CHAPTER 14

Preparation of Blood Compatible Hydrogels by Preirradiation Grafting Techniques

J. Chen*, L. Yang, Y.C. Nho and A.S.Hoffman

Summary

Some kinds of hydrophilic monomers such as acrylamide (AAm), N,N-Dimethylacrylamide (DMAAm), N-(3-dimethylaminopropyl) methacrylamide (DMAPMAAm), polyethylene glycol methylacrylate (PEGMA) and 2-methacryloyloxyethyl phosphorylcholine (MPC) were grafted onto preirradiated polymer substrates of polypropylene (PP) and cellulose acetate films. The effects of irradiation dose, solvent and co-solvent system, monomer concentration and reaction time on the yield of grafting were studied. The surface of the grafted films gave typical properties of hydrogels, which was measured by water contact angle method. The grafted samples were characterized by FTIR in ATR mode. The blood compatibility of the grafted samples was roughly evaluated by the platelet adsorption and thrombus tests, respectively and the results showed that the blood compatibility of the sample films was relatively improved.

KEYWORDS: hydrogels; blood compatibility; radiation grafting; AAm; DMAAm; DMAPMAAm; PEGMA; MPC

*Correspondence to: Jie CHEN, Department of Chemical Engineering and Technology, Shanghai University, P.R.CHINA.
E-mail: jchen@shu.edu.cn

Topics in Tissue Engineering, Vol. 4. Eds. N Ashammakhi, R Reis, & F Chiellini © 2008.

INTRODUCTION

Usually, when blood is in contact with a polymeric substrate, plasma proteins are rapidly adsorbed and subsequent adherence of platelets leads to thrombus formation. It is one of the main problems of the biomaterials[1, 2]. After proteins adsorb to the surfaces, platelets adhere and release α-granule contents (Figure 1), including platelet factor 4 (PF4) and β-thromboglobulin (βTG), and dense granule contents including adenosine diphosphate (ADP). Thrombin is generated locally through coagulation reactions catalyzed by procoagulant platelet surface phospholipids. Thromboxane A_2 (TxA_2) is then synthesized. ADP, TxA_2, and thrombin recruit additional circulating platelets into an enlarging platelet aggregate. Thrombin-generated fibrin stabilizes the platelet mass[3].

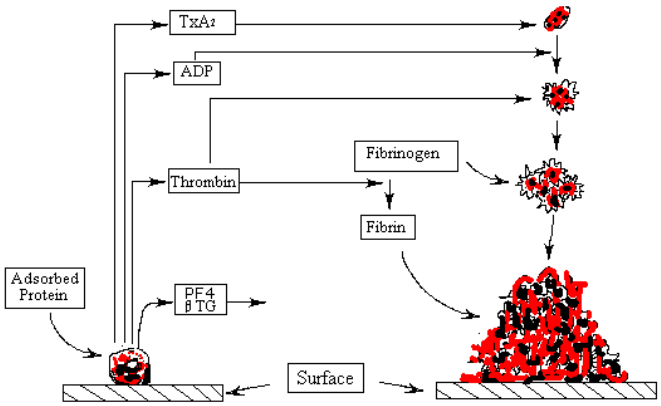


Fig.1. Schematic diagram of thrombus formation on artificial surfaces

In the application of polymers as artificial vessels or implants, surface modification is very important in the prevention of protein adsorption and platelet adhesion[4, 5]. It was reported that polymers with hydrophilic surface have low adsorption of serum proteins and do not have strong interactions with cells[6-9].

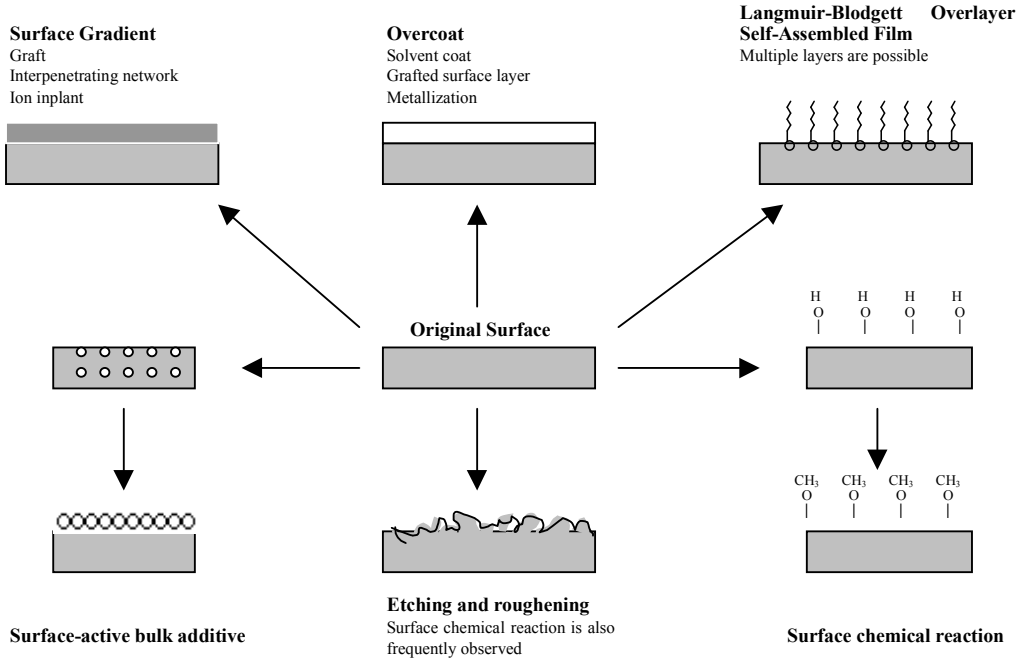


Fig. 2. Schematic representations of methods to modify surfaces

There are several methods of surface modification as shown in Figure 2[3]. Among them, grafting copolymerization is one of the widely used methods. Grafting modification can be achieved by UV irradiation[10-14], plasma treatment[15], ionizing irradiation with EB and γ–ray, etc. [16-29].

Radiation grafting by γ-ray or electron beam is a most widely used method for the bonding of hydrophilic monomers onto the surface of hydrophobic polymers[30-33]. Simultaneous radiation grafting and preirradiation grafting are the two common methods in grafting copolymerization[34-38].

I. Simultaneous radiation grafting

In the simultaneous radiation grafting, the substrate materials are irradiated in the presence of monomers or monomer solutions. While grafted chain growing during the irradiation, homopolymerization sometimes takes place at the same time, and some inhibitors are usually

needed for stopping the homopolymerization. The main reactions of simultaneous radiation grafting are illustrated as Figure 3. Where R• is radical of macromolecule A fraction or monomer B, which formed during irradiation.

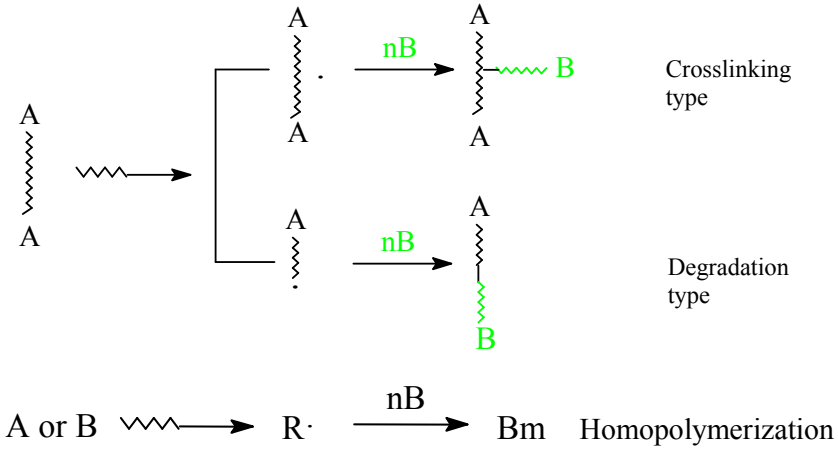


Fig. 3. Schematic reactions of simultaneous radiation grafting.

The advantages of simultaneous radiation grafting can be summarized as follows: easier operation, faster reaction and lower dose. And more, the presence of monomers can protect polymer substrates from degradation during irradiation. Usually, homopolymerization is a serious problem in simultaneous radiation grafting. It decreases the grafting efficiency and, the homopolymer anchored on the substrates is sometimes difficult to be removed. The following methods are commonly used for inhibiting homopolymerization and increasing the grafting yields:

1) Adding inhibitors, such as Cu^{2+} and Fe^{2+}.
2) Absorbed dose is closely related to the density of substance. Therefore, the homopolymer is controlled by the dilution of monomer B. For example, to use the monomers gas, monomers vapor and monomers solution etc.
3) Some solvent or mixture of solvents can not only inhibit the homopolymerization, but also control the depth of grafting.

II. Preirradiation grafting

Preirradiation grafting is that the substrate irradiated firstly, and then the grafting reaction is performed by contacting preirradiated substrate with monomer (in gas, liquid or solution state) at a certain temperature with oxygen free in the system. In this method, monomer is not irradiated, so the homopolymerization is easier to be avoided. On the other hand, grafting reaction happens at the outside of irradiation source, so the grafting reaction can be performed in any place even if there is no irradiation source.

Irradiation in the absence of oxygen

In non-oxygen irradiation, macromolecular substrates are irradiated in nitrogen atmosphere or in vacuum, then oxygen removed monomer is introduced. The free radicals in the polymer can be trapped and retained for a period of time, which may initiate the grafting reaction under certain conditions. The characteristics of this method are: 1) Relatively higher irradiation dose is needed and the degradation of the substrate sometimes is possible; 2) It needs relatively long life of free radicals or trapped radicals on the substrates. Usually trapped radicals in higher crystalline polymer, such as PE, PP etc. can be kept for a long time. In general, the lifetime of the trapped radicals is much longer at lower temperatures. Accordingly, irradiation at low temperature can increase the grafting yield.

Irradiation in the presence of oxygen

Radiation peroxidized polymers are formed by irradiating the polymer substrates in air. The diperoxides or hydroperoxides usually are stable at room temperature. Figure 4 shows the formation of diperoxides and hydroperoxides, as well as the main reactions during the grafting.

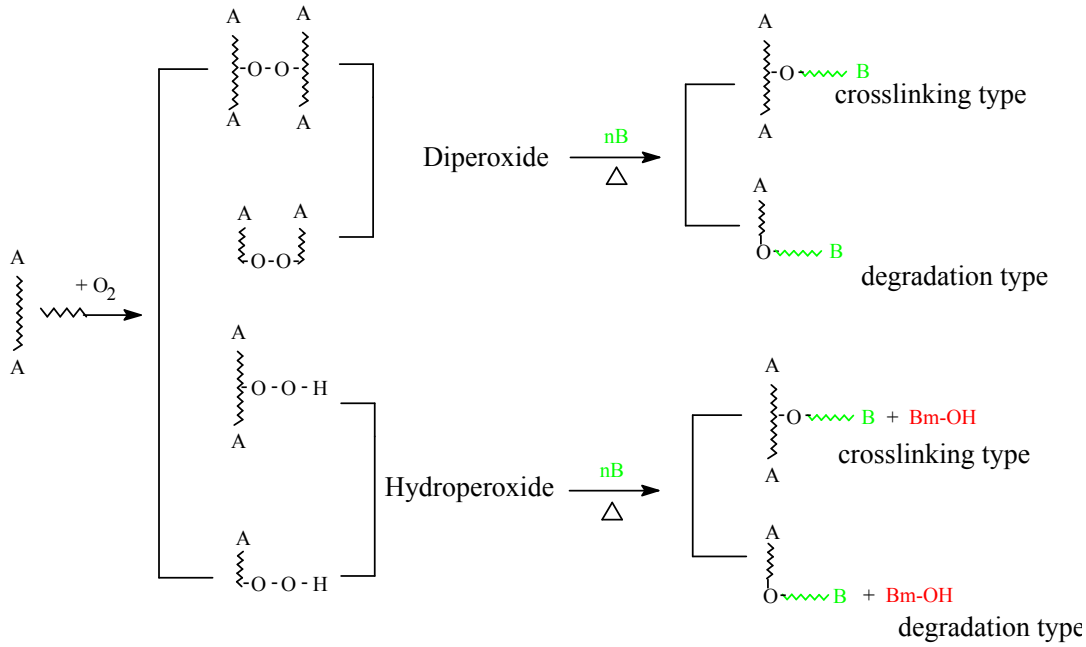


Fig. 4. Schematic reactions of preirradiation grafting.

It shows the thermal dissociation of the hydroperoxide gives rise to an equivalent number of graft copolymer and homopolymer molecules. The latter one is from the initiation of the polymerization of monomer B by •OH radicals. Usually the homopolymerization can be avoided to a large extent by decomposing the hydroperoxides at low temperature in a redox system, such as in the following example:

$$ROOH + Fe^{2+} \longrightarrow RO\cdot + OH^{-} + Fe^{3+}$$

Besides the substrate itself, there are also some factors that influence the preirradiation grafting reactions, such as absorbed dose, monomer concentration, additives, reaction temperature and reaction time etc. A new method of two or more times grafting reaction on the preirradiated polypropylene (PP) and polyethylene (PE) film were reported recently. Two different kinds of monomers were grafted on the preirradiated polymeric substrates respectively by the intermittent grafting method, and novel interpenetrate networks (IPN) hydrogels were obtained[39-41]. Three kinds of hydrophilic monomers were applied to modify the blood compatibility of the surface of PP and cellulose films.

Acrylamide polymers and copolymers were studied earlier as biomaterials[42-45]. However, the mechanical strength of acrylamides hydrogels sometimes is not high enough for the application of blood-contacting implant. Some methods were reported on the modification of polymeric substrates by grafting of AAm, for preparing the new materials with good blood and bio-compatibility, as well as good mechanical strength[46-54]. Copolymerization of acrylamides with some other monomers was also an efficient method in the preparation of biomaterials and drug delivery system[55-58].

Polyethylene glycol methylacrylate (PEGMA) is widely used in medicine and pharmacy because it has a good biocompatibility and no toxicity[60, 61]. PEGMA sometimes is copolymerized for the preparation of hydrogel and drug delivery systems[62-66]. It can be used to endow the hydrophobic polymer with hydrophilic surface[67-71]. Many research works were reported on the graft of polyethylene glycol methylacrylate (PEGMA) polymeric substrate for the modification blood and biocompatibility[72-79].

The 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer having phospholipid polar group shows excellent blood compatibility. That suppression of platelet adhesion and activation was observed even when the MPC polymer contacted human whole blood without anticoagulant. This is due to the reduction of protein adsorption on the MPC polymer surface from human plasma[80, 81]. The MPC can be polymerized in water or grafted onto cellulose membrane surface even in a heterogeneous system. It was reported that the introduction of poly(MPC) chains on the membrane surface was effective in preventing platelet adhesion and activation[82-84]. Some methods have been reported on the introducing of poly(MPC) onto the surface of cellulose and chitosan membrane and other polymeric substrates[85-94]. The co-polymers of hydrophilic monomer (MPC) with hydrophobic monomers were reported on other applications such as biomaterial and drug delivery etc.[95-105]

The above three types of hydrophilic monomers grafted onto preirradiated PP films and cellulose acetate films are introduced for the modification of blood compatibility, respectively. The modified surfaces were characterized by FTIR and water contact angle; The comparison of blood compatibility between the grafted and non-grafted sample substrates were made by evaluating the platelet adsorption, plasma protein adsorption and the amount of thrombus respectively.

EXPERIMENTAL STUDIES

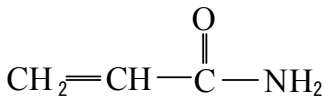
Materials

Commercial polypropylene (PP) film with the thickness of 0.20mm and commercial cellulose acetate film with the thickness of 0.01mm from Hanjung Chemical Co. (Korea) were used as a substrate for the grafting reaction. These polymeric substrate films were cut into $2\times5cm^2$ pieces and ultrasonically cleaned twice in methanol for 1h each time, and then dried in a vacuum oven.

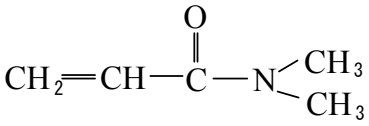
Chemicals

1. *Acrylamides*: acrylamide (AAm), from Junsei Chemical Co., Ltd. (Japan); N,N-Dimethylacrylamide (DMAAm), from Aldrich Chemical Company, Inc. (USA); N-(3-dimethylaminopropyl) methacrylamide (DMAPMAAm), from Tokyo Kasei Co. Ltd. (Japan), were used without further treatment. The structures of the monomers are as follows:

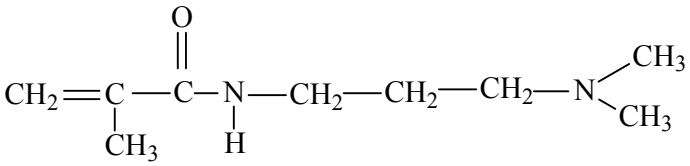
AAm



DMAAm



DMAPMAAm



2. Monomers of polyethylene glycol methylacrylate (PEGMA) with different polyethylene oxide repeat units were supplied by the Nippon Oil & Fats Co. The structure of PEGMA is as follows and the three kinds of PEGMA used in this experiment are shown in Table 1.

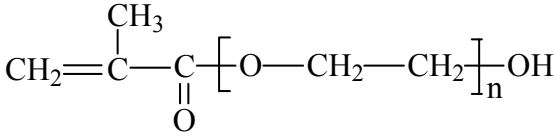
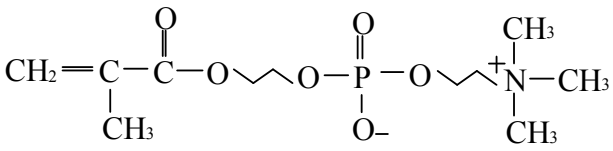


Table 1. The Structure of PEGMA.

Abbreviation	Ethylene Oxide No (n)	Molecular Weight
PEO90	2	163~173
PEO200	4-5	261~283
PEO350	7-9	387~468

2-methacryloyloxyethyl phosphorylcholine (MPC) (from Biocompatibles Ltd. UK) was used without further treatment.

Structure of MPC



Acetone, Methanol (MeOH), tetrahydrofuran (THF) and other chemicals were reagent grades and used without further treatment. Other chemicals were reagent grades and used without further treatment.

EXPERIMENT

1. Grafting procedure

The gamma ray irradiation from Co-60 source was carried out at an exposure rate of 4.56kGy/h in the presence of air to a total dose of 10~60 kGy. The irradiated polymeric substrate films were stored in a refrigerator and kept at -130°C until the grafting reaction was performed. The grafting reaction was conducted in a Pyrex ampoule having a vacuum cock. Solvent or solvents mixture (e.g. acetone, THF/MeOH, THF/H_2O or water) as the diluent was added first, followed by monomer. Irradiated sample film was immersed in the Pyrex ampoule containing 30ml monomer solution, and purged by bubbling nitrogen for 20min. The grafting reaction was carried out by placing the ampoule in a water bath at the designed temperature. After the grafting reaction, the

film was taken out of the monomer solution in the ampoule and washed with methanol and distilled water to remove the remaining homopolymer. The degree of grafting was determined by the following equation:

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

Where W_g and W_o were the weights of the grafted and starting sample, respectively.

2. FTIR Verification

The grafted sample films were verified by Fourier transform infrared (FTIR) spectroscopy in the attenuated total reflectance mode (ATR). A Nicolet 5SXC spectrophotometer with a nominal 45 degree attenuated total reflectance was used to examine the functional group of >C=O on the grafted PP films.

3. Water Contact Angle Measurement

The grafted polypropylene film surface was characterized by assessing water contact angle using an optical contact angle goniometer (Erma, Japan). Water contact angle for each sample was measured by a sessile drop method five times at room temperature. Drops of purified water (3μl) were dropped onto the grafted PP surface, and the direct microscopic measurement of the contact angles for polypropylene stored at room temperature during the 3 min after deposition was done with a goniometer.

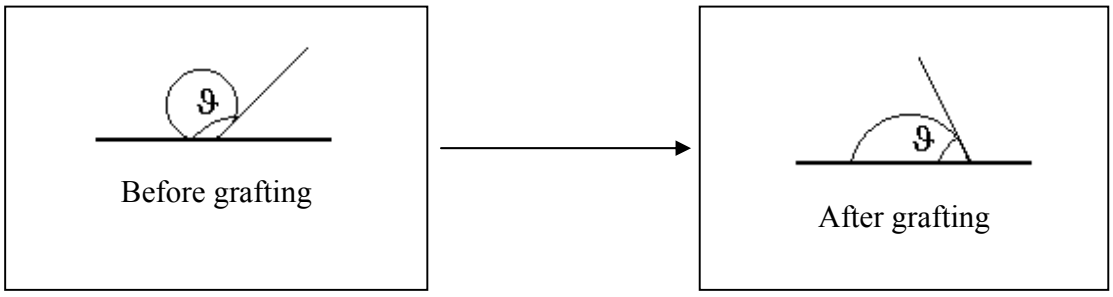


Fig. 5. Schematic diagram of water contact angle

4. Platelet Adhesion

Human blood from healthy volunteers was collected with a polypropylene syringe containing a 3.8% sodium citrate solution. Platelet rich plasma (PRP) having a 2.43×10^5 cell/μl concentration was obtained by centrifuging the human blood at 2300 rpm for 4 min at 4°C. Non-grafted control and grafted sample films were hydrated by soaking in phosphate-buffered saline (PBS, pH 7.4) filled polystyrene 24 wall vials five times for 10 min. Each hydrated film was transferred into a PRP pre-warmed to 37°C for 30 min. After incubation at 37°C, the samples were washed carefully with PBS to remove weakly adhered platelets. Platelets adhered on the sample surfaces were fixed with a 2.5% glutaraldehyde in PBS for 10 min at room temperature, and then were dehydrated in an ethanol-grade series (50, 60, 70, 80, 90, and 100%) for 10 min. after each was washed with PBS, and followed to dry on a clean hood at room temperature. The platelets attached on the sample films were examined by a scanning electron microscope (SEM, JSM-840A, JEOL Co., Japan) with a tilt angle of 45 degrees after gold deposition in vacuum.

5. Determination of the amount of thrombosis

The amount of thrombus formed on grafted samples and non-grafted control sample was evaluated by an *in vitro* method following Imai and Nose[59] technique using ACD human whole blood which was supplied from Blood Bank of the Korea Red Cross. Before the clot test, the samples (1.5×1.5 cm^2) were hydrated to constant weight in saline water (0.9% NaCl) and kept at 37°C in a constant temperature water bath in watch glasses. ACD human blood (0.05 ml) was added to each sample. The reaction was then started by adding 0.1M calcium chloride (0.005 ml) to each sample of blood and the blood with calcium chloride solution were mixed at once by stir of a Teflon stick. After 30 min, distilled water (1 ml) was added to stop the reaction and separate the thrombus, which was formed on various samples. After 5 min, the thrombus formed was taken out of sample film with a spatula and was fixed by soaking in 37% formaldehyde (1 ml) solution for 5 min at room temperature, and then washed by soaking in water for 5 min. The fixed thrombus obtained was blotted between two pieces of cellulose based filter papers and weighed on a chemical balance. To obtain the exact data, the amount of thrombus formed on sample at the same condition was measured 3 times for each sample. The percentage of thrombus on PP films is relatively to the thrombus on glass, which is supposed as 100% at the same condition.

6. Plasma Protein Adsorption

Human plasma protein (Sigma Chemical Co., St. Louis, MO, USA) was diluted with PBS to make a 1% solution. The non-grafted control and grafted PP films were hydrated in PBS 5 times at 37°C first, and then were placed to contact with the above plasma protein solution in 24 wall vials of polystyrene at the same temperature for 1 hr incubation. Then the samples were washed with PBS, followed by purified water to remove non-adsorbed proteins. After vacuum drying, the change in protein adsorption of the control and PEGMA grafted polypropylene surfaces were investigated by electron spectroscopy for chemical analysis (ESCA). The changes in the nitrogen 1s peaks from the X-ray photoelectron spectroscopy survey scan spectra were examined.

RESULTS AND DISCUSSION

1. The effects of reaction conditions on the degree of grafting

The effect of irradiation dose on the degree of grafting

It is known that free radicals created by irradiation in solid polymers are immobilized, and may remain trapped for a period of time. In the preirradiation process, the polymeric material is irradiated, and subsequently the de-aerated monomer is contacted with the irradiated polymer. Virtually little homopolymer is produced by this method, and there is no limitation to any particular polymer/monomer combination since the monomer itself is not irradiated. Although preirradiation method has been successfully used for grafting of various vinyl monomers onto polymer, the grafting yield obtained by this method usually depends on the efficiency of the trapped radicals. The main factor governing the trapping of radicals is the physical state of the irradiated polymer. In the case of rubbery and non-crystalline polymers, the mobility of radicals is fairly significant, and their survival time after irradiation is not so long compared with the polymers having high crystallinity. The validity of the grafting method depends largely on the crystallinity of the polymer, and the relative reaction rates of the monomer with trapped radicals and the thermal decay of radicals at the required temperature for grafting. The crystallinity of polypropylene used in this experiment was about 48.6%, based on 147 Joule/mole of polypropylene having 100% crystallinity.

The effect of irradiation dose on the degree of grafting of DMAAm and DMAPMAAm on PP film was determined in the aqueous solutions of DMAAm and DMAPMAAm, respectively. Figure 6 shows that the degree of grafting of DMAAm and DMAPMAAm increased with the irradiation dose. Under the same reaction condition, the grafting yield of DMAAm was higher than that of DMAPMAAm.

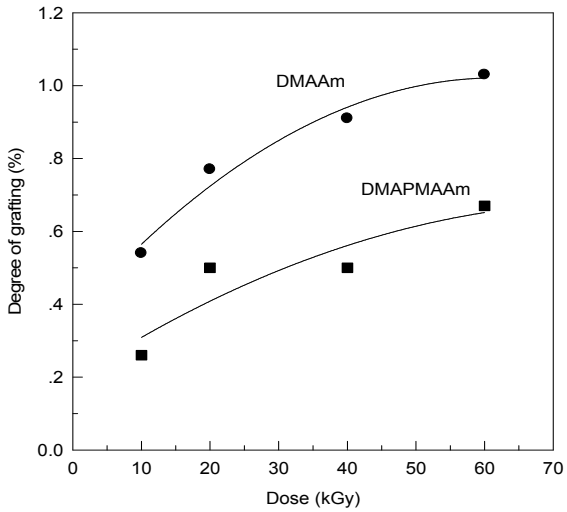


Fig. 6. The effect of irradiation dose on the degree of grafting (Grafting reaction was performed in aqueous solution with 10% (v/v) monomer at 70°C for 3hr).

The effect of solvents on the degree of grafting

i) The effect of solvents on the degree of grafting of acrylamides

Acetone and water were used as the diluents for the grafting reaction, respectively. It was found that AAm and DMAAm were easier to be grafted onto PP films in both acetone and aqueous solution than that of DMAPMAAm. Figure 7 shows the effect of reaction time on the degree of grafting of AAm onto PP films. It shows that he higher grafting yield could be easily be obtained in acetone solution than that in aqueous solution. The authors attributed the behavior to the swelling of the polymer. It is also plausible that wetting solvent for polypropylene in this experiment leads to the enhanced access of monomers to the grafting sites in polypropylene. Therefore, acetone as the solvent is more favorable for grafting yield of acrylamide than water in this experiment.

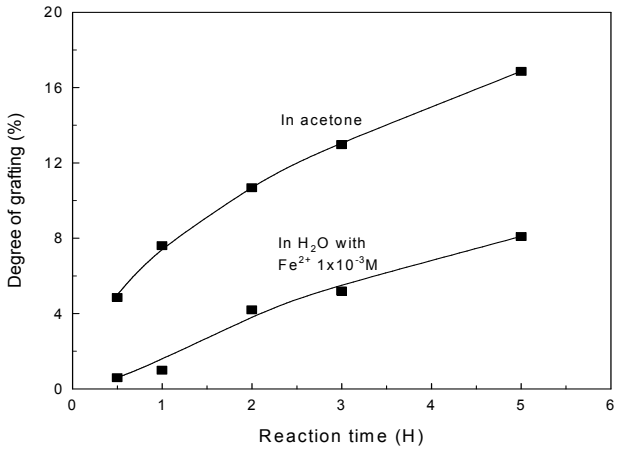


Fig. 7. The effect of reaction time on the degree of grafting of AAm onto PP film with the comparison of different grafting solution (Dose 20kGy; Temp. 60°C for acetone solution and 70°C for aqueous solution).

ii). The effect of solvent mixture on the degree of grafting of PEGMA

Figure 8 shows the effect of different co-solvent systems on the grafting yield of PEGMA. It shows that the samples grafted in the co-solvent system of THF/MeOH (2:1, v/v) gave higher grafting yield than that of MeOH/H_2O (1:1, v/v) under the same reaction condition. It may be attributed that the good wetting and swelling properties of the solvent mixture for polypropylene film enhanced the yield of grafting.

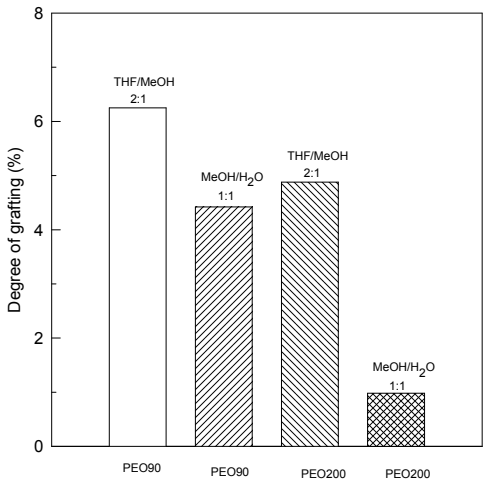


Fig. 8. The comparison of grafting yield at different co-solvent system. (Grafting condition: PEGMA concentration: 20% v/v; Temperature: 70°C; Time: 5h; Dose: 20kGy)

iii) The effect of H_2O fraction in MeOH/H_2O on the degree of grafting of PEGMA and MPC.

Distilled water and co-solvent system of MeOH/H_2O were used as diluents for MPC and PEGMA. Figure 9 shows that the grafting yield of MPC/PEGMA and PEGMA increased with the increase of H_2O fraction in the co-solvent system. In the MPC grafting, the higher grafting yield was obtained when the composition of the MeOH/H_2O co-solvent was about 60/40 (v/v).

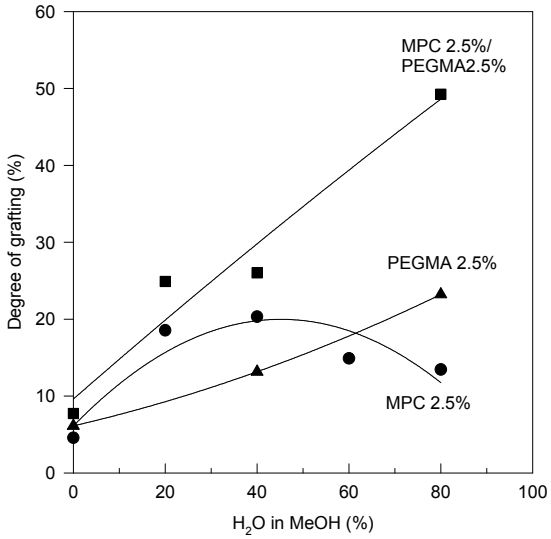


Fig. 9. The effect of H_2O fraction in MeOH/H_2O on the degree of grafting of various monomer system (grafting reaction was performed at 70^{o}C for 5h).

The effect of reaction time on the degree of grafting

i) The effect of reaction time on the degree of grafting of AAm on to PP film

Figure 7 can be used to explain the effect of reaction time on the degree of grafting of AAm onto the γ-ray pre-irradiated PP film in acetone and water. It shows that the degree of grafting of AAm increased with the reaction time in this experiment condition.

ii) The effect of reaction time on the degree of grafting of MPC

Figure 10 shows the effect of reaction time on the degree of grafting of MPC when the grafting reaction was performed in MeOH/H_2O (1:1, v/v) co-solvent system. It shows that the degree of grafting increased with the reaction time. It means that the extension of reaction time is a useful method for getting the higher grafting yield when other grafting conditions were fixed.

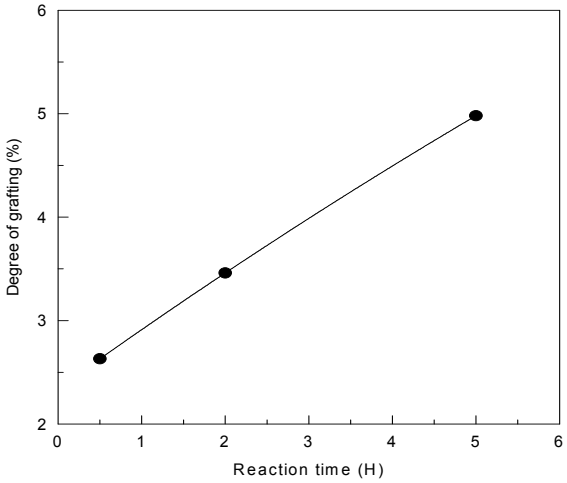


Fig.10. The effect of reaction time on the degree of grafting (Grafting reaction was performed in MeOH/ H_2O 1:1 solution system with 1% MPC at 70^oC)

The effect of monomer concentration on the degree of grafting

Figure 11 is the effect of monomer concentration on the degree of grafting of MPC and MPC/PEGMA onto preirradiated cellulose acetate film. It shows that the grafting yield increased very fast with the increase of monomer concentration, when the grafting reaction was performed in MeOH/ H_2O (1:1, w/w) co-solvent system with the monomer mixture of MPC/PEGMA (1:1 w/w). In the case of aqueous solution, the grafting yield of MPC increased slowly with the increase of MPC at first and then went to terminate when the monomer concentration of MPC was above 3%.

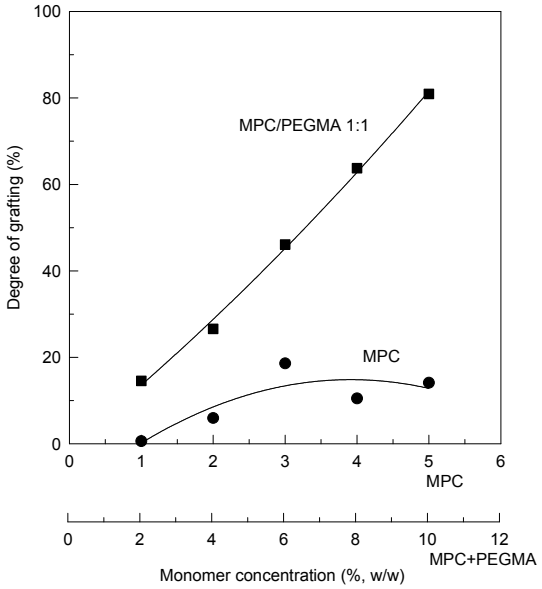


Fig.11. The effect of monomer concentration on the degree of grafting (Grafting reaction was performed in: a. aqueous solution of MPC with $1.0x10^{-3}$M $FeSO_4$ and b. MPC+ PEGMA MeOH/ H_2O 1:1 70°C for 5h)

2. Verification of surface properties

The FTIR spectra

i) The FTIR spectra of AAm DMAAm grafted PP films

Carbonyl peak of each grafted film was checked by FTIR spectroscopy in the attenuated total reflectance (ATR) mode when AAm and DMAAm were grafted onto PP film in acetone solution and in aqueous solution, respectively (Figure 12 and 13). The sample grafted in aqueous solution gave much stronger carbonyl (>C=O) peak than that of the sample grafted in acetone.

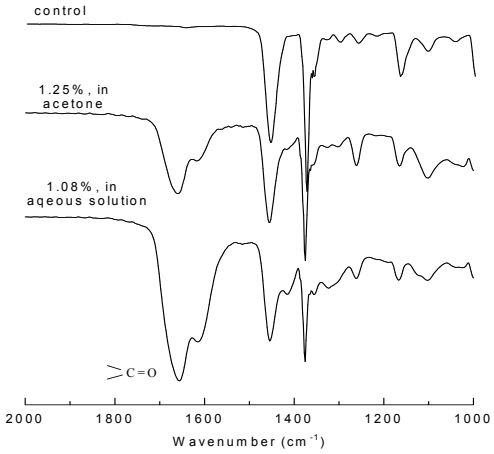


Fig.12. The FTIR-ATR spectra of AAm grafted PP film with the comparison of different grafting solution.

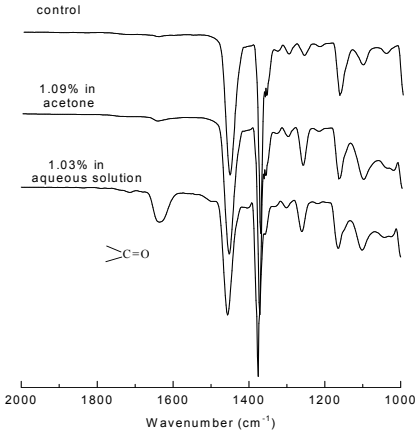


Fig.13. The FTIR-ATR spectra of DMAAm grafted PP film with the comparison of different grafting solution.

ii) The FTIR spectra of PEGMA grafted PP samples

When the grafting reaction was carried out in MeOH/H_2O solution system, the stronger carbonyl (>C=O) peak on FTIR spectra appears even though the grafting yield is much lower than that of the sample grafted in THF/MeOH solution system (Figure 14).

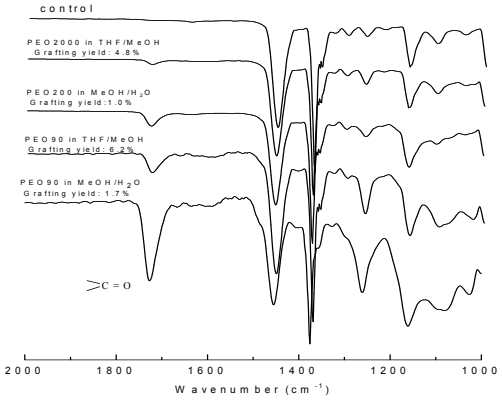


Fig.14. The comparison of carbonyl (>C=O) group on FTIR-ATR spectra of PEGMA grafted PP films from two different co-solvent systems.

iii) The FTIR spectra of MPC grafted cellulose acetate films

Figure 15 and Figure 16 are the FTIR-ATR spectra of MPC grafted cellulose acetate films, which were grafted in MeOH/H_2O co-solvent system and aqueous solution, respectively. Compared with the peak of ~1650 (cm^{-1}), the strength of carbonyl group (>C=O) peak with the wavenumber of ~1720 (cm^{-1}) increased with grafting yield. When the grafting reaction was carried out in aqueous solution (Figure 16), the carbonyl peak on the FTIR spectra (wavenumber ~1720 cm^{-1}) is relatively stronger compared to the similar grafting yield, which was carried out in MeOH/H_2O (Figure 15).

Figure 17 shows FTIR-ATR spectra of MPC/PEGMA grafted cellulose acetate films. It shows that the carbonyl group (wavenumber ~1720 cm^{-1}) peak is clearly appeared after the grafting of MPC/PEGMA. It also shows that the strength of the carbonyl peek with 1720cm^{-1} increased with grafting yield.

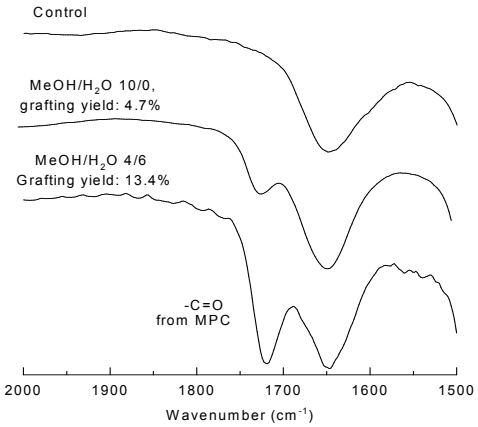


Fig. 15. The FTIR spectra of MPC grafted cellulose acetate films (Grafting reaction was performed in MeOH/ H_2O co-solvent system with 2.5% w/w MPC at 70^oC for 5h; Dose: 30kGy)

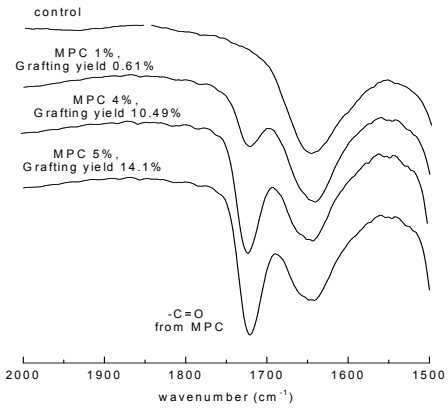


Fig.16. The FTIR spectra of MPC grafted cellulose acetate films (Grafting reaction was performed in aqueous solution in the presence of 1×10^{-3}M $FeSO_4$ at 70^oC for 5h; Dose: 30kGy)

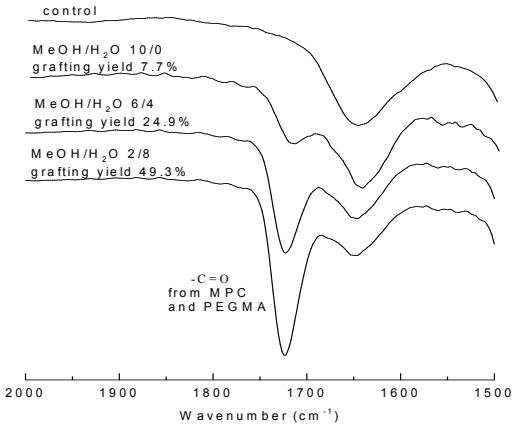


Fig. 17. FTIR spectra of MPC/PEGMA grafted cellulose acetate films (Grafting reaction was performed in MeOH/ H_2O co-solvent system with MPC 2.5% /PEGMA 2.5% at 70^oC for 5h; Dose: 30kGy)

The water contact angle

i) The water contact angle of AAm grafted PP films

Figure 18 is the relationship between grafting yield and the water contact angle. It shows that the water contact angle of AAm-grafted sample is lower than that of DMAPMAAm. It can be attributed to the higher hydrophilicity of AAm than that of DMAPMAAm. Regardless of the monomer of DMAPMAAm, AAm and DMAAm, water contact angle of PP samples grafted in the presence of water was lower than that in the presence of acetone (Figure 19). For example, water contact angle of the surface of AAm-grafted PP film with the yield of 1.08% was around 20^o when the grafting yield was carried out in aqueous solution of AAm. However, the water contact angle of AAm-grafted PP film with the yield of 1.05% was around 90^o when the grafting **yield was carried out in acetone solution of AAm.**

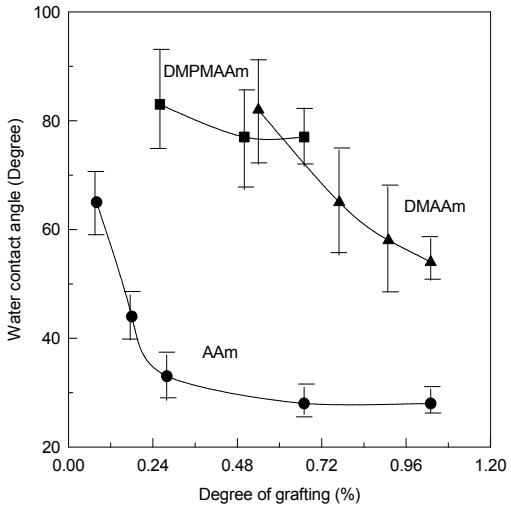


Fig.18. The relationship between grafting yield and water contact angle (Grafting reaction was performed in aqueous solutions).

Figure 20 shows the schematic illustration of the grafted surface property when the grafting reaction was performed in aqueous solution system and acetone solution system, respectively. Acrylamides (used in this experiment) are consisted of hydrophilic and hydrophobic groups. During the grafting process, the hydrophilic group of acrylamides is greatly oriented to the outside surface of grafted PP in the presence of water, because it is more compatible with water than acetone. Therefore, either hydrophobicity or hydrophilicity was greatly influenced by the type of solvent in grafting reaction.

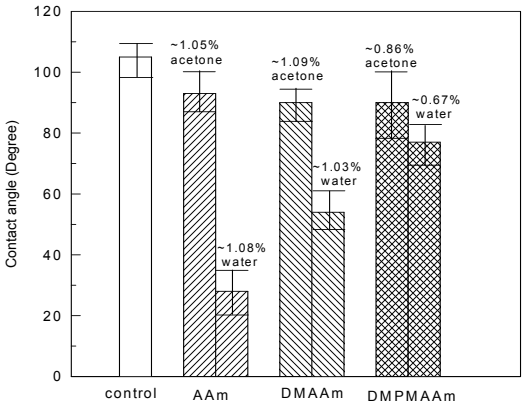


Fig.19. The comparison of water contact angle of acrylamides grafted PP films at different solution

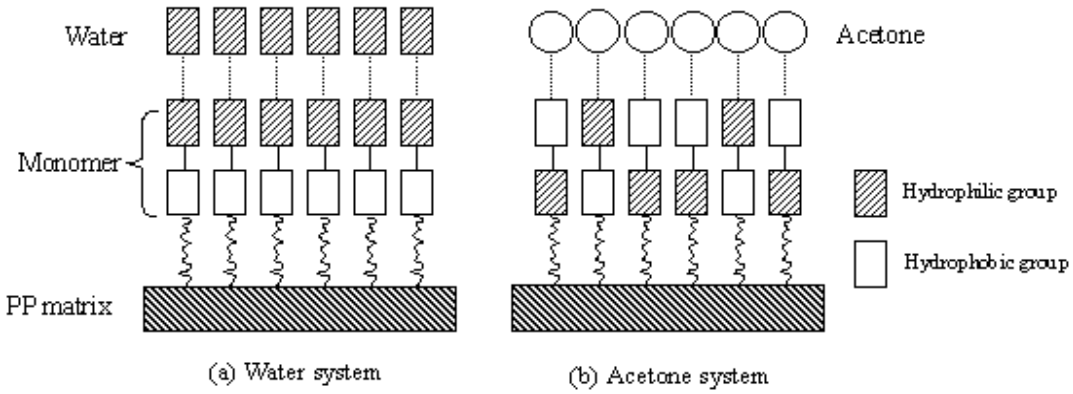


Fig. 20. Schematic illustration of the grafted surface property when the grafting reaction was performed in different solution system.

Water is a good solvent not only for hydrophilic monomer AAm, but also for the homopolymer of PAAm. When the grafting reaction was performed in aqueous solution of AAm, the concentration of PAAm is relatively higher on the surface of PP film. Crosslinking reaction could easily take place between the well-extended grafting sections of PAAm in water. In this way, PAAm was mostly coated on the surface. Therefore, many of the carbonyl groups

were detected by FTIR spectrometer. The acetone is relatively hydrophobic and not a good solvent for AAm, but it is relatively a good solvent for PP film. On the other hand, homopolymer of PAAm is not dissolved in acetone. During the grafting reaction of AAm in acetone solution, the monomer diffused easily to the surface of PP film due to the precipitation of PAAm. This reaction condition seems beneficial for the growing of grafting chain but not for crosslinking reaction between the grafted chains. In our experiment, the crosslinked homopolymer of AAm was not found in acetone solution after grafting reaction. Therefore, the grafted surface may be comb like but not well coated. The trend of inner part grafting may also be relatively greater when the grafting reaction was performed in acetone solution. Some carbonyl groups may be covered and not detected by FTIR spectrometer.

ii) The water contact angle of PEGMA grafted PP films

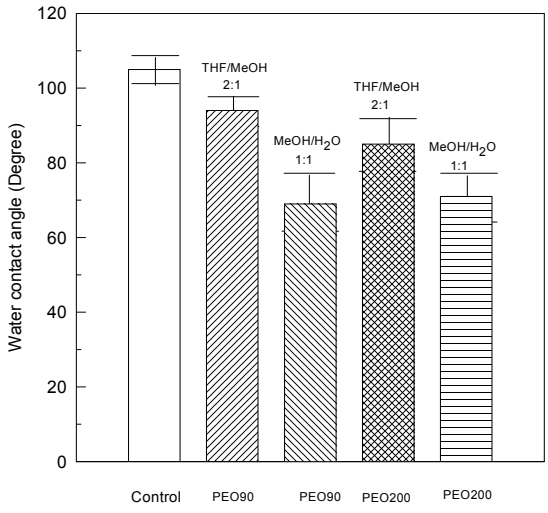


Fig. 21. The comparison of water contact angle of PEGMA grafted PP films at different co-solvent system (Grafting yield of PEO90: ~3%, PEO200: ~1%).

The water contact angle of the samples grafted in the $MeOH/H_2O$ was clearly lower, compared with the non-grafted film sample, while ones grafted in THF/MeOH was almost not change (as shown in Figure 21). For example, water contact angle of the surface of 4.5% PEGMA (PEO90)-grafted PP film was around 65° when the grafting yield was carried out in

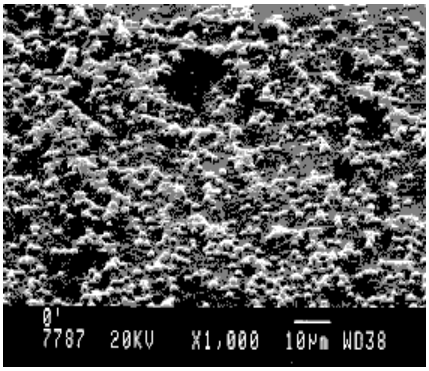
MeOH/H_2O as the solvent. However, the water contact angle of 6.2% PEGMA (PEO90)-grafted PP film was around 92° when the grafting yield was carried out in THF/MeOH. This phenomenon may be explained as follows: PEGMA molecule consists of hydrophilic and hydrophobic groups. During the grafting process, the hydrophilic group of PEGMA is greatly oriented to the surface of grafted layer when MeOH/H_2O was used as the solvent, because hydrophilic group of PEGMA is more compatible with MeOH/H_2O than that of THF/MeOH. Therefore, it is possible to explain that the stronger carbonyl (>C=O) peak identified by FTIR spectra in MeOH/H_2O system is related with the arrangement of PEGMA molecule during grafting reaction, depending on the type of solvent.

3. Blood compatibility of the samples

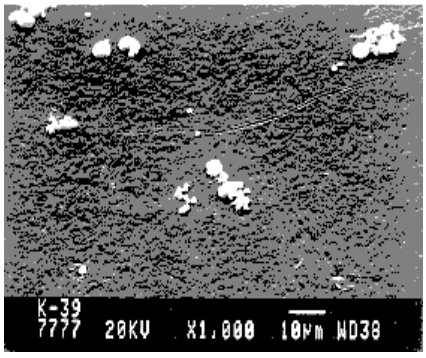
3.1. Platelet adhesion

i) Platelet adhesion to AAm, DMAAm and DMAPMAAm grafted PP films

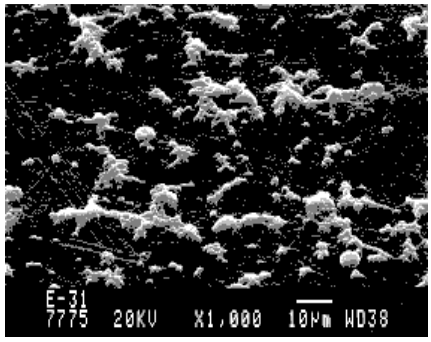
Figure 22 is the scanning electron microscope (SEM) pictures of non-grafted control and grafted PP samples after platelet adhesion test. It shows that the AAm, DMAAm and DMAPMAAm grafted samples have lower platelet adsorption compared with non-grafted control sample. Among them, the samples grafted with AAm (b), or DMAAm (d) in aqueous solutions show the lowest platelet adsorption and the shape of the hangover platelet was well kept. In the case of AAm grafted PP films, the platelet adsorption on the surface of the sample grafted in aqueous solution (b) was much lower than that in acetone solution (c).



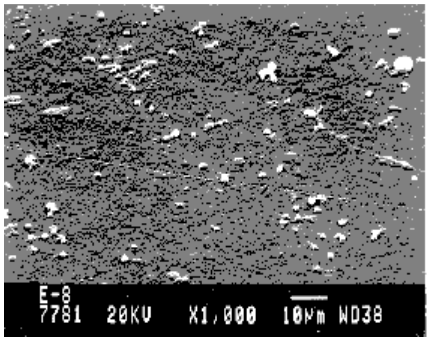
a. non grafted control



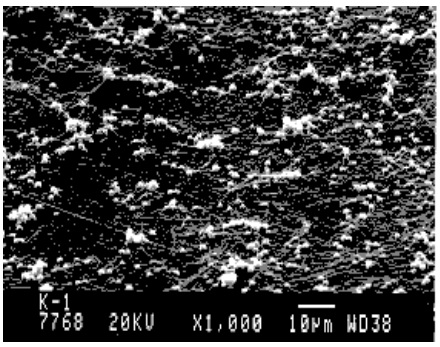
b. AAm grafted in aqueous solution (grafting yield: ~0.21%)



c. AAm grafted in acetone
(grafting yield: ~4.68%)



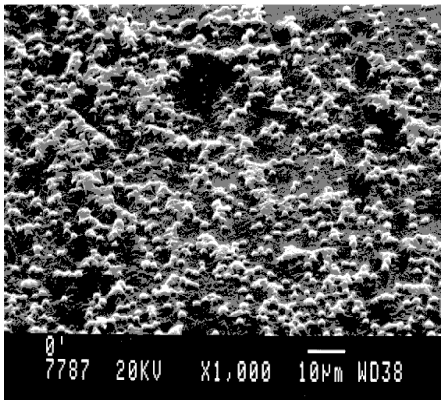
d. DMAAm grafted in aqueous solution
(grafting yield: ~0.77%)



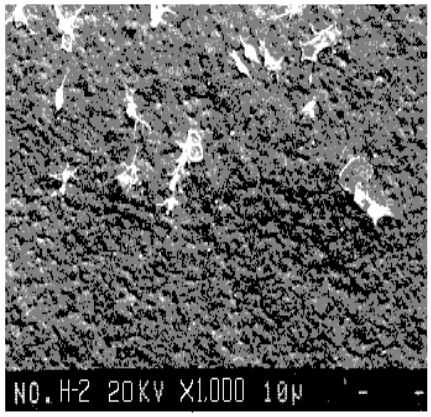
e. DMAPMAAm grafted in aqueous solution
(grafting yield: ~0.44%)

ii) The platelet adsorption of PEGMA grafted PP films

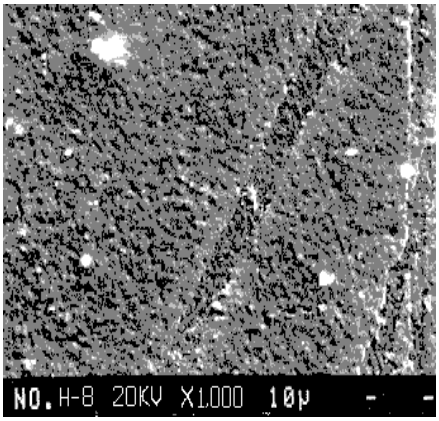
Figure 23 shows the SEM pictures of platelets which adhered to the non-grafted control and the grafted samples. The samples were grafted in the co-solvent system of THF/H_2O. It shows that the PEGMA-grafted samples have lower platelet adsorption. There is almost no platelet adsorption on the PEGMA (PEO200 and PEO350)-grafted sample. Compared with the control sample, there is less amount of platelet on the PEO-90 grafted sample.



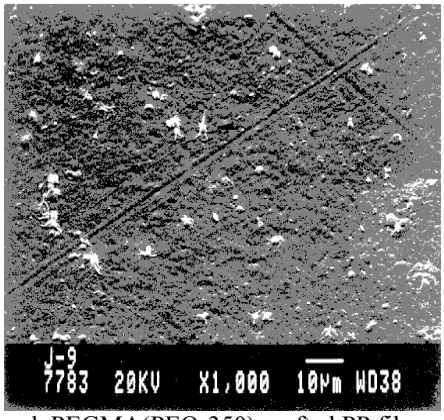
a. control



b. PEGMA(PEO-90) grafted PP film (grafting yield: ~3%)



c. PEGMA(PEO-200) grafted PP film (grafting yield: ~3%)

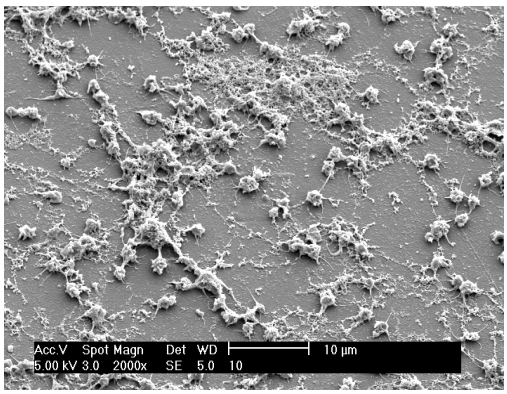


d. PEGMA(PEO-350) grafted PP film (grafting yield: ~0.4%)

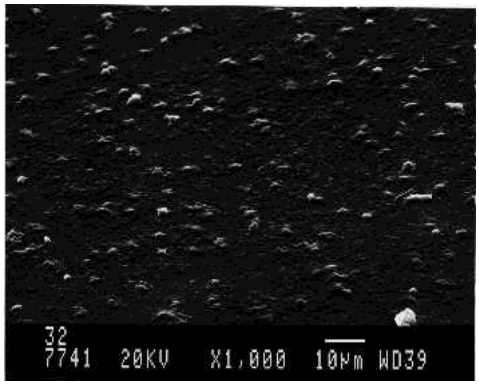
Fig. 23. The SEM picture of platelet adhesion on the PP films in the comparison of grafted and non-grafted samples. (grafting reaction was performed in THF/H_2O solution)

iii) Platelet adsorption of MPC MPC/PEGMA grafted cellulose acetate films

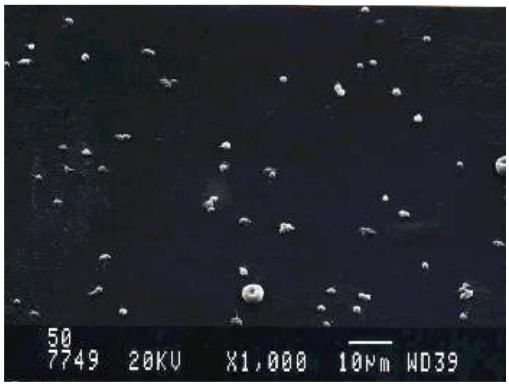
Figure 24 is the scanning electron microscope (SEM) picture of MPC and MPC/PEGMA grafted samples after interacted with PRP. It shows that the adhesion of platelet on the MPC and MPC/PEGMA grafted sample was much decreased compared to the control sample. There is almost no platelet adhesion on the MPC grafted cellulose acetate films with the yield of 13.45% carried out in aqueous solution (b) and 10.49% carried out in MeOH/H_2O (c). There is almost no platelet on the sample of MPC/PEGMA grafted cellulose acetate film with the yield of 4.58% (d).



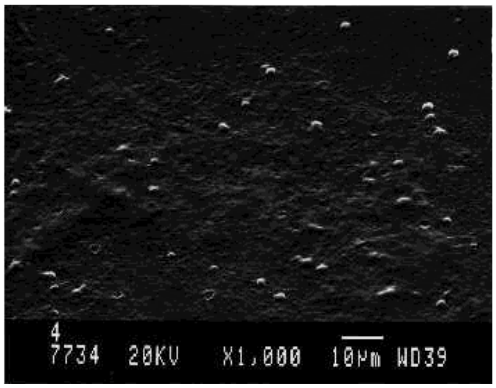
Control



b. MPC grafted sample
(grafting yield: ~13.45% in H_2O)



c. MPC grafted sample
(grafting yield: ~10.49% in MeOH/H_2O)



d. MPC grafted sample
(grafting yield: ~4.58% in H_2O)

Fig. 24. The SEM picture of platelet adhesion on the cellulose acetate films of MPC and MPC/PEGMA grafted sample.

3.2 The thrombus formed on the surface of the samples

i) The thrombus formed on the sample surface of non-grafted control and acrylamides grafted PP films

Figure 25 is the thrombus formed on the sample surface of non-grafted control and acrylamides grafted PP films in the comparison of different solutions. It shows that the thrombus formed on the acrylamides-grafted sample is lesser than that of control sample. On the other hand, the thrombus formed on the samples grafted in aqueous solution was relatively lesser than that of in acetone solution.

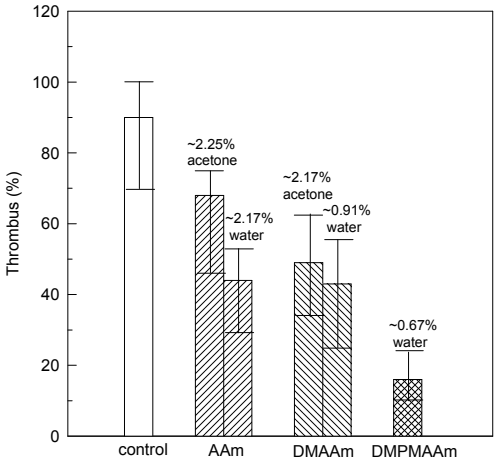


Fig. 25. The comparison of thrombus percentage on acrylamides grafted PP films at different solutions

ii) Thrombus formed on the sample surface of non-grafted control and PEGMA grafted PP films

Figure 26 shows the thrombus percentage formed on the surface of various PP samples. The thrombus is clearly decreased with the grafting of PEGMA on the PP film. There is only around 40% thrombus on the PP surface with the grafting yield of 3% (PEO200), while about 80%

thrombus was formed on the control sample. Among the grafted samples, the PEO-350 grafted sample shows the lowest thrombus percentage.

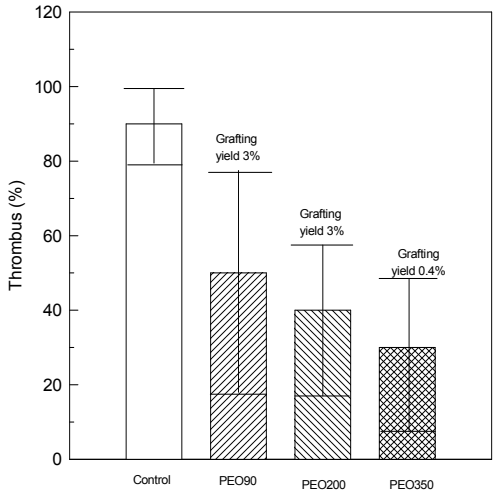


Fig. 26. Comparison of thrombus percentage on PEGMA grafted PP films with different PEO repeat units and the non-grafted control

iii) Thrombus formed on the sample surface of non-grafted control and MPC, MPC/PEGMA grafted PP films

Figure 27 shows the thrombus percentage formed on the grafted cellulose acetate film compared to the control sample. Relatively, the thrombus percentage of MPC, MPC/PEGMA grafted cellulose acetate films were much lower due to the phospholipid polar group on the grafted sample surface.

3.3 Plasma protein adsorption

It is known that proteins are complex macromolecules with molecular weight ranging from thousands to millions and that they adsorb on practically all interfaces during the first few minutes of blood or biological fluid exposure[3]. Generally, the adsorption process results in platelet adsorption and follows the thrombus formation. The surface that repels all proteins is the desirable blood compatibility. In this experiment, the human plasma protein adsorption on the non-grafted control and PEGMA grafted PP film samples were detected by ESCA (Figure 28). The nitrogen peak (N-1s: 399.3eV) on the control PP film surface was obviously higher than that

of the PEGMA-grafted polypropylene surface, indicating a large amount of protein adsorption on the control surface. It may be explained that this is due to the hydrophobic interaction of protein molecules with the hydrophobic polypropylene film surface. The sample with higher grafting yield had the lower plasma protein adsorption. As shown in Figure 28, when the grafting yield was about 1.15%, no N-1s peak was found. That means there is almost no plasma protein adsorption on the surface of this grafted substrate.

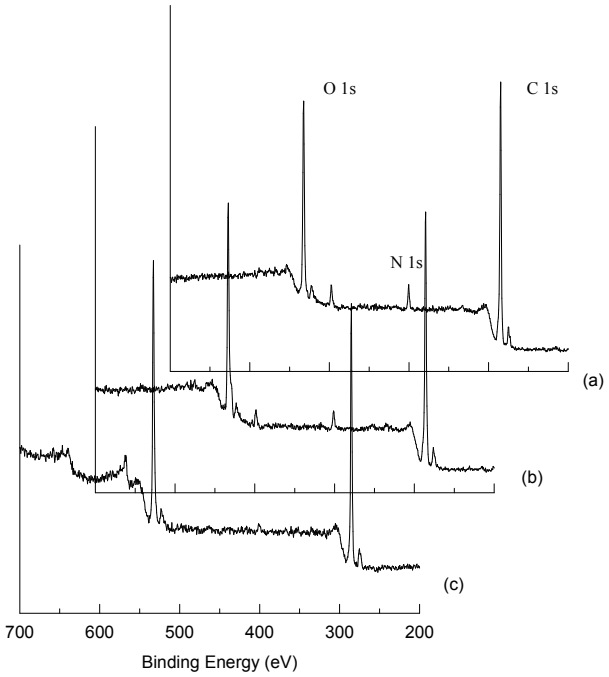


Fig. 28. ESCA survey scan spectra of (a) control, (b) 0.69% and (c) 1.15% of PEGMA (PEO200) grafted PP surface after plasma protein adsorption (Grafting reaction was performed in 10% PEGMA in MeOH/H_2O solution at 70°C for 5h; Dose: 20kGy).

CONCLUSIONS

1. Pre-irradiation grafting technique was used for the modification of polypropylene and cellulose acetate films. Three kinds of acrylamides monomers and PEGMA with three different polyethelene oxide repeat units were grafted onto preirradiated PP films in different

co-solvent system, respectively; MPC and MPC/PEGMA were grafted onto cellulose acetate films in both aqueous solution and MeOH/H_2O co-solvent system, respectively.

2. The degree of grafting was affected by irradiation dose, monomer concentration, solvents, reaction time etc.
3. Hydrophobic or hydrophilic property of the grafted sample surface is greatly influenced by the property of solvent in grafting reaction. For example, the water contact angle of the PP film grafted with AAm, DMAAm and DMAPMAAm in aqueous solution is much smaller than that of the samples grafted in acetone.
4. The blood compatibility of the PP films and cellulose acetate films were improved, related to the platelet adhesion, plasma protein adsorption and thrombus, by the grafting of acrylamides, PEGMA and MPC respectively.
5. Pre-irradiation grafting is a good method and easier to be performed for the modification of polymeric surface. It can be applied in the preparation and modification of biomaterials and tissue engineering scaffolds etc.

REFERENCES

1. Hoffman AS. Blood-biomaterial interactions: an overview. In: Cooper SL, eds. Biomaterials: Interfacial Phenomena and Applications. Washington, DC: American Chemical Society 1982; 199: 3-8.
2. Andrade JD., Nagaoka S, Cooper SL., Okano T, Kim SW. Surface and blood compatibility. Current hypothesis. Trans Am Soc Artif Intern Organs 1987; 33: 75-84.
3. Ratner BD, Hoffman AS, Schoen, FJ and Lemons JE. Blood coagulation and blood-materials interaction. Biomaterials Science (An introduction to materials in medicine, second edition), Academic Press 2004; pp332-338.
4. Lee JH., Kopecek J and Andrade JD. Protein-resistant surfaces prepared by PEO-containing block copolymer surfactants. J. Biomed. Mater. Res.1989; 23: 351-368.
5. Lee JH, Kapeckova P, Kopecek J and Andrade JD. Surface properties of copolymers of alkyl methacrylates with methoxy(polyethylene oxide) methacrylates and their application as protein-resistant coatings. Biomaterials 1990; 11: 455-464.
6. Baier RE, Gott V and Furuse A. Surface chemical evaluation at thrombus resistant materials before and after venous implantation, trans. Am. Soc. Artf. Intern. Organs 1970; 16: 50-59.

7. Ikada Y, Suzuki M, Taniguchi M and Iwata H. Interaction of blood with radiation-grafted materials, Radiat. Phys. Chem.1981; 18(5-6): 1207-1216.
8. Amiji M and Park K. Provention of protein adsorption and platelet adsorption on surfaces by PEO/PPO/PEO triblock copolymers. Biomaterials 1992; 13(10): 682-692.
9. Sun YH, Gombotz WR, and Hoffman AS. Synthesis and Characterization of Non-Fouling Polymer Surfaces: I. Radiation grafting of hydroxyethyl methacrylate onto silastic film. J. Bioact. Compat. Polym. 1986; 1: 316-334.
10. Dunkirk SG., Gregg SL, Dinan LW, Monfils JD, Haapala JE, Marcy JA, Chapper DL, Ammos RA, and Gere PE. Photochemical coating for the protection of bacterial colonization, J. Biomater. Appl. 1991; 6: 131-156.
11. Casimiro MH, Botelho ML, Leal JP and Gil MH. Study on chemical, UV and gamma radiation-induced grafting of 2-hydroxyethyl methacrylate onto chitosan. *Radiation Physics and Chemistry* 2005; *72(6): 731-735.*
12. Ebara M, Hoffman JM, Stayton PS and Hoffman AS. Surface modification of microfluidic channels by UV-mediated graft polymerization of non-fouling and 'smart' polymers. Radiation Physics and Chemistry 2007; 76(8-9): 1409-1413.
13. Khan MA, Bhattacharia SK, Kader MA and Bahari K. Preparation and characterization of ultra violet (UV) radiation cured bio-degradable films of sago starch/PVA blend, Carbohydrate Polymers 2006; 63: 500–506.
14. Shanmugharaj AM, Kim JK and Ryu SH. Modification of rubber surface by UV surface grafting. *Applied Surface Science* 2006; *252(16*): *5714-5722.*
15. Raffaele-Addamo A, Selli E, Barni R, Riccardi C, Orsini F, Poletti G, Meda L, Massafra MR and Marcandalli B. Cold plasma-induced modification of the dyeing properties of poly(ethylene terephthalate) fibers. Applied Surface Science 2006; 252(6): 2265-2275.
16. Jeong BJ, Lee JH and Lee HB. Preparation and characterization of comb-like PEO gradient surfaces. Journal of Colloid and Interfaces Science 1996; 178: 757-763.
17. Hoffman AS. Biomedical application of plasma gas discharge processes. J. Appl. Polym. Sci. Appl. Polym. Symp. 1988; 42: 251-267.
18. Haddadai-asl V, Burford RP, and Garnett JL. Radiation graft modification of ethylene-propylene rubber-II. effect of additives. Radiat. Phys. Chem. 1995; 45(2): 191-198.
19. Wilson JE. Radiation grafting of chloromethylstyrene on polyethylene, followed by quanternization and heparinization. J. Macromol. Sci-Chem. 1977; A11(11): 2113-2122.
20. Hegazy E.A, AbdEl-Rehim HA, Kamal H and Kandeel KA. Advances in radiation grafting. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with*

Materials and Atoms 2001; *185(1-4*): *235-240.*

21. El-Sawy NM and Sagheer FA. Radiation-induced graft polymerization of acrylic acid onto poly(tetrafluoroethylene-perfluorovinyl ether) copolymer films: complexation with some transition metals and biological activity. *European Polymer Journal* 2001; *37(1): 161-166.*

22. Nho YC and Kwon OH. Blood compatibility of AAc, HEMA, and PEGMA-grafted cellulose film. *Radiation Physics and Chemistry* 2003; *66(4*): *299-307.*

23. Nurkeeva ZS, Aala A-SA. Kupchishin AI, V.Khutoryanskiy V, Mun GA and Beksyrgaeva AG. Introduction of O-butyrylchitosan with a photosensitive hetero-bifunctional crosslinking reagent to silicone rubber film by radiation grafting and its blood compatibility. *Colloids and Surfaces B: Biointerfaces* 2003; *30(4): 299-306.*

24. Mao C, Yuan J, Mei H, Zhu A, Shen J and Lin S. Introduction of photocrosslinkable chitosan to polyethylene film by radiation grafting and its blood compatibility, *Materials Science and Engineering: C*, 2004; *24(4): 479-485.*

25. Nho YC, Lim YM and Lee YM. Preparation, properties and biological application of pH-sensitive poly(ethylene oxide) (PEO) hydrogels grafted with acrylic acid (AAc) using gamma-ray irradiation. *Radiation Physics and Chemistry* 2004; *71(1-2)*: *239-242.*

26. Mao C, Qiu YZ, Sang HB, Mei H, Zhu AP, Shen J and Lin SC. Various approaches to modify biomaterial surfaces for improving hemocompatibility, *Advances in Colloid and Interface Science* 2004; *110(1-2*): *5-17.*

27. Terada A, Yuasa A, Tsuneda S, Hirata A, Katakai A and Tamada M. Elucidation of dominant effect on initial bacterial adhesion onto polymer surfaces prepared by radiation-induced graft polymerization. *Colloids and Surfaces B: Biointerfaces* 2005; *43(2*): *99-107.*

28. Johnell M, Larsson R and Siegbahn A. The influence of different heparin surface concentrations and antithrombin-binding capacity on inflammation and coagulation. *Biomaterials 2005; 26(14*): *1731-1739.*

29. Nho YC, Park SE, Kim HI and Hwang TS. Oral delivery of insulin using pH-sensitive hydrogels based on polyvinyl alcohol grafted with acrylic acid/methacrylic acid by radiation. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 2005; 236(1-4): 283-288.*

30. Adem E, Avalos-Borja M, Bucio E, Burillo G, Castillon FF and Cota L. Surface characterization of binary grafting of AAc/NIPAAm onto poly(tetrafluoroethylene) (PTFE). *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 2005; *234(4): 471-476.*

31. Clochard M-C, Betz N, Goncalves M, Bittencourt C, Pireaux J-J, Gionnet K, Déléris G and

Le Moël A. Peptide immobilization onto radiation grafted PVDF-g-poly(acrylic acid) films. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 2005; 236(1-4): 208-215.*

32. Wada Y, Seko N, Nagasawa N, Tamada M, Kasuya KI and Mitomo H. Biodegradability of poly(3-hydroxybutyrate) film grafted with vinyl acetate: Effect of grafting and saponification. *Radiation Physics and Chemistry* 2007; *76(6): 1075-1083.*
33. Çaykara T, Alaslan ŞŞ, Gürü M, Bodugöz H and Güven O. Preparation and characterization of poly(isobutyl methacrylate) microbeads with grafted amidoxime groups. *Radiation Physics and Chemistry* 2007; *76(10): 1569-1576.*
34. Chapiro A. Preparation of graft copolymers with the aid of ionizing radiations. Radiation Chemistry of Polymeric Systems. Interscience Publishers, New York 1962; pp 596-686.
35. Kaetsu I. Radiation techniques in the formulation of synthetic biomaterials. In: Singh A., Silverman J. (Eds.), Radiation Processing of Polymers. Hanser, New York 1992; pp. 150-185.
36. Lugao A. B., Machado L. D. B., Miranda L. F., Alvarez M. R. and Rosiak J.M., Study of wound dressing and hydration/dehydration properties, Radiat. Phys. Chem. 1998; 52(1-6): 319-322.
37. Ulanski P, Janik I and Rosiak J.M, Radiation formation of polymeric nanogels, Radiat. Phys. Chem. 1998; 52(1-6): 289-294.
38. Fang Y. E. And Shi T. Y., Polypropylene dialysis membrane prepared by cobalt-60 gamma-radiation-induced grafting copolymerization. J. Membrane Sci. 1988; 39(1): 1-9.
39. Chen J, Yang LM, Wu MH, Xi Q, He SM and Li YW. Preparation of interpenetration polymer networks by two times grafting of monomers on preirradiated polypropylene film. Radiation Physics and Chemistry 2000; 59: 313~316.
40. Chen J, Yang LM, Chen LQ, Wu MH, Nho YC and Kaetsu I. An interesting grafting reactivity of EB preirradiated polypropylene film. Radiation Physics and Chemistry 2004; 69 (2): 149-154.
41. Hui BJ, Chen J, Yang LM, Li J, Pei Y and Shi LL. Preparation of pH sensitive hydrogel by two times grafting of acrylamide and acrylic acid onto preirradiated polyethylene film. Journal of Radio Analytical and Nuclear Chemistry 2004; 260(3): 673-677.
42. Hicks GP and Updike SJ. The preparation and characterization of lypholized polyacrylamide enzyme gels for chemical analysis, Anal. Chem. 1966; 38, 726-731.
43. Merrill EW. Pakala PW and Mahmud NA. Hydrogels for blood contact. in Hydrogels in Medicine and Pharmacy. N. A. Peppas ed. CRC Press Boca Raton, FL, 1987;, Vol. 3, pp 1-16.

44. Kawashma K and Umeda K. Immobilization of enzymes by radiopolymerization of acrylamide, Biotechnol. Bioeng. 1974; 16: 609-616.
45. Rosiak J, Burezak K and Pakala W. Polyacrylamide hydrogels as sustained release drug delivery dressing materials, Radiat. Phys. Chem.1983; 22(3-5): 907-915.
46. Saraydın D, Ünver-Saraydın S, Karada E, Koptagel E and Güven O. In vivo biocompatibility of radiation crosslinked acrylamide copolymers. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 2004; 217(2): 281-292.
47. Mirzadeh H, Katbab AA, Khorasani MT, Burford RP, Gorgin E and Golestani A. Cell attachment to laser-induced AAm-and HEMA-grafted ethylenepropylene rubber as biomaterial: in vivo study. Biomaterial 1995; 16(8): 641-648.
48. Yao KJ, Shen SC, Yun JX, Wang LH, Chen F and Yu XM. Protein adsorption in supermacroporous cryogels with embedded nanoparticles. Biochemical Engineering Journal 2007; 36(2): 139-146.
49. Ilkhanizadeh S, Teixeira AI and Hermanson O. Inkjet printing of macromolecules on hydrogels to steer neural stem cell differentiation. Biomaterials 2007; 28(27): 3936-3943.
50. Salmaso S, Bersani S, Pennadam SS, Alexander C and Caliceti P. Avidin bioconjugate with a thermoresponsive polymer for biological and pharmaceutical applications. International Journal of Pharmaceutics 2007; 340(1-2): 20-28.
51. Ebara M, Hoffman JM, Stayton PS and Hoffman AS. Surface modification of microfluidic channels by UV-mediated graft polymerization of non-fouling and 'smart' polymers. Radiation Physics and Chemistry 2007; 76(8-9): 1409-1413.
52. Biswal J, Kumar V, Bhardwaj YK, Goel NK, Dubey KA, Chaudhari CV and Sabharwal S. Radiation-induced grafting of acrylamide onto guar gum in aqueous medium: Synthesis and characterization of grafted polymer guar-g-acrylamide. Radiation Physics and Chemistry 2007; 76(10): 1624-1630.
53. Benke N, Takács E, Wojnárovits L and Borsa J. Pre-irradiation grafting of cellulose and slightly carboxymethylated cellulose (CMC) fibres. Radiation Physics and Chemistry 2007; 76(8-9): 1355-1359.
54. Zhao GW, Chen YS and Wang XL. Surface modification of polyethylene film by acrylamide graft and alcoholysis for improvement of antithrombogenicity. Applied Surface Science 2007; 253(10): 4709-4714.
55. Rokhade AP, Patil SA and Aminabhavi TM. Synthesis and characterization of semi-interpenetrating polymer network microspheres of acrylamide grafted dextran and chitosan for controlled release of acyclovir. Carbohydrate Polymers 2007; 67(4): 605-613.

56. Sarbu A, Pinho MN, Freixo MR, Goncalves F and Udrea I. New method for the covalent immobilization of a xylanase by radical grafting of acrylamide on cellulose acetate membranes. Enzyme and Microbial Technology 2006; 39(1): 125-130.
57. Tu CY, Liu YL, Lee KR and Lai JY. Surface grafting polymerization and modification on poly(tetrafluoroethylene) films by means of ozone treatment. Polymer 2005; 46(18): 6976-6985.
58. Zhao JC, Xie ZH, Guo Z, Liang GZ and Wang JL. Enzyme-mediated radical initiation of AM graft onto the HDPE film. Applied Surface Science 2004; 229(1-4): 124-131.
59. Imai Y and Nose Y. A new method for evaluation of Antithrombogenicity of materials, J. Biomed. Mater. Res. 1972; 6: 165-172.
60. Harris JM. Introduction to biotechnical and biomedical applications of poly(ethylene glycol), in Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical application. Plenum, New York 1992; pp. 127-136.
61. Grainger D, Feijien J and Kim SW. Poly(dimethylsiloxane)-poly(ethylene oxide)-heparin block copolymers, I: Synthesis and characterization. J. Biomed. Mater. Res. 1998; 22: 231-242.
62. Podual K, Doyle FJ and Peppas NA. Preparation and dynamic response of cationic copolymer hydrogels containing glucose oxidase. Polymer 2000; 41(11): 3975-3983.
63. Schwarte LM and Peppas NA. Novel poly(ethylene glycol)-grafted, cationic hydrogels: preparation, characterization and diffusive properties. Polymer 1998; 39(24): 6057-6066. (copolymer)
64. Abraham S, Brahim S, Ishihara K and Guiseppi-Elie A. Molecularly engineered p(HEMA)-based hydrogels for implant biochip biocompatibility. Biomaterials 2005; 26,(23): 4767-4778.
65. Kim HW, Chung CW, Hwang SJ and Rhee YH. Drug release from and hydrolytic degradation of a poly(ethylene glycol) grafted poly(3-hydroxyoctanoate). International Journal of Biological Macromolecules 2005; 36(1-2): 84-89.
66. Deshpande MC, Garnett MC, Vamvakaki M, Lindsey Bailey, Armes SP and Stolnik S. Influence of polymer architecture on the structure of complexes formed by PEG–tertiary amine methacrylate copolymers and phosphorothioate oligonucleotide. Journal of Controlled Release 2002; 81(1-2): 185-199.
67. Gregonis D, Van Wagonen R and Andrade JD. Poly(ethylene glycol) surfaces to minimize protein adsorption, Trans. Sec. World Congr. Biomater. 1984; 7: 266-274.
68. Lee JH, Jeong BJ and Lee HB. Plasma protein adsorption and platelet adhesion onto comb-

like PEO gradient surfaces. J. Biomed. Mat. Res. 1997; 34: 105-114.

69. Kim SW. Nonthrombogenic Treatments and Strategies, in Biomaterials Science: An introduction to Materials in Medicine, Ratner BD, Hoffman AS, Schoen FJ and Lemons JE ed. Academic Press, New York 1996; pp. 305-307.
70. Jeong BJ, Lee JH and Lee HB. Preparation and characterization of comb-like PEO gradient surfaces. Journal of Colloid and Interfaces Science 1996; 178: 757-763.
71. Susanto H, Balakrishnan M and Ulbrich M.Via surface functionalization by photograft copolymerization to low-fouling polyethersulfone-based ultrafiltration membranes. Journal of Membrane Science 2007; 288(1-2): 157-167.
72. Chen YJ, Kang E.T, Neoh KG, Wang P and Tan KL. Surface modification of polyaniline film by grafting of poly(ethylene glycol) for reduction in protein adsorption and platelet adhesion. Synthetic Metals 2000; 110(1): 47-55.
73. Beyer M, Felgenhauer T, Bischoff FR, Breitling F and Stadler V. A novel glass slide-based peptide array support with high functionality resisting non-specific protein adsorption. Biomaterials 2006; 27(18): 3505-3514.
74. Kim HW, Chung CW and Rhee YH. UV-induced graft copolymerization of monoacrylate-poly(ethylene glycol) onto poly(3-hydroxyoctanoate) to reduce protein adsorption and platelet adhesion. International Journal of Biological Macromolecules 2005; 35(1-2): 47-53.
75. Li YL, Neoh KG and Kang ET. Poly(vinyl alcohol) hydrogel fixation on poly(ethylene terephthalate) surface for biomedical application. Polymer 2004; 45(26): 8779-8789.
76. Li YL, Neoh KG and Kang ET. Plasma protein adsorption and thrombus formation on surface functionalized polypyrrole with and without electrical stimulation. Journal of Colloid and Interface Science 2004; 275(2): 488-495.
77. Nho YC and Kwon OH. Blood compatibility of AAc, HEMA, and PEGMA-grafted cellulose film. Radiation Physics and Chemistry 2003; 66(4): 299-307.
78. Zou XP, Kang ET and Neoh KG. Plasma-induced graft polymerization of poly(ethylene glycol) methyl ether methacrylate on poly(tetrafluoroethylene) films for reduction in protein adsorption. Surface and Coatings Technology 2002; 149(2-3): 119-128.
79. Zhang F, Kang ET, Neoh KG, Wang P and Tan KL. Surface modification of stainless steel by grafting of poly(ethylene glycol) for reduction in protein adsorption. Biomaterials 2001; 22(12): 1541-1548.
80. Ishihara K, Ueda T, Nomura H, Kurita K and Nakabayashi N. An extraordinary water structure in biocompatible phospholipid polymers. Fifth World Biomaterials Congress 1996; May 29-June 2, Toronto Canada, pp. 219.

81. Lewis AL, Hughes PD, Kirkwood LC, Leppard SW, Redman RP, Tolhurst LA and Stratford PW. Synthesis and characterisation of phosphorylcholine-based polymers useful for coating blood filtration devices. Biomaterials 2000; 21(18): 1847-1859.
82. Ishihara k, Fukomoto K, Aoki J and Nakabahashi N. Improvmant of blood compatibility on cellulose dialysis membrane. 1. Grafting of 2-methacyloyloxyethyl phosphorylcholine onto a cellulose membrane surface. Biomaterials 1992; 13: 145-149.
83. Fukomoto K, Ishihara k, Takayama R, Aoki J. and Nakabahashi N. Improvmant of blood compatibility on cellulose dialysis membrane. 2. Blood compatibility of phospholipid polymer grafted cellulose membrane surface, Biomaterials 1992; 13: 235-239.
84. Ishihara K, Fukomoto K, Miyazaki T. and Nakabahashi N. Improvmant of hemocompatibility on a cellulose dialysis membrane with a novel biomedical polymer having a phospholipid polar group. Artif. Organs 1994; 18: 559-564.
85. Lee MK, Park HS, Kim EY and Park SM. Development and application of biocompatible polymers(II) ---Biocompatibility of chitosan graft copolymer with phosphoryl choline groups, J. of Kor. Soc. of Dyers and Finishers 1995; 7(2): 63-69.
86. Ishihara K, Tsuji T, Sakai Y and Nakabayashi N.(1994), Synthesie of graft copolymer having phospholipid polar group by macromonomer method and their properties in water, J. Polym. Sci., Part A: Polym. Chem. 1994; 32: 859-867.
87. Ye SH, Watanabe J, Iwasaki Y and Ishihara K. In situ modification on cellulose acetate hollow fiber membrane modified with phospholipid polymer for biomedical application. Journal of Membrane Science 2005; 249(1-2): 133-141.
88. Morimoto N, Watanabe A, Iwasaki Y, Akiyoshi K and Ishihara K. Nano-scale surface modification of a segmented polyurethane with a phospholipid polymer. Biomaterials 2004; 25(23): 5353-5361.
89. Inoue Y, Watanabe J and Ishihara K. Dynamic motion of phosphorylcholine groups at the surface of poly(2-methacryloyloxyethyl phosphorylcholine–random–2,2,2-trifluoroethyl methacrylate). Journal of Colloid and Interface Science 2004; 274(2): 465-471.
90. Iwasaki Y, Takamiya M, Iwata R, Yusa SI and Akiyoshi K. Surface modification with well-defined biocompatible triblock copolymers: Improvement of biointerfacial phenomena on a poly(dimethylsiloxane) surface. Colloids and Surfaces B: Biointerfaces 2007; 57(2): 226-236.
91. Yokoyama R, Suzuki S, Shirai K, Yamauchi T, Tsubokawa N and Tsuchimochi M. Preparation and properties of biocompatible polymer-grafted silica nanoparticle. European Polymer Journal 2006; 42(12): 3221-3229.

92. Okajima Y, Saika S and Sawa M. Effect of surface coating an acrylic intraocular lens with poly(2-methacryloyloxyethyl phosphorylcholine) polymer on lens epithelial cell line behavior. Journal of Cataract & Refractive Surgery, Volume 32, Issue 4, April 2006, Pages 666-671.

93. Yasuhiko Iwasaki, Shin-ichi Sawada, Nobuo Nakabayashi, Gilson Khang, Hai Bang Lee and Kazuhiko Ishihara. The effect of the chemical structure of the phospholipid polymer on fibronectin adsorption and fibroblast adhesion on the gradient phospholipid surface. Biomaterials 1999; 20(22): 2185-2191.

94. Furuzono T, Ishihara K, Nakabayashi N and Tamada Y.. Chemical modification of silk fibroin with 2-methacryloyloxyethyl phosphorylcholine. II. Graft-polymerization onto fabric through 2-methacryloyloxyethyl isocyanate and interaction between fabric and platelets. Biomaterials 2000; 21(4): 327-333.

95. Ishihara K, Fujiike A, Iwasaki Y, Kurita K and Nakabayashi N.(1996), Synthesis of polymers having a phospholipid polar group connected to a poly(oxyethylene) chain and their protein adsorption-resistance propties, J. Polym. Sci., Part A: Polym. Chem. 1996; 34: 199-205.

96. Licciardi M, Giammona G, Du JZ, Armes SP, Tang YQ and Lewis AL. New folate-functionalized biocompatible block copolymer micelles as potential anti-cancer drug delivery systems. Polymer 2006; 47(9): 2946-2955.

97. Abraham S, Brahim S, Ishihara K and Guiseppi-Elie A. Molecularly engineered p(HEMA)-based hydrogels for implant biochip biocompatibility. Biomaterials 2005; 26(23): 4767-4778.

98. Lam JKW, Ma Y, Armes SP, Lewis AL, Baldwin T and Stolnik S. Phosphorylcholine–polycation diblock copolymers as synthetic vectors for gene delivery. Journal of Controlled Release 2004; 100(2): 293-312.

99. Ogawa R, Watanabe J and Ishihara K. Domain-controlled polymer alloy composed of segmented polyurethane and phospholipid polymer for biomedical applications. Science and Technology of Advanced Materials 2003; 4(6): 523-530.

100. Long SF, Clarke S, Davies MC, Lewis AL, Hanlon GW and Lloyd AW. Controlled biological response on blends of a phosphorylcholine-based copolymer with poly(butyl methacrylate). Biomaterials 2003; 24(23): 4115-4121.

101. Uchiyama T, Watanabe J and Ishihara K. Biocompatible polymer alloy membrane for implantable artificial pancreas. Journal of Membrane Science 2002; 208(1-2): 39-48.

102. Lewis AL, Cumming ZL, Goreish HH, Kirkwood LC, Tolhurst LA and Stratford PW. Crosslinkable coatings from phosphorylcholine-based polymers. Biomaterials 2001; 22(2): 99-111.

103. Ishihara K. Bioinspired phospholipid polymer biomaterials for making high performance artificial organs. Science and Technology of Advanced Materials 2000; 1(3): 131-138.

104. Ishihara K, Iwasaki Y and Nakabayashi N. Novel biomedical polymers for regulating serious biological reactions. Materials Science and Engineering: C 1998; 6(4): 253-259.

105. Salvage JP, Rose SF, Phillips GJ, Hanlon GW, Lloyd AW, Ma IY, Armes SP, Billingham NC and Lewis AL. Novel biocompatible phosphorylcholine-based self-assembled nanoparticles for drug delivery. Journal of Controlled Release 2005; 104(2): 259-270.