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Extraction of Protein from Skim Natural Rubber Latex Using PEG as a Surfactant via Low Speed Centrifugation and Continuous Flow

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ABSTRACT: Protein extractions from skim natural rubber latex using 3 %w/v polyethylene glycol (PEG6000) via both low speed centrifugation and continuous flow were investigated. In centrifugal extraction, when the speed was 1000 rpm, the extractable protein (EP) content in serum increased with processing time from 5 to 30 min and when the time was fixed at 5 min, EP content increased with centrifugal speed. In addition, further washing deproteinized chips with 2 %w/v SDS solution could remove proteins with efficiencies corresponding to the efficiencies of protein removals in latex phase, implying the role of PEG in protein reduction in both steps. In continuous flow extraction, EP content increased with increasing Reynolds number or increasing mean residence time of the flow to a maximum and then dropped. The efficiencies of the centrifugal extraction and continuous flow extraction were 55.2 and 33.7%, respectively. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39900.

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INTRODUCTION

Natural rubber latex (NRL) is obtained from rubber trees (*Hevea brazilenses*) mostly grown in Malaysia, Indonesia, and Thailand. Natural rubber latex is composed of rubber particles which are mainly *cis*-1,4-polyisoprene, proteins, phospholipid, carbohydrates, and inorganic substances such as potassium, magnesium, zinc, copper, and iron.[1] Field latex containing 30–33 wt % rubber is centrifuged to yield a concentrate latex with a rubber content of 60 wt %[1,2] and skim latex with rubber fraction of 5–8 wt %.[3] Due to an increasing interest in this significant amount of skim rubber, the basic characteristics of skim rubber and cream or concentrate rubber were investigated. It was reported that skim rubber particles were smaller and spread more easily when forming a film, yielding smoother film product. Additionally, they had lower amount of protein-phospholipid materials.[2] These findings suggest potential uses of skim rubber in protective products such as masks, gloves, and medical products.

It was shown that hydrophilic protein-phospholipid materials coated the surface of hydrophobic core of the rubber particles, of which the protein fraction was approximated to be 0.84.[4] There are more than 250 kinds of proteins in the latex, with a total amount between 1–1.8 wt % depending on the latex source and 30–60 kinds are believed to cause allergic reactions to human.[5] Some people are allergic to proteins in products made of natural rubber latex when they are exposed to human skin. Allergic reactions are shown in the form of asthma, life-threatening anaphylaxis, rashes, and skin infections.[5–8]

Therefore, several methods have been applied to reduce extractable proteins in either natural rubber latex or films. Proteins on rubber particles in filed latex could be depolymerized by applying ^{60}Co irradiation[9] or using proteolytic enzyme,[10] yielding low-molecular-weighted proteins that could be soluble in serum, thereby increasing the extractable protein (EP) in the latex. The denaturant such as urea can change the protein morphology so that the adsorbed proteins are easily removed. Applying urea together with sodium dodecyl sulfate as a surfactant in field and high ammonia natural rubber latex (HANR) yielded highly purified rubber.[11] In addition, bases such as potassium hydroxide[12] and sodium hydroxide together with Triton-X as a surfactant,[10] which involved in saponification with phospholipid on rubber particles could also remove the adsorbed proteins. Recently, the group of Chaikumpollert has reported a novel procedure in which the combination of using alcohols to remove phospholipid and using urea in the presence of surfactants to denature proteins could produce protein-free natural rubber.[13]

Only a few works applied polymers to extract proteins in rubber latex. A water-soluble polymer and ^{60}Co irradiation were used in extracting protein from HANR.[14] In addition, polyethylene glycol (PEG) was investigated as a non-ionic surfactant for a

