# CHAPTER IV

RESULTS AND DISCUSSION

4.1 Grafting Yield

In this particular study, photo-induced graft polymerization in the presence of benzophenone, a well-known photosensitizer was selected as an approach for grafting hydrophilic monomers onto NR latex films. The films were soaked in the solution containing benzophenone to allow the photosensitizer to permeate into the substrate before starting graft copolymerization process. After being irradiated, the molecule of benzophenone is excited to the triplet state that can abstract hydrogen atom from polyisoprene of NR and subsequently yields polyisoprene radicals capable of initiating graft copolymerization of hydrophilic monomers.
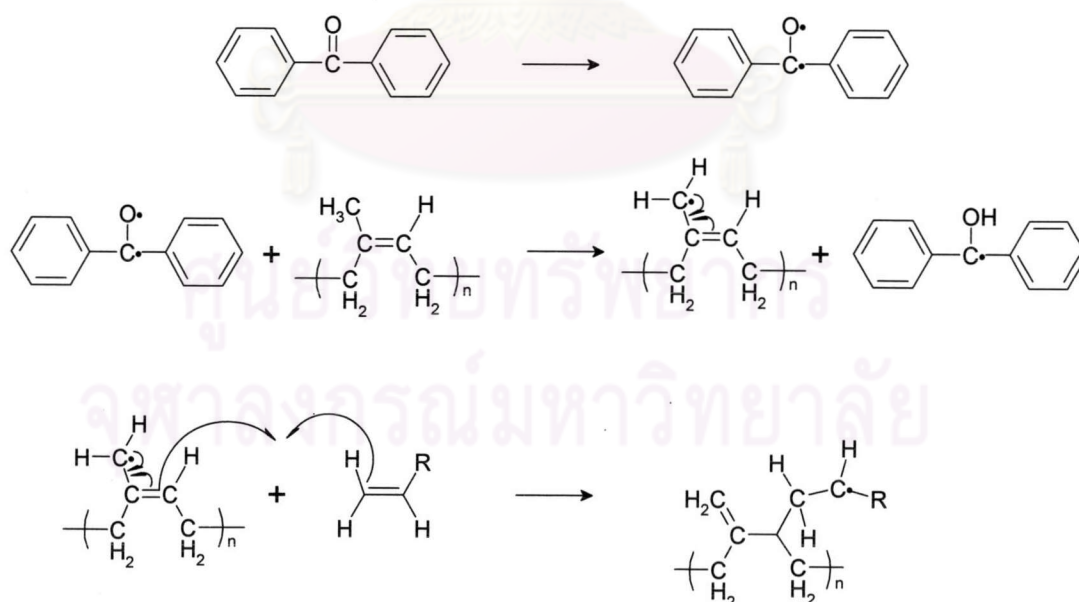
Scheme 4.1 Mechanism of UV-induced graft copolymerization of hydrophilic monomer in the presence of benzophenone.

4.1.1 Effect of Time

The first grafting parameter to be determined was grafting time. The average grafting yield as a function of time using 0.5 M of PEGMA and VPy is shown in Figure 4.1. The magnitude of the grafting yield increased as a function of time for the vulcanized NR latex film was less than that of the unvulcanized NR films. This implies that the crosslinked network of vulcanized NR prohibits the permeation of benzophenone as well as the monomer and thus suppresses the extent of grafting. The grafting yield of the vulcanized rubber leveled off at the value ~1.0 % in the case of PEGMA and at the value ~ 0.2 % in the case of VPy after 150 min. In the case of MPC, the grafting yield of ~ 1.3 % was obtained using the same period of time for grafting.
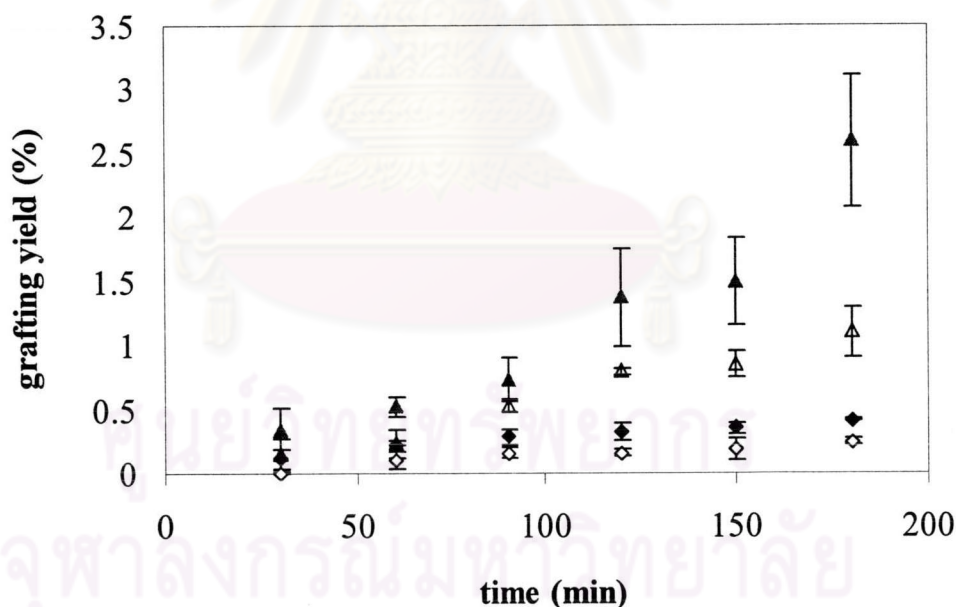

Figure 4.1   The grafting yield as a function of time: vulcanized-g-PEGMA (▲), unvulcanized-g-PEGMA (Δ), vulcanized-g-VPy (◆) and unvulcanized-g-VPy (◇).

It can be observed that the maximum grafting yields of both unvulcanized and vulcanized NR using VPy as a monomer were quite low (< 0.5%) in comparison with PEGMA. We explained these results as a consequence of its reactivity being lower than PEGMA and MPC. Since lone-pair electrons on nitrogen atom of

pyrolidone help stability the radical. This may cause higher stability than the other two monomers leading to the less extent of surface grafting.

4.1.2 Effect of Monomer Concentration

The effect of monomer concentration on the grafting yield was determined by varying the concentration from 0.1 M to 0.5 M using the grafting time of 150 min. The grafting yield plotted as a function of monomer concentration is shown in Figure 4.2. As the higher monomer concentration used, the greater extent of grafting was obtained.
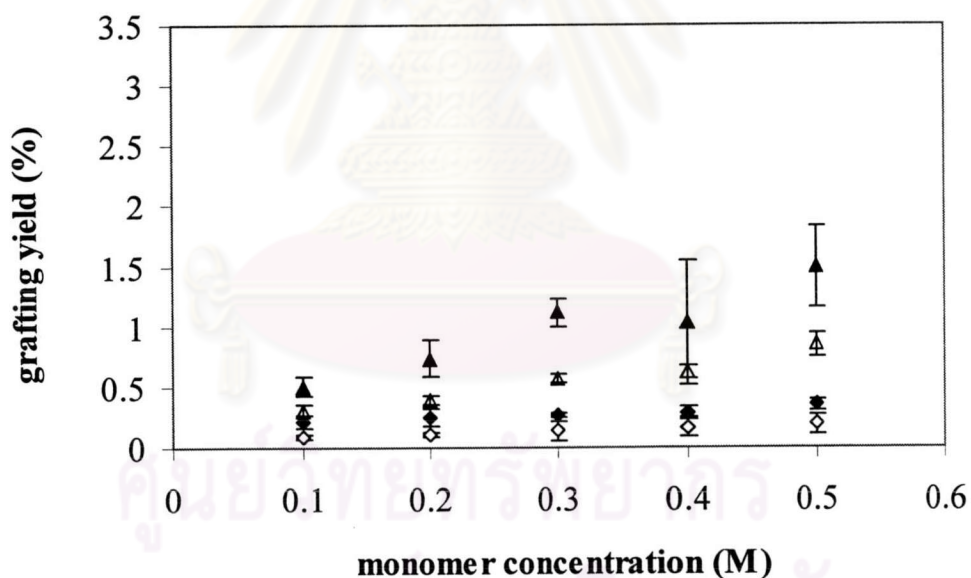

