extensively used for biosensors, in testing devices, and for bio-regulation [3]. Such products suitable for a biomedical application must be biocompatible, beginning with their surface. Evaluating of the biomaterials biocompatibility has been a complex task. This complexity arises from the fact that biomaterials are made from a diversity of materials having various uses, with body contact ranging from transient skin contact, to contact with blood to permanent implantation. Biocompatibility is generally demonstrated by testing device materials, and their leachable chemicals, using toxicological principles [5].

One of the first steps in the manufacture of a medical device involves the selection of suitable biocompatible materials. This is an essential step because the types of tests required for the evaluation of a device depend on the physical
and chemical nature of its materials in addition to the nature of the device's exposure to the body. A specific material may appear suitable on the basis of its physical properties, cost, and availability, but might contain toxic chemical components. Therefore, it is advisable to screen the candidate materials at an early stage to eliminate those that are toxic, and select those that are sufficiently biocompatible or nontoxic for their possible uses [4, 6, 7].

The term “biocompatibility” refers to two different aspects that are strongly intercorrelated: – the tissues’ high tolerance vs. a foreign body, and – the chemical and especially physical stability of the materials during its entire presence in the organism [8].

The aim of this work is to present the elaboration of biocompatible materials based on macromolecular structures and a protein.

The used macromolecular matrices were (homo)-polymer of methyl methacrylate (MMA) and three (co)-polymers of methyl methacrylate with different percentage of 2, 3-epoxypropyl methacrylate (GMA) 3%, 9% and 25% respectively. The procedure for the macromolecular compounds synthesis is radical emulsion polymerization. The used bioactive product is albumin.

2. EXPERIMENTAL

2.1. MATERIALS

The monomers methyl methacrylate (c > 99 wt%, Fluka), 2, 3-epoxypropyl methacrylate (c > 97 wt%, Fluka) were freshly distilled before syntheses.

Sodium lauryl sulfate (C_{12}H_{25}O_{4}SNa from Sigma provenience (c > 95 wt%) was used as tensioactive agent without further purification.

Potassium persulphate was twice recrystallized from twice distilled water.

In all experiments the used water was twice distilled and contained no foreign ions.

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

2.2. SYNTHESIS PROCESS

A radicalic emulsion polymerization procedure of MMA and MMA with different fraction of GMA (3 wt%, 9 wt%, 25 wt%) has been used in order to obtain particles with narrow and uniform size. The selected comonomers have appropriate reactivity constants (Table 1), so the obtained copolymers are considered as random ones.

The presence of the functional comonomers has influenced the polymerization process (Fig. 1). Thus, small percentage of the GMA (variant with
Table 1
Reactivity constants data of MMA and GMA

<table>
<thead>
<tr>
<th>Monomer 1</th>
<th>Monomer 2</th>
<th>$r_1$</th>
<th>$r_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA</td>
<td>GMA</td>
<td>0.688</td>
<td>0.501</td>
</tr>
</tbody>
</table>

Fig. 1 – The conversion curves for PMMA and its copolymers.

3%) determines an increase of the conversion from 71.7% to 83%. At the same time, unexpected, higher content of the comonomer (variant with 25% GMA) decrease the polymerization rate with consequent extended reaction time.

Table 2 gives some values of the momentary polymerization rate.

Supplementary data concerning the macromolecular matrices synthesis, as well as their characterization were presented before [9]. Some of the characteristics of the synthesized polymers are listed in Table 3.

Table 2
The momentary polymerization rate value

<table>
<thead>
<tr>
<th>Momentary Polymerization Rate</th>
<th>PMMA</th>
<th>P(MMA-co-GMA): 97/3</th>
<th>P(MMA-co-GMA): 75/25</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{100}$</td>
<td>4.66</td>
<td>0.0033</td>
<td>0.0033</td>
</tr>
<tr>
<td>$v_{500}$</td>
<td>3</td>
<td>0.0033</td>
<td>0.005</td>
</tr>
<tr>
<td>$v_{900}$</td>
<td>3.55</td>
<td>0.29</td>
<td>0.0055</td>
</tr>
<tr>
<td>$v_{1800}$</td>
<td>35.55</td>
<td>22.22</td>
<td>0.0055</td>
</tr>
<tr>
<td>$v_{2700}$</td>
<td>25.93</td>
<td>30.074</td>
<td>3.22</td>
</tr>
</tbody>
</table>
In order to purify the synthesized macromolecular matrices to remove the tensioactive agent and eventually residual monomer, the obtained polymers were gently dried and then washed with twice-distilled water until the water used for dialyze have an UV spectrum correspondingly to pure water.

For a better protein immobilization the polymeric matrices was primary swollen in chloroform (the maxim swelling degree attained is also presented in Table 3) and then was again washed with twice-distilled water until the water used for dialyze have an UV spectrum correspondingly to pure water.

### 2.3. BIOCOMPATIBLE COMPOSITES

The procedure used to synthesizes the biocompatible the polymeric matrices, consists in the immersing the correspondingly quantity of polymer into a albumin aqueous solution having pH = 8 (made with NaOH and determined with a Digital pH-meter 100 device), was previously described [11]. The physical coupling procedure during 24 h at 25°C was made in two different manners: (a) through classical stirring (100 rot/min) (b) with a pre-treatment of the polymeric matrix before the coupling with the protein. The ratio between macromolecular compound and protein was 1:2. Then the obtained bioactive compounds were three times washed with a NaOH water solution having pH = 8, and was gently drying.