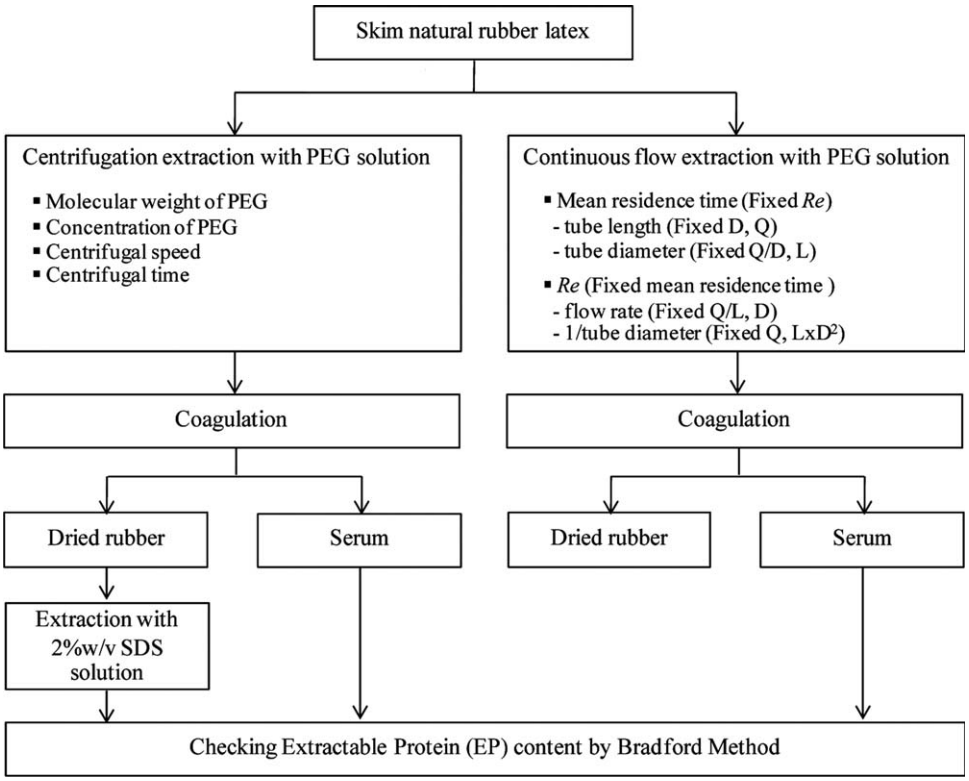

Figure 1. Schematic representation of experimental procedure.

reduction of EP content without any compromise on the mechanical properties.[15] PEG has been reported to potentially precipitate proteins[16,17] and the attachment of proteins on PEG-*grafted*-natural rubber product could be prevented.[18] Importantly, PEG is commercially available, non-toxic, and biocompatible so it is widely used in cosmetics and in biotechnological and medical applications.[19–21]

Unlike other works in which the rubber latices were incubated with surfactants and/or denaturants, for a day or many hours before centrifugation at a high speed up to almost 20,000 rpm for a centrifugal time up to almost an hour[3,9,11,13,15,22–24], we are interested in short time and low speed extractions, which could be important in understanding the mechanisms of mass transfer in protein extraction of skim natural rubber latex. Since all the researches about protein extraction from rubber latex have not been focused on the process parameters, it is of interest to investigate more about them when using PEG for the production of low-protein rubber, especially from the skim latex about which few works have been studied.[3,16] Thus, this study explores the effect of process parameters in centrifugal processes including centrifugal speed and time and in continuous flows including tube length, tube diameter and flow rates of both skim rubber and the PEG solution.

EXPERIMENTAL

Materials

Skim natural rubber latex was obtained from Rubber Estate Organization (total solid content of 7.67 wt %, dry rubber content of 4.23 wt %, initial protein content in the serum of 0.66 mg/mL and pH of 9.03). Polyethylene glycol with molecular weights of 6000 (PEG6000) and 20000 (PEG20000) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co.LLC. (Germany). Sulfuric acid and sodium dodecyl sulfate (SDS) were purchased from Ajax Finechem Pty Lty. Toluene was purchased from Panreac Quimica S.L.U. Dye reagent concentrate was purchased from Bio-Rad. The silicone tube was purchased from Dura.

Methods

The experiments were designed as shown in Figure 1. The extractions were carried out by both centrifugation and continuous flow. Only the dried samples from the centrifugation were further extracted by surfactants. The detail of each step is as followed.