Figure 4.2 The grafting yield as a function of monomer concentration using 150 min of grafting : vulcanized-g-PEGMA (▲), unvulcanized-g-PEGMA (Δ), vulcanized-g-VPy (◆) and unvulcanized-g-VPy (◊).

4.2   Surface Characterization of Modified NR Latex Films

In order to confirm the success of surface grafting, two major surface characterization methods were used. The wettability of modified NR latex films can be assessed by water contact angle measurements. ATR-IR was used to determine the chemical functionality of the modified NR latex films. The water contact angle data of NR-g-PEGMA as shown in Figure 4.3 revealed that the surface of modified NR latex film became more hydrophilic as the grafting yield increased as a function of time for both unvulcanized and vulcanized NR. From Figure 4.4, a similar trend was also observed in the case of NR-g-VPy with a slightly higher contact angle as a consequence of the lower grafting yield.

Since the sampling depth of contact angle is generally in a range of a few angstroms, the contact angle of a modified surface should theoretically be independent of the depth of modification. The declining trend of water contact angle implied that not only the modification may proceed to the greater depth, but the graft density became higher as the grafting yield was elevated as a function of time. A slight difference of contact angle between the modified vulcanized and unvulcanized NR films suggested that the lightly crosslinked NR allowed the denser and the thicker modified layer to be formed.

Unlike the grafting time, the monomer concentration did not significantly affect the wetting behavior of the modified NR latex films. Interestingly, the contact angle of unvulcanized NR can almost be superimposed on the one of vulcanized NR regardless of their different grafting yields.
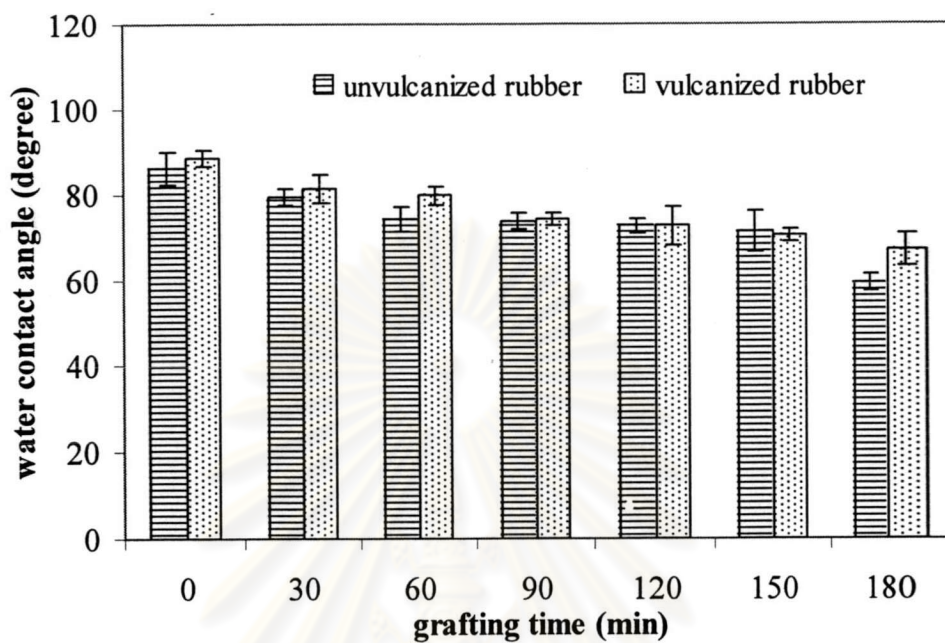
Figure 4.3 Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by 0.5M PEGMA as a function of time.
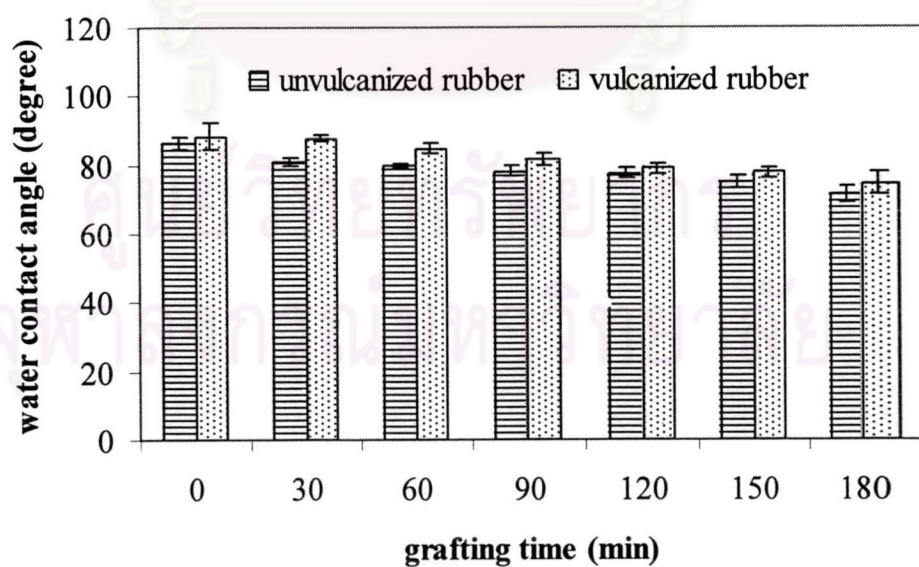

Figure 4.4 Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by 0.5M VPy as a function of time.
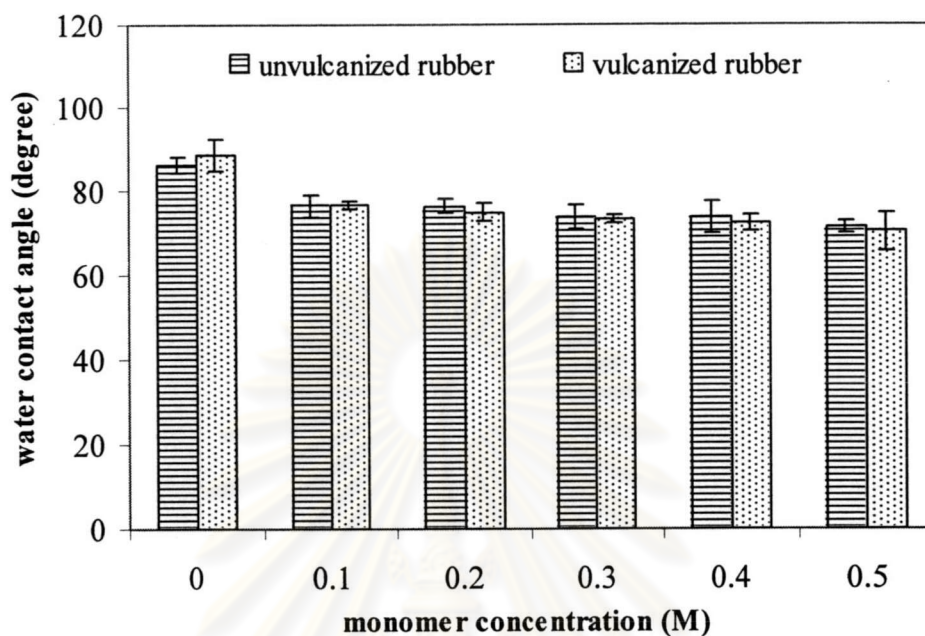
Figure 4.5 Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by PEGMA for 150 min as a function of monomer concentration.
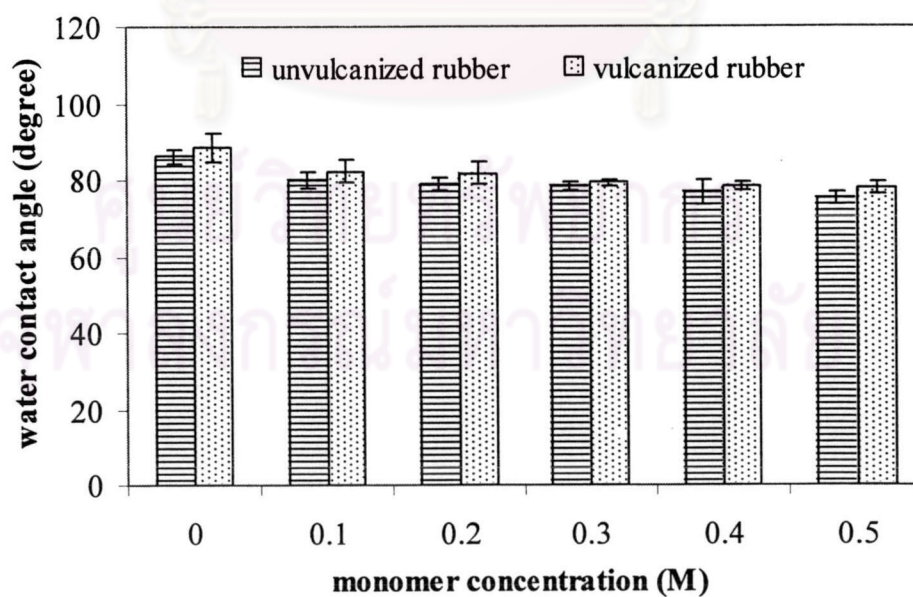

Figure 4.6 Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by VPy for 150 min as a function of monomer concentration.

Even though the depth of modification increased, as a function of monomer concentration (grafting yield increase) the graft density was not much affected. From a practical perspective, it is not necessary to obtain a great depth of modification. Thus, the grafting time seems to be a more effective parameter in controlling the grafting yield as well as the grafting density.

The success of grafting was also confirmed by ATR-IR analysis. Figures 4.7 and 4.8 show ATR-IR spectra of unvulcanized and vulcanized NR latex films before and after graft copolymerization respectively. The appearance of a characteristic peak of carbonyl stretching at 1720 cm^{-1} in the spectra of both vulcanized and unvulcanized NR latex films after surface grafting indicated that PEGMA was incorporated into the depth of at least 1-2 μm from the top surface of both NR substrates. The less intensity of carbonyl stretching of vulcanized NR after graft copolymerization suggested the smaller depth of modification as compared with vulcanized NR.
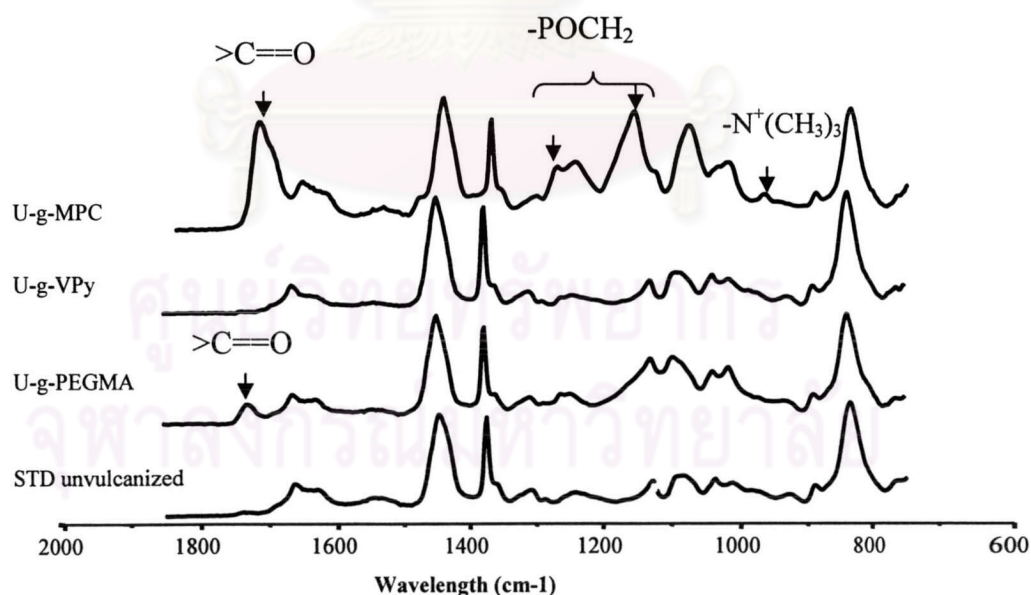

Figure 4.7 ATR-IR spectra of unvulcanized NR before and after being graft copolymerized by 0.5 M PEGMA, VPy and MPC for 150 min.
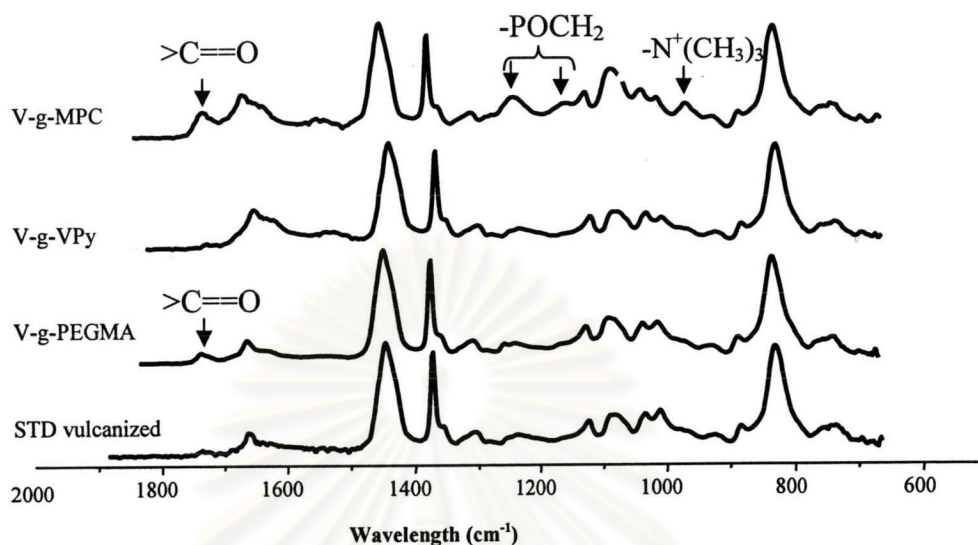
Figure 4.8 ATR-IR spectra of vulcanized NR before and after being graft copolymerized by 0.5 M PEGMA, VPy and MPC for 150 min.

As a consequence of the low grafting yield, we did not observe a characteristic peak of carbonyl stretching in the spectra of both vulcanized and unvulcanized NR latex films after being graft copolymerized with VPy. This observation suggests that the depth of modification is less than the sampling depth of ATR-IR.

For the NR latex films grafting with MPC, in addition to the carbonyl stretching at 1720 cm^{-1}, the stretching due to the phosphate group at 1150, 1240 cm^{-1}, and 970 cm^{-1} stretching due to the ammonium group of MPC were observed in both vulcanized and unvulcanized films. This result also confirms the success of graft copolymerization of MPC and that the modification has proceeded to the depth of at least 1-2 μm.

4.3 Determination of Benzophenone Residue after Graft Copolymerization

For a medical application, it is necessary to assure that there is no photosensitizer initially incorporated leaching out after graft copolymerization. The amount of benzophenone dissolving out from both unvulcanized and vulcanized NR by measuring UV absorbance of ethanolic solution after soaking the NR latex films before and after graft copolymerization. According to Figure 4.9, the amount of benzophenone adsorbed and/or absorbed on NR latex films that can be soluble in ethanol was ~30 and ~45 $\mu g/cm^2$ in the case of unvulcanized and vulcanized NR, respectively. After graft copolymerization, it was found that the amount of dissolved benzophenone was below 5 $\mu g/cm^2$ indicating that most benzophenone was washed out after graft copolymerization.
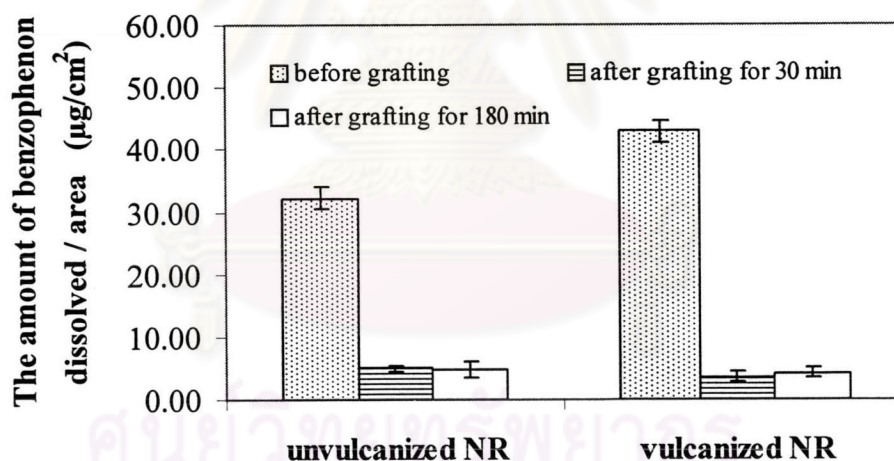

Figure 4.9 The amount of benzophenone per surface area ($\mu g/cm^2$) dissolving out from vulcanized and unvulcanized NR latex films before and after graft copolymerization.

4.4 Blood Compatibility

In this study, the blood compatibility of modified NR latex films was determined with an attention to investigate the interaction between the surface with blood components which are plasma proteins in platelet-poor plasma (PPP) and platelets in platelet-rich plasma (PRP). The effects of grafting yield as a function of grafting time and the type of monomer on blood compatibility.

4.4.1 Plasma Protein Adsorption

The amount of plasma protein adsorbed on a material surface is a primary factor in evaluating the blood compatibility of the material. When polymeric biomaterial is in contact with blood, surface induced thrombosis is initiated by the adsorption of plasma proteins, followed by adhesion and activation of platelets. The amount of plasma protein adsorption was determined by using BCA microassay. The calibration curve using albumin as a standard is displayed in Appendix B.

Figures 4.10 and 4.11 show the amount of plasma protein adsorbed on NR latex films after graft copolymerization with PEGMA as a function of time and monomer concentration, respectively. The amount of plasma protein adsorbed was significantly decreased after surface grafting. Obviously, the reduction of protein adsorption depended on the grafting yield which can be varied as a function of grafting time and monomer concentration. As a result of the lower grafting yield, the protein reduction of vulcanized NR was not as effective as that of unvulcanized NR using the same grafting time and concentration. The complete absence of protein adsorption on NR latex films required as high as 1% grafting yield (See Figure 4.1 for comparison). Eventhough a similar trend was also observed in the case of VPy, the reduction of protein adsorption was not so effective due to its grafting yield being lower than 0.5% (see Figures 4.12-4.13).
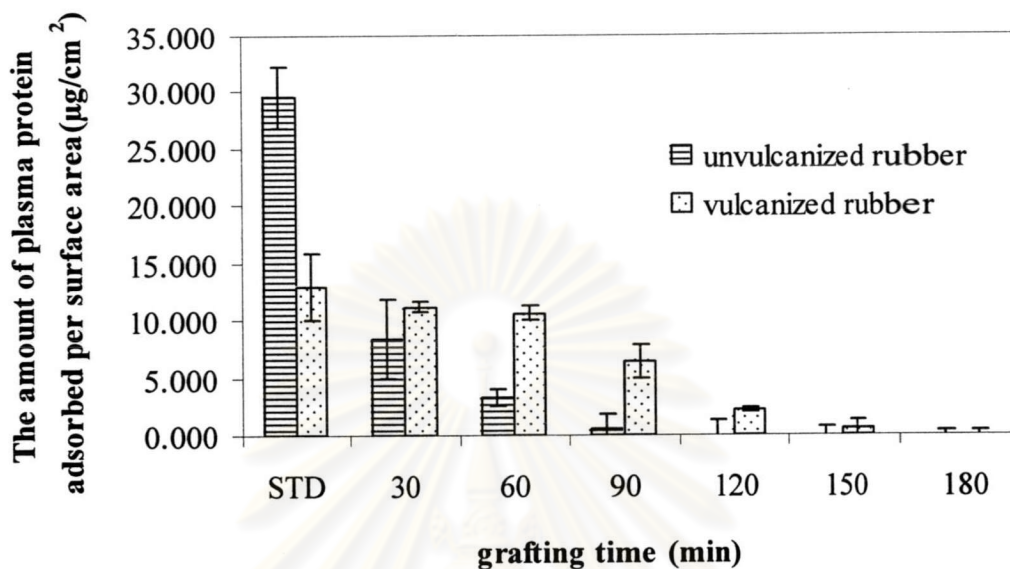
Figure 4.10 The amount of plasma protein adsorbed per surface area of vulcanized NR and unvulcanized NR before and after graft copolymerization by 0.5 M PEGMA as a function of grafting time.
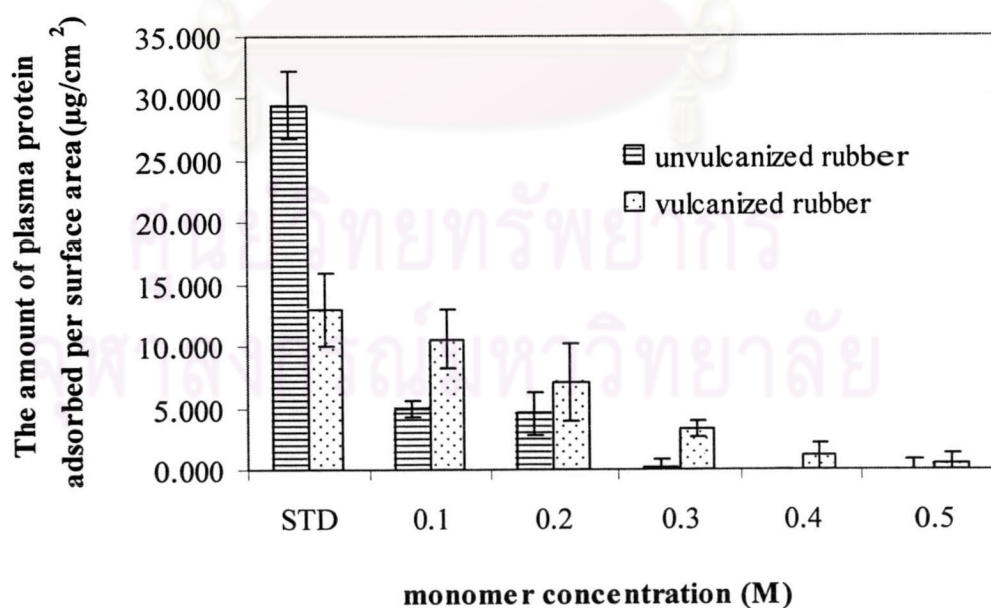

Figure 4.11 The amount of plasma protein adsorbed per surface area of vulcanized NR and unvulcanized NR before and after graft copolymerization by PEGMA for 150 min as a function of monomer concentration.

Figure 4.12 The amount of plasma protein adsorbed per surface area of vulcanized NR and unvulcanized NR before and after graft copolymerization by 0.5 M VPy as a function of grafting time.

Figure 4.13 The amount of plasma protein adsorbed per surface area of vulcanized NR and unvulcanized NR before and after graft copolymerization by VPy for 150 min as a function of monomer concentration.
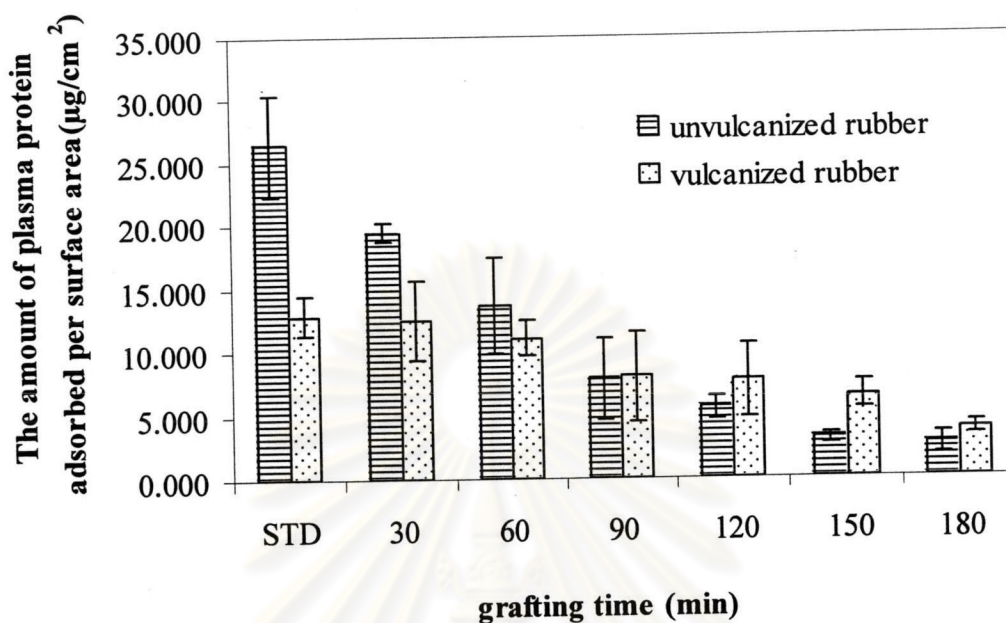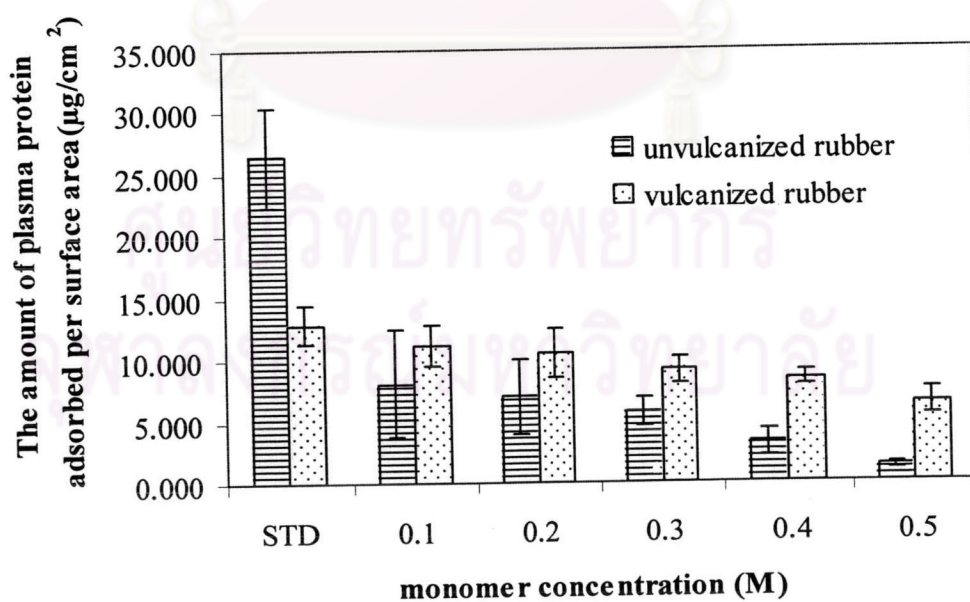
Considering comparative results of plasma protein adsorption on NR latex films after graft copolymerization with PEGMA, VPy and MPC as shown in Figure 4.14, it can be implied that PEGMA and MPC are the monomers suitable for graft copolymerization in order to reduce protein adsorption. Hydrophilic polymers being highly effective in repelling protein adsorption can be regarded as its chain flexibility and its large excluded volume in the aqueous environment.
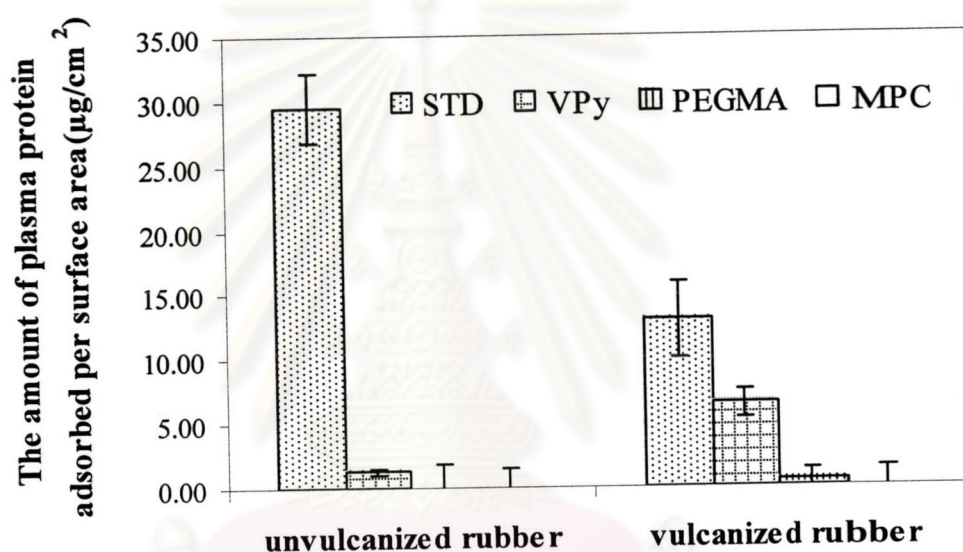

Figure 4.14 The amount of plasma protein adsorbed per surface area of vulcanized and unvulcanized rubber before and after graft copolymerization by 0.5 M PEGMA, VPy and MPC at 150 min grafting time.

4.4.2 Platelet Adhesion

It is well known that platelets also contribute to the thrombus formation. In general, a foreign substrate induces adhesion and activation of platelets with the adsorbed protein layer serving as a controlling factor of the platelet response. The extents of platelet adhesion on the modified NR latex films were monitored by scanning electron microscope. Figures 4.15-4.18 show SEM micrographs displaying the responses to platelet adhesion of unvulcanized and vulcanized NR latex films before and after graft polymerization after being exposed to platelet-rich plasma (PRP). There are a few platelets attached on the unmodified NR latex films indicating their poor blood compatibility. Fewer platelets were able to adhere on the surface of NR latex films as the grafting yield increased as a function of both grafting time and monomer concentration. The decreasing of platelet adhesion on the modified surfaces represents the success of improvement of blood compatibility of NR latex films. The results corresponded quite well with protein adsorption data as previously shown.
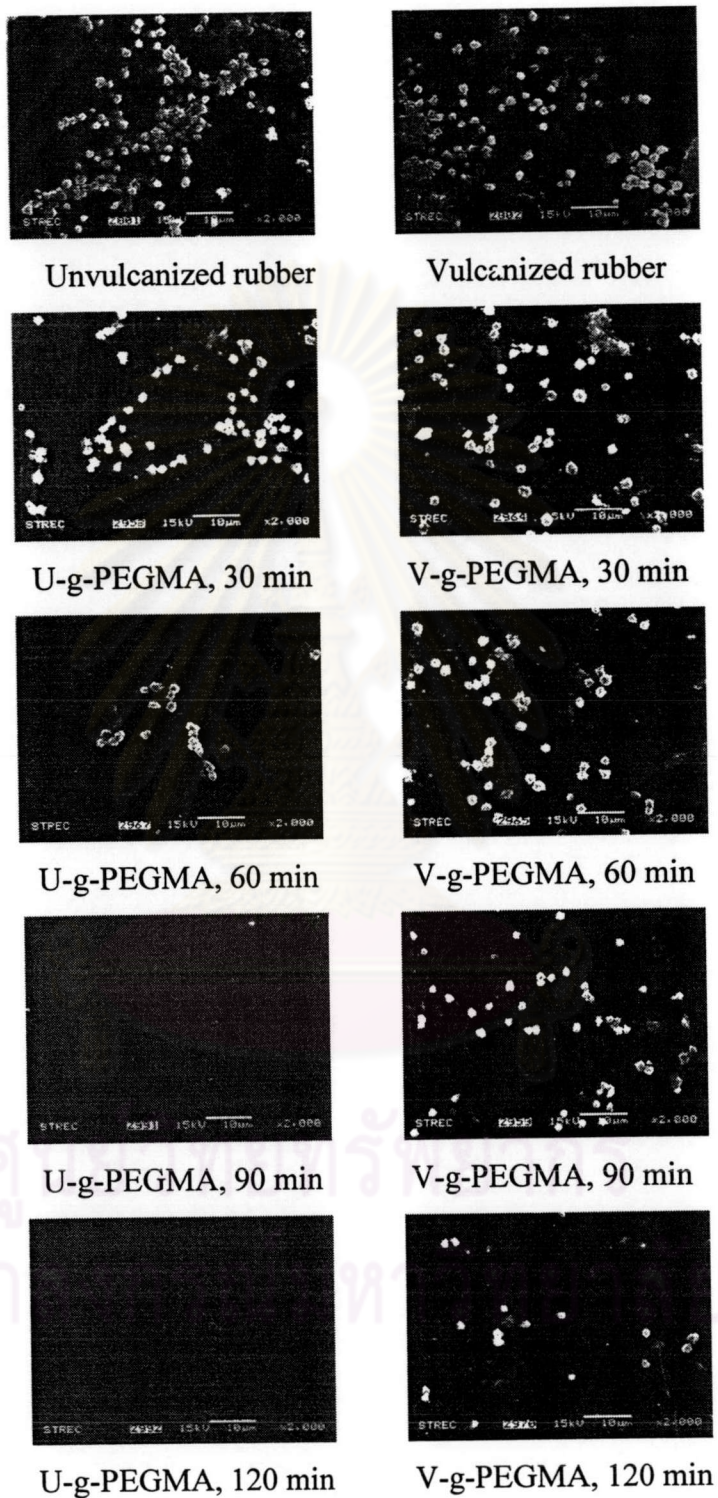
Figure 4.15 SEM micrographes of unvulcanized NR (U) and vulcanized NR (V) before and after graft copolymerization with 0.5 M PEGMA as a function of time after contacting with PRP

Unvulcanized rubber

Vulcanized rubber

U-g-PEGMA, 0.1 M

V-g-PEGMA, 01 M

U-g-PEGMA, 0.2 M

V-g-PEGMA, 0.2 M

U-g-PEGMA, 0.3 M

V-g-PEGMA, 0.3 M
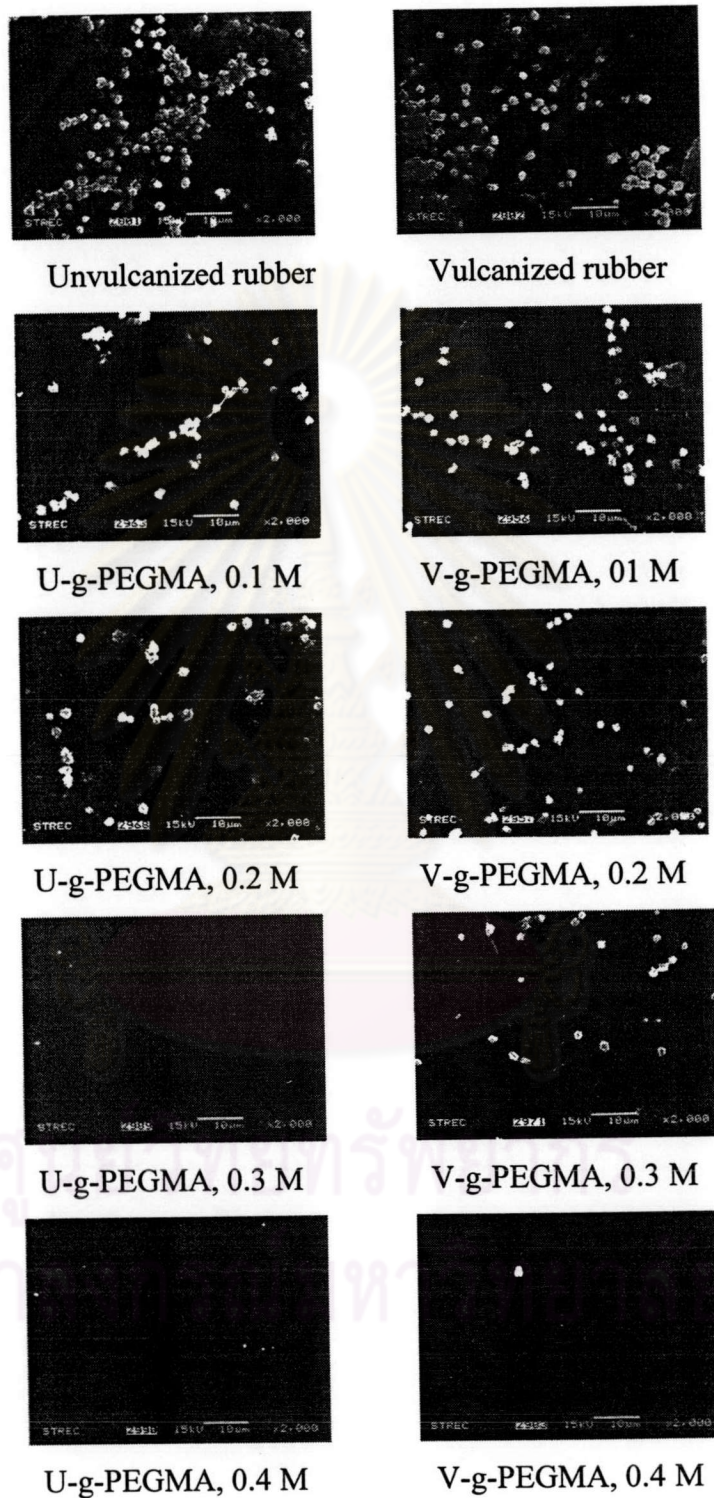
U-g-PEGMA, 0.4 M

V-g-PEGMA, 0.4 M

Figure 4.16 SEM micrographes of unvulcanized NR (U) and vulcanized NR (V) before and after graft copolymerization with PEGMA for 150 min as a function of monomer concentration after contacting with PRP.
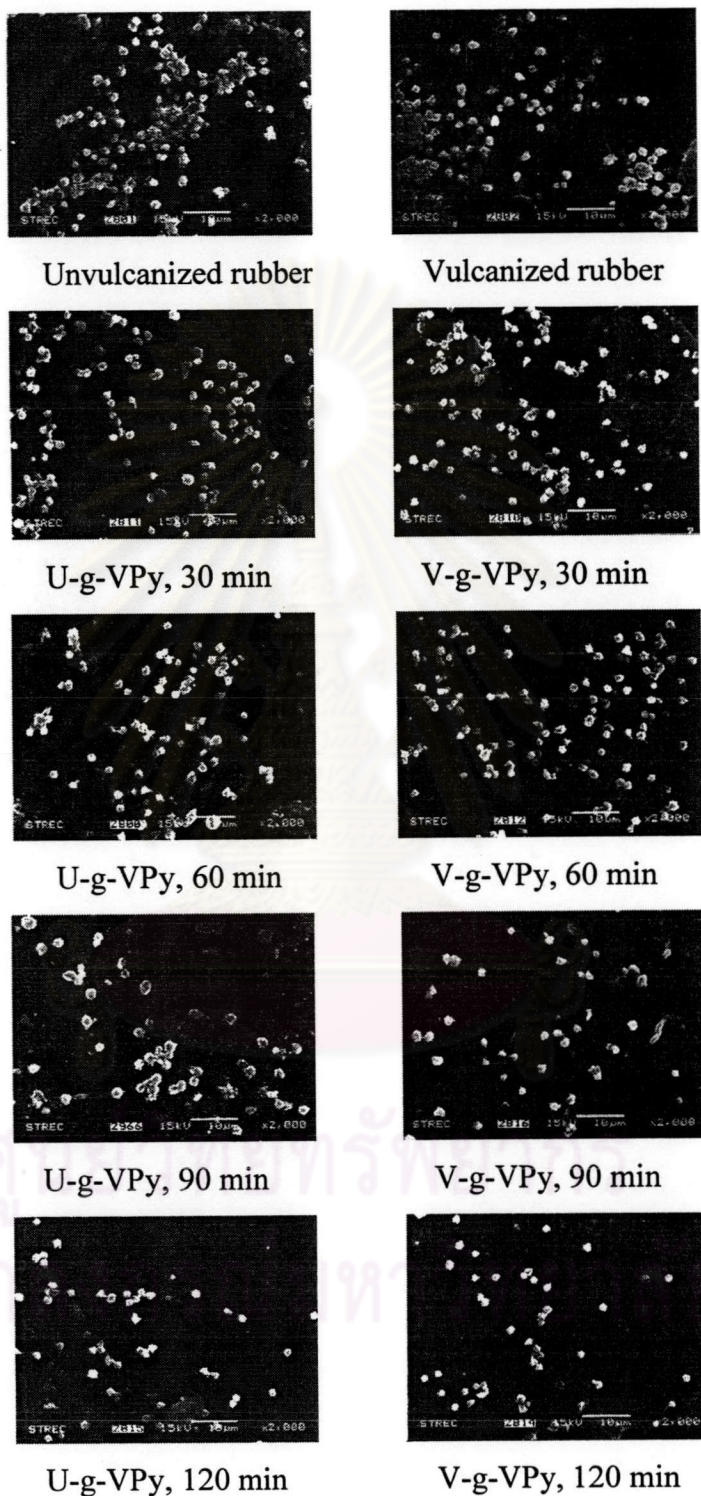
Figure 4.17 SEM micrographes of unvulcanized NR (U) and vulcanized NR (V) before and after graft copolymerization with 0.5 M VPy as a function of time after contacting with PRP.

Unvulcanized rubber

Vulcanized rubber

U-g-VPy, 0.1 M

V-g-VPy, 0.1 M

U-g-VPy, 0.2 M

V-g-VPy, 0.2 M

U-g-VPy, 0.3 M

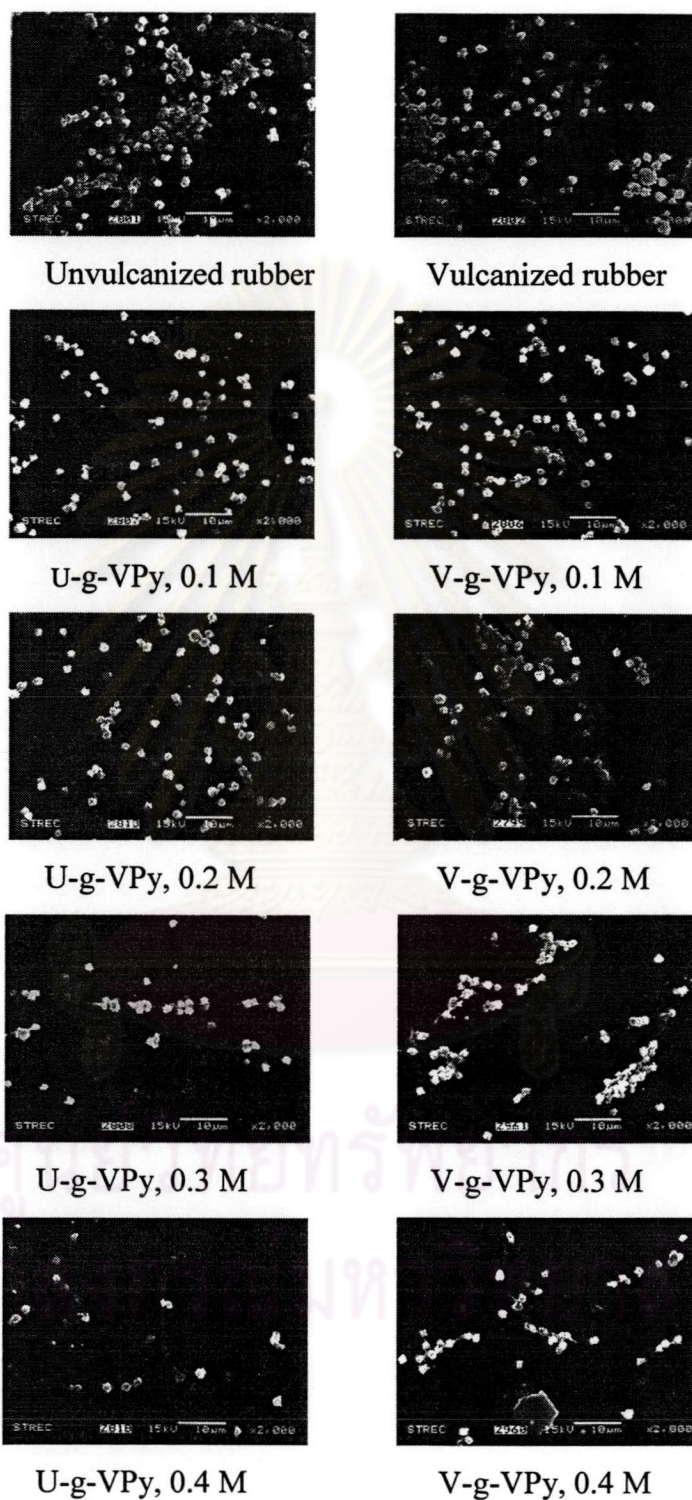V-g-VPy, 0.3 M

U-g-VPy, 0.4 M

V-g-VPy, 0.4 M

Figure 4.18 SEM micrographes of unvulcanized NR (U) and vulcanized NR (V) before and after graft copolymerization with VPy for 150 min as a function of monomer concentration after contacting with PRP.

Figure 4.19 shows the comparative results of platelet adhesion on NR latex films after graft copolymerization with 0.5 M PEGMA, VPy and MPC for 150 min. All three monomers can be used to improve the blood compatibility of NR latex films. But PEGMA and MPC are more effective than VPy for unvulcanized and vulcanized NR latex films.
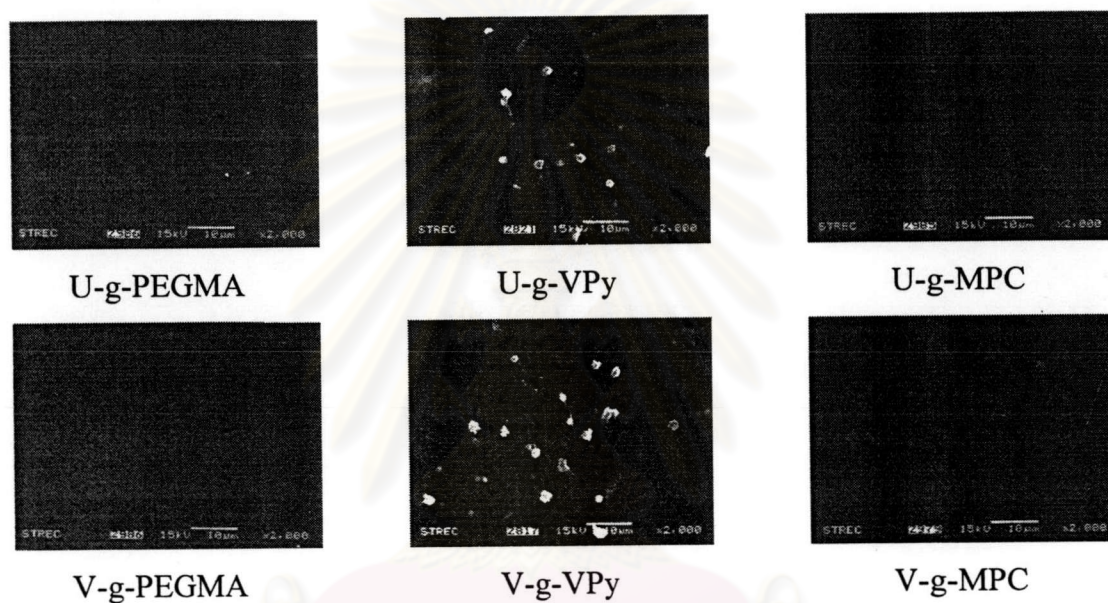

| U-g-PEGMA | U-g-VPy | U-g-MPC |
| V-g-PEGMA | V-g-VPy | V-g-MPC |

Figure 4.19 SEM micrographes of unvulcanized NR (U) and vulcanized NR (V) after graft copolymerization by 0.5 M PEGMA, VPy and MPC for 150 min.

4.5 Mechanical Properties

In order to determine the feasibility of graft copolymerization for practical purposes, the mechanical properties of modified NR latex films were determined Dumbbell test pieces were cut from the vulcanized NR latex sheets before graft copolymerization for tensile testing.

As outlined in Table 4.1, it can be observed that graft copolymerization unexpectedly caused a significant increase of vulcanized NR tensile strength, especially in the case of PEGMA whose grafting yield was about 1%. Since UV-exposure alone did not affect the tensile strength of the vulcanized NR latex film, the improvement of tensile strength should originate from the existence of the surface modified layer, not by the extra crosslinking possibly induced during UV-exposure. Slight increase of elongation at break and modulus was also evidenced as a consequence of surface grafting.

Table 4.1 Tensile strength, modulus (at 100 300 and 500 %) and elongation at break of vulcanized NR latex films before and after graft copolymerization with 0.5 M PEGMA and VPy for 150 min.

Rubber type	Tensile Strength (N/ mm^2)	Tensile Modulus (%)			Elongation at break (%)
		100	300	500	
Standard rubber	19.65 ± 1.53	0.30±0.01	0.89±0.02	1.48±0.04	950.80 ± 28.47
Rubber with UV irradiation[1]	19.69 ± 5.33	0.29±0.04	0.87±0.11	1.45±0.21	958.80 ± 60.87
Vulcanized-g-PEGMA	35.69 ± 1.82	0.38±0.01	1.14±0.02	1.94±0.04	985.46 ± 8.71
Vulcanized-g-VPy	28.73 ± 3.86	0.36±0.04	1.07±0.12	1.84±0.32	989.90 ± 59.25

[1]NR latex films were subjected to benzophenone soaking before UV irradiation

The tensile properties suggest that UV exposure and grafting be caused adverse effect on mechanical properties of NR latex film. UV-induced graft copolymerization is thus a feasible method that can be used to improve blood compatibility of NR latex films.