The method for the albumin immobilization at the polymeric matrix by classic stirring procedure was described before. [10] The purpose of this paper is to compare other activities with the obtained data achieved from the procedure with the pre-treatment of the matrices in order get the new biocompatible composites. The new method consists in two stages: first a physical pre-treatment

---

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Latex density g/cm³</th>
<th>Latex pH</th>
<th>Particles Dimension [µm]</th>
<th>α₁₅₀₀ [%]</th>
<th>EG%</th>
<th>Tg [°C]</th>
<th>Ti [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0207</td>
<td>4</td>
<td>&lt; 3</td>
<td>80.5</td>
<td>–</td>
<td>97</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>1.046</td>
<td>3.5</td>
<td>&lt; 3</td>
<td>148</td>
<td>0.9</td>
<td>0.036</td>
<td>116</td>
</tr>
<tr>
<td>3</td>
<td>1.0411</td>
<td>4</td>
<td>&lt; 3</td>
<td>129</td>
<td>2.7</td>
<td>0.057</td>
<td>118</td>
</tr>
<tr>
<td>4</td>
<td>1.0256</td>
<td>5</td>
<td>&lt; 3</td>
<td>77</td>
<td>7.57</td>
<td>0.103</td>
<td>122</td>
</tr>
</tbody>
</table>

1 – PMMA; 2 – P(MMA-c-GMA) 97/3; 3 – P(MMA-co-GMA) 81/9; 4 – P(MMA-co-GMA) 75:25; S_c – Chloroform Solubility; - partial soluble, – insoluble, α₁₅₀₀ – the swelling degree after 1500 min time of swelling, * microscopic determination, EG – epoxy group – ** Theoretic, *** Determinate; T_g – glass transition temperature; T_i – temperature corresponding to the beginning of the thermogravimetric decomposition.
procedure of the polymeric matrix and second the so-called albumin immobilization.

I – As physical pre-treatment procedure of the polymeric particles suspended in the distilled water we used:

A – their maintenance under an IR lamp (VEB LABORTECHNIK ILM LABOR Type 4) for 30 min at 30 cm distance, and

B – their preservation under an UV source (Brilliancy 400 µm, wavelength 365 nm) for 10 min at 10 cm distance.

After these procedures the polymeric particles were gently dried and were characterized by FTIR spectroscopy (on a DIGILAB, Scimitar Series, USA, spectrophotometer, the resolution recording was 4 cm⁻¹) in order to observe and evidence the physical treatment effect upon the macromolecular chains (Fig. 2 A-C). The FTIR spectra demonstrate that the pre-treatment with the IR or UV radiation maintain the structural integrity of the macromolecular matrices.

II – In the second step the pre-treated polymeric particles were immersed in the albumin solution accordingly to the before described procedure.

Data obtained from UV-vis spectra performed on a Specord 42 M were used to evidence the presence of albumin in the obtained biocompatible compounds. The used method was also previously described [10, 11].
RESULTS AND DISCUSSIONS

Interest in coupling of the protein layer has double purposes: (1) to confer biocompatibility to the macromolecular structure, and (2) to introduce more functional groups to participate at the immobilization reactions of further bioactive products.

Copolymerization allows systematic variation of the active units distribution along to a polymer chain. Moreover, copolymers can be tailor-made in order to vary the hydrophilic or hydrophobic character of the entire molecule or of a single domain (block systems).

Fig. 3 represents the amount of albumin immobilization through physical links, after stirring in usual conditions. Meantime Fig. 4 exemplifies the amount of albumin immobilized on the polymeric matrices pretreated under UV radiation, and Fig. 5 illustrates the albumin content immobilized on the polymeric matrices pretreated under IR radiation.
Bio-structures based on macromolecular compounds

As can be observed in usual conditions of stirring the presence of the functional (co)monomer determines the increase of the albumin immobilization.

The procedures with the polymers pre-treatment were conceived as moderate healing of the matrices capable to generate a relaxed network and new positions for the albumin physical capture. Unfortunately it was not obtained a rule between the physical treatment and the catching capacity. Anyway these physical treatments determine a better capacity of coupling for the polymeric matrices in respect with the protein, in order to obtain bioactive compounds.

Thus, UV radiation treatment allows similar capacity of coupling between homo- and copolymers. Higher content of functional comonomer near to the physical treatment do not offers a clear dependence.

Concerning IR treatment of the polymeric matrices it permits a growth ability of coupling with reference to the variant with UV radiation treatment. At the same time, a proportional dependence between the content of functional monomer and the capacity of coupling, it is observed.
CONCLUSIONS

Poly(methyl methacrylate) as well as methyl methacrylate copolymers with a monomer bearing functional groups – 2, 3-epoxypropyl methacrylate –, with controlled dimension and size of particles, have been obtained. The addition of the comonomer determines modifications of the thermal stability of the synthesized polymeric structures. At the same time the addition of GMA enables the increase of functional groups number in order to obtain polymeric networks capable to catch biologic active substances.

In conditions of usual stirring the capacity of coupling is directly dependent on the amount of functional (co)monomer, the bioactive compounds immobilization being better in case of the copolymers. It is also observed a direct dependence between the GMA content and the yields of albumin immobilization in case of IR radiation pre-treatment of the polymeric matrices. UV pre-treatment do not offer a strictly interdependence between polymeric composition, the coupling capacity and the method used for the obtainment of bioactive compounds. We must also notice the methods with polymeric matrices pre-treated offer better conditions for coupling of bioactive substances.

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