Protein Extraction in Skim Latex with PEG Solution by Centrifugation. First, PEG6000 and PEG20000 were tested for their extracting performance by varying the concentration from 0.6 %w/v to 6 %w/v. The equal volumes of 30 mL of skim latex and PEG solution were mixed and the mixtures were centrifuged at 3000 rpm for 5 min to extract proteins from rubber particles. Before coagulation, the mixture was stirred by an overhead stirrer at 150 rpm for 12 min. The rubber was coagulated with sulfuric acid solution and the coagulum was dried in an oven at 60°C for 24 h. The serum was immediately checked for the extractable protein (EP) content based on the standard curve of BSA. The results are the average from at least three samples. The amount of protein is reported as EP content in liquid (mg/g), which is defined as

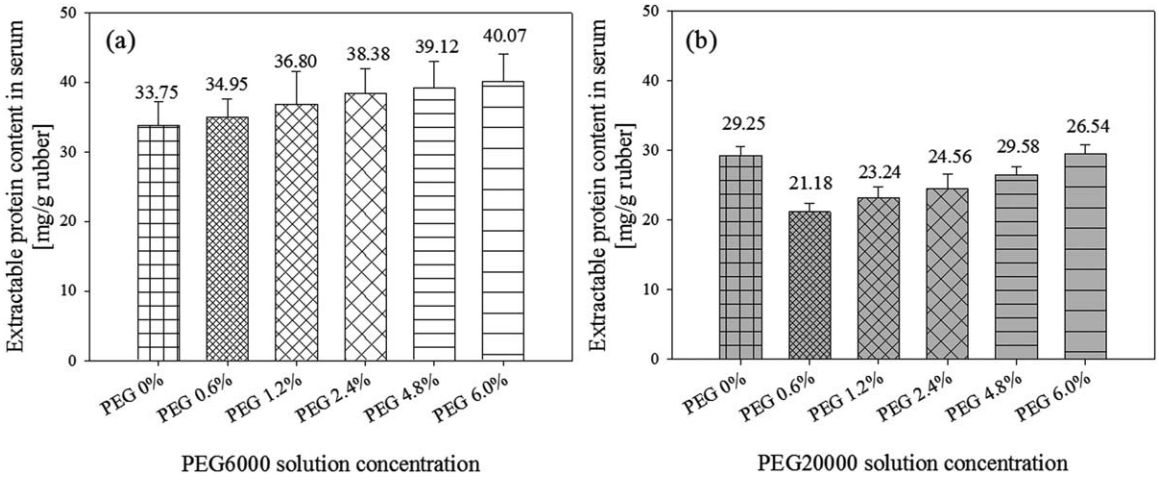

Figure 2. The effect of PEG concentration on EP content in serum (a) PEG6000 and (b) PEG20000.

$$\text{Extractable protein Content(EP)} = \frac{\text{Total content of protein in liquid(mg)}}{\text{Weight of skim rubber(g)}} \quad (1)$$

Second, the effects of centrifugal speed and time were investigated. In this experiment, 30 mL of 6 %w/v PEG6000 solution were added to 30 mL of skim rubber latex so that the concentration of PEG in latex was 3 %w/v. This concentration is much higher than those used in another work[15] since we would like to observe the short time effect of centrifugation on extraction. The mixture was centrifuged at a speed of 1000, 2000, or 3000 rpm for 5, 15, or 30 min.

Protein Extraction in Skim Latex with a PEG Solution by Continuous Flow. In these experiments, the flow rate of skim rubber latex was varied in the range of 11.0–38.5 mL/min and the flow rate of 6 %w/v PEG6000 solution was varied in the range of 7.6–26.6 mL/min. The tubes with diameter ranged from 0.4 to 0.7 cm and length ranged from 14.69 to 135 cm were used.

The skim natural rubber latex and 6 %w/v PEG solution flowed in separate tubes and were forced to mix in T-connector before continuing flowing along a same tube. The mixture had been collected at the tube end until the total volume reached 60 mL, which is the same volume obtained in the centrifugal extraction experiments, and collecting time was then recorded. The mixture was stirred by an overhead stirrer at 150 rpm for 12 min. After that the rubber was coagulated with sulfuric acid solution and dried in an oven at 60°C for 24 h. The serum was immediately checked for the extractable protein content. The results are the average from at least three samples.

Preparation of Rubber Chips. The rubber films from the centrifugal extraction experiments were cast from the solution of coagulated rubber in toluene on a glass plate. Subsequently, the cast film was allowed to dry at room temperature, removed from the plate, and cut to 0.5 cm × 0.5 cm chips with the thickness of 0.034 ± 0.05 mm. Some samples were chosen to be characterized by Fourier transform infrared (FTIR) spectrometer (Perkin Elmer).

Protein Extraction from Rubber Chips. All chips were washed with an extracting medium, which is 2 %w/v SDS solution. Each chip was put in centrifuge tube and 2 mL of extracting medium was added. The tube was centrifuged at 3000 rpm for 5 min and then the protein content in the medium was measured by diluting 10 μL of medium with 1990 μL of distilled water. The EP content in the medium can be calculated according to eq. (1).

Determination of the Extractable Protein (EP) Content by Bradford Micro-Assay. Determination of water-soluble protein content was done by Bradford micro-assay using BSA as a standard protein.[25] In this method, the color of protein solution changed into blue after adding a Bio-Rad dye (reagent). A calibration curve of BSA was prepared from the BSA solution with the concentration of 1.25, 2.5, 5, 7.5, and 10 μg/mL and the corresponding absorbance was measured by UV–VIS spectrophotometer at a wavelength of 595 nm. The amount of protein in an unknown sample can be determined by comparison with the calibration curve of the standard BSA.

RESULTS AND DISCUSSION

The Effect of PEG Concentration in Centrifugal Extraction

Figure 2 clearly shows that EP content in serum after rubber latex was extracted with PEG solution increased with an increase in concentration of both PEG6000 and PEG20000. In contrast to the previous work[15] in which high-molecular-weighted PEG could extract proteins from rubber particles more efficiently than low-molecular-weighted PEG, it was seen in this study that PEG with higher molecular weight was less efficient in removing proteins from skim rubber particles. Moreover, for PEG6000, increasing PEG concentration could increase EP content and it was better than using only pure water but using PEG20000 at concentration up to 4.8 %w/v was worse than the case of pure water.

Kumar et al.[26] reported the effect of PEG in precipitation of BSA, a model protein used in their study. It was found that adding more PEG in BSA solution resulted in more decrease in solubility of BSA. It was hypothesized that protein molecules

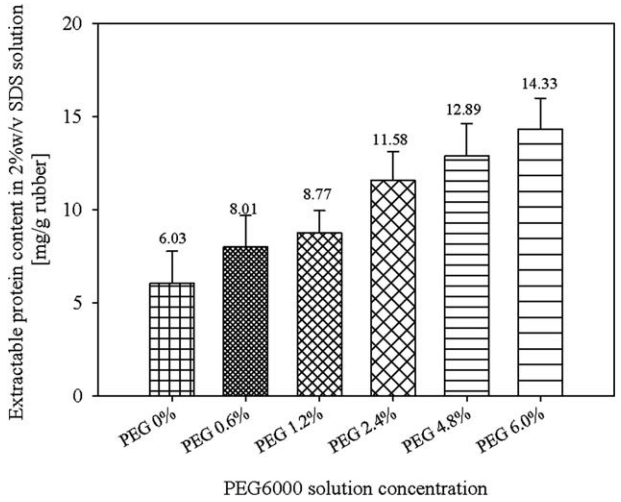

Figure 3. The EP content in 2 %w/v SDS solution after leaching deproteinized rubber chips obtained from the experiment of using PEG solutions with various concentrations.

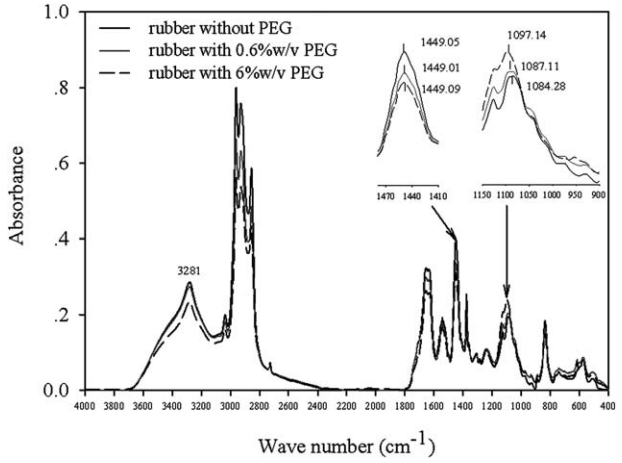

Figure 4. FTIR Spectra of untreated rubber and rubbers treated with 0.6 % w/v and 6 % w/v PEG solutions.

are sterically excluded from the regions of the water occupied by PEG molecules. Therefore, proteins are concentrated and precipitated.[16,17] PEG, when being grafted to the natural rubber molecules, was also reported to increase blood compatibility and prevent protein adsorption on natural rubber samples.[18] It was also proposed that PEG interact with protein molecules by hydrophobic interactions and this was thought to be responsible for the destabilization of protein structure.[27] In our case, proteins on the rubber particles may be excluded by the adsorbed PEG molecules or be attracted to PEG molecules via hydrophobic interactions so that protein structure was partly denatured and finally proteins could be removed from the rubber surface along with some PEG molecules. More amount of PEG adsorbed on the rubber particles, more proteins could be extracted to the serum. If hydrophobic interactions are of importance, it is possible that PEG molecules could interact firstly with soluble proteins originally in serum. PEG molecules coupled with proteins are then transported to the surface of the rubber particle and take some time to interact with the adsorbed proteins in order to remove them. Figure 2 suggests that for large PEG molecules (PEG20000), the kinetics of interactions with adsorbed proteins is slower than PEG6000 molecules at the same extraction condition (centrifugal speed of 3000 rpm and centrifugal time of 5 min). Therefore, there is less protein amount in the serum. When the concentration of PEG20000 is higher, the kinetics could be improved, leading to more proteins in serum as shown in Figure 2(b). In the experiments that follow, 6% w/v PEG6000 solution is then chosen to be used as an extracting agent to study the effects of centrifugal speed and time.

It is interesting to investigate protein removal from rubber chips obtained from deproteinized rubber in previous treatments. The results are the average from nine samples. All rubber chips were subjected to 2 %w/v SDS solution. The results are shown in Figure 3, where the EP content in the extracting medium showed similar trend to the EP content in serum depicted in Figure 2. These results imply that there were some amounts of PEG left on the surface of rubber particles when they were coagulated. Once the solid rubber ship was washed in 2 %w/v SDS solution, SDS which is an anionic surfactant could be adsorbed on the chip surface and denatured the remaining proteins[25] while the remaining PEG molecules could be dissolved back to SDS solution and also interact with proteins in the same way as they did in the latex phase. The confirmation of reducing protein content and increasing PEG amount in dried skim rubber is shown by FTIR spectra in Figure 4.

In Figure 4, the principal peak of PEG is observed for skim rubber without PEG, for rubber with 0.6 %w/v PEG and rubber with 6 %w/v PEG at 1084.28, 1087.11, and 1097.14 cm^{-1}, respectively, which corresponds to C–O–C (ether group). The absorbance increased as the amount of PEG increased, confirming that more PEG molecules were adsorbed on the surface of rubber particles. In addition, the peak corresponding to –NH in secondary amides in polypeptides is seen almost at the same position of 1449 cm^{-1} for all samples. The absorbance decreased as the amount of PEG increased, confirming the removal or proteins by PEG. Another peak representing proteins is observed at 3281 cm^{-1} corresponding to NH deformation. Both peaks of proteins clearly show the reduction of protein after extraction.

The Effect of Centrifugal Speed and Time in Centrifugal Extraction

In Figure 5, it is observed that at the lowest speed of 1000 rpm, EP content increased with centrifugal time and for the shortest centrifugal time of 5 min, EP content increased with increasing centrifugal speed. These results reflect the diffusion rate of PEG molecules through mass transfer layer around rubber particles before removing the protein molecules. If more PEG molecules could be accommodated on the surface, more effective they would be in removing proteins.

According to Bird et al.,[28] the mass transfer rate to or from sphere surface is dependent on the mass transfer coefficient at the interface (k), which is expressed in terms of Sherwood number (Sh), which is a function of Reynolds number (Re), and Schmidt number (Sc) as shown in eq. (2).

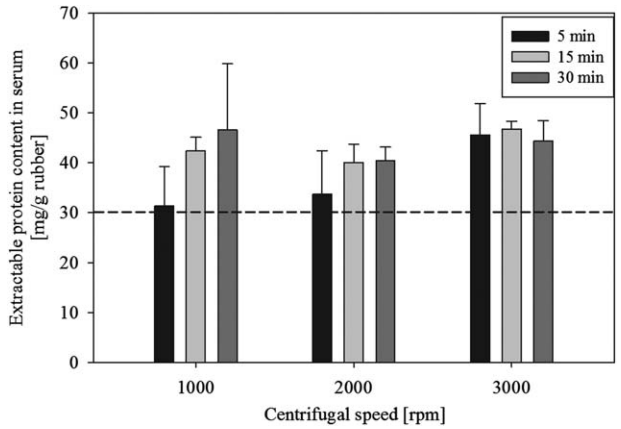

Figure 5. The effects of centrifugal speed and time on EP content in serum after extraction with PEG.

$$Sh=2+0.60\,Re^{1/2}Sc^{1/3} \tag{2}$$

Here,

$$Sh=\frac{kD_p v_\infty}{\mu} \tag{3}$$

$$Sc=\frac{\mu}{\rho D_{AB}} \tag{4}$$

$$Re=\frac{\rho D_p v_\infty}{\mu} \tag{5}$$

In these equations, D_p is the spherical particle diameter, v_∞ is the approaching velocity of liquid, μ is the liquid viscosity, ρ is the liquid density, and D_{AB} is the diffusivity of substance A in liquid B. The Reynolds number (Re) in this case is linearly related to the centrifugal speed, i.e., increasing centrifugal speed could increase Re, thereby increasing k in Sherwood number. As a result, more proteins could be extracted. Another theoretical aspect also discussed by Bird et al.,[29] is the transfer through boundary layer covering the surface while there is a flow around the submerged objects. The transfer rate depends on the diffusion coefficient as well as the thickness of the boundary layer. The flow with higher velocity causes boundary layer to be thinner, leading to higher rate of mass transfer. This was recently reported in the study of the nitrification rate of submerged tubular filter in the recirculating system. It was found that boundary later thickness derived from the experimental data was lower when the fluid flow rate through tubular filter was higher and they were qualitatively consistent with the theoretical predicted values.[30] Similarly, it could be expected that the layer around the rubber particle was thinner as the centrifugal speed increased. The estimation of boundary layer thickness could be done by eq. (6).[29]

$$\delta=\sqrt{\frac{12\mu x}{\rho v_\infty}} \tag{6}$$

Where, δ is boundary layer thickness, x is the size of rubber particles, and other variables are already defined. Based on eq. (6), the thickness is 1.64, 1.15, and 0.94 μm when the centrifugal speed is 1000, 2000, and 3000 rpm, respectively. In case of varying centrifugal time while maintaining the speed at 1000 rpm, the amount of PEG adsorbed at rubber surface increased with time as clearly seen in Figure 5 as long as there were enough PEG molecules diffusing from the bulk liquid phase.

However, at higher speeds of 2000 and 3000 rpm, the phenomena were different. The EP content tended to increase and leveled off with time at 2000 rpm while the EP content tended to decreased with time at 3000 rpm. The results are similar to those reported in the study of the effect of surfactants on extraction of phenanthrene in spiked sand.[31] The amount of phenanthrene that could be extracted increased with mixing speed up to a maximum and then dropped when continuing increasing the speed but the reason behind this was not provided. The optimum of the extracted amount was also reported in another work where the effect of mixing history on gluten protein recovery from flour-water batters was studied.[32] The authors proposed that the product of speed and mixing time was a governing parameter. The amount of gluten protein recovery (extraction from the flour batters) also showed an increasing trend with the product at first and then dropped when the product was greater than an optimum.

In the extraction performed in colloidal systems such as rubber latex systems and others,[31,32] the colloidal stability may be of importance. Applying high stirring speed and longer stirring time could disturb latex stability, which would result in coagulation of the colloidal particles.[33] In addition, the presence of a polymer at a low concentration could cause bridging effect, decreasing stability of the system.[33] Therefore, the results of protein extraction at a higher speed and for a longer time may be attributable to the competition between mass transfer rate of PEG, which promotes the extraction efficiency, and coagulation rate of rubber particles, which lowers the extraction efficiency. Prolonged centrifugation with higher degree of turbulence may induce the closeness of deproteinized rubber particles, which were likely to have less repulsive forces among negative charges from proteins at the surface.[15] This could generate microscopic clusters of rubber particles whose size grows larger with time, leading to less active surface for PEG adsorption as well as protein removal.

The rubber chips coagulated from this experiment were also subjected to 2 %w/v SDS solution. The results are shown in Figure 6. Similar to Figure 5, EP content in SDS solution showed the similar trend to that in the serum as shown in Figure 3. Therefore, if the condition was so beneficial that more amount of PEG could be adsorbed onto rubber particles, it would result in removal of more proteins to the serum as well as removal of more proteins from the rubber chips.

Extractions in Continuous Flow Processes

As discussed above for the centrifugal processes, it is expected that the degree of turbulence in mixing governed by the Reynolds number for flow in the tube (Re) and the mixing time

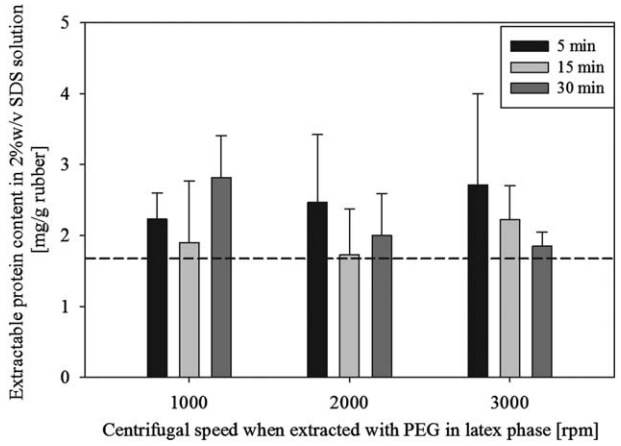

Figure 6. The EP content in 2 %w/v SDS solution after leaching deproteinized rubber chips obtained from the experiment studying the effects of centrifugal speed and time.

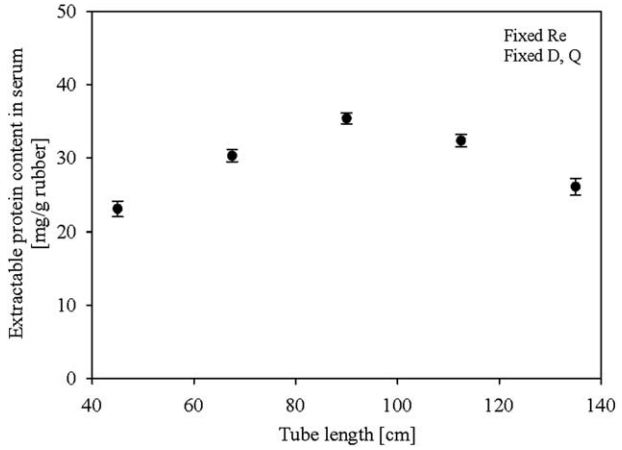

Figure 7. The effect of mean residence time on EP content in serum in continuous flow extraction by fixing Reynolds number in Scheme 1 (keeping D and Q constant and varying L).

during the extraction process are important to protein extraction efficiency of rubber latex. In the continuous flow process, Reynolds number is defined as

$$Re = \frac{\rho D v}{\mu} \tag{7}$$

Where, v is the average flow velocity, D is the tube diameter, ρ is the fluid density, and μ is the fluid dynamic viscosity. For a flow in a tube, the volumetric flow rate (Q) is defined as

$$Q = vA = V/t \tag{8}$$

Here, V is the collected mixture volume, t is the collecting time, and A is the cross-sectional area of the flow, which is $\pi D^2/4$, where D is the diameter of the tube. Therefore, the equation of Re in terms of Q could be written as

$$Re = \frac{4\rho Q}{\pi \mu D}. \tag{9}$$

For all cases, Reynolds numbers are so small that the flows were in laminar flow region. In addition, the mixing time (extraction time) in the tube or the mean residence time (t_R) of the mixture could be defined as

$$t_R = \left(\frac{\pi D^2}{4}\right)\frac{L}{Q} \tag{10}$$

Where L is the length of the tube. As seen from eqs. (9) and (10), the tube diameter and the total flow rate can influence both Reynolds number and mean residence time but in the opposite ways. Therefore, the effects of tube diameter and total flow rate cannot be separately studied. It was then decided that experiments were done to study these parameters by fixing either Reynolds number or mean residence time and considering the effect of the group of parameters instead. Up to present, there has been only one group studying the flow process in protein extraction of field latex and HANR by using urea together with SDS.[23] An open pipe used in that work was so long that the incubation time is quite long, yielding satisfactory results. However, there were no studies about the effects of process parameters on the extraction efficiency.

The Effect of Mean Residence Time

In this part, Reynolds number of the flow was fixed in order to study the effect of mean residence time. According to eqs. (9) and (10), fixing D and Q can fix Re and t_R can be increased by increasing the tube length. Otherwise, fixing the ratio Q/D and L, and increasing D can also increasing t_R while Re is fixed. Since the mixture viscosities and densities are in close range between 18–20 cP and 0.98–1.10 g/cm^3, respectively, these should not affect Re much.

To perform the first scheme, PEG flow rate was fixed at 7.6 mL/min and that of skim natural rubber latex was fixed at 11 mL/min. The tube diameter of 4 mm was chosen while the tube length was varied to be 45, 67.5, 90, 112.5, and 135 cm. In the second scheme, the ratio between the total flow rate of PEG and skim natural rubber latex to tube diameter was fixed at 46.5 mL/min-cm. The tube length of 45 cm was chosen while the tube diameter was varied to be 0.4, 0.5, 0.6, and 0.7 cm. The results of both schemes are shown in Figures 7 and 8, respectively.

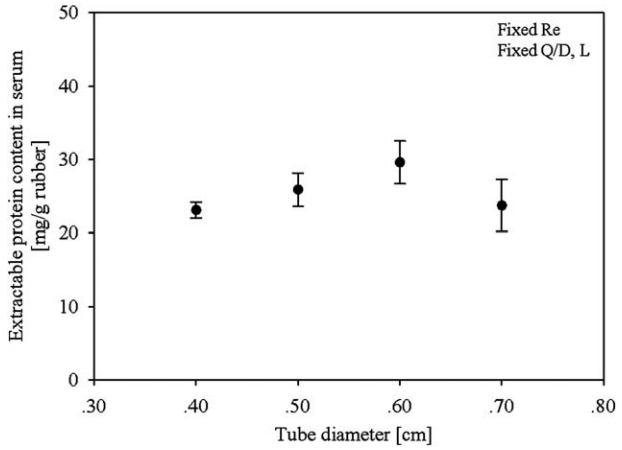

Figure 8. The effect of mean residence time on EP content in serum in continuous flow extraction by fixing Reynolds number in Scheme 2 (keeping Q/D and L constant and varying D).

Figures 7 and 8 show that when mean residence time increased, EP content increased at first since increasing mean residence time means increasing the mixing time for both skim latex and PEG solution to be in contact. However, the EP finally reached a maximum and then dropped as can be seen in both figures. This finding shows that there has to be another factor influencing extraction efficiency. This factor is also related to the degree of mixing which is called the axial dispersion in laminar flow as in our case, where *Re* is in the range of 2–6. Taylor[34] was the first studying this effect in laminar flow in pipe where the combination of axial and radial molecular diffusion, convection along the non-uniform velocity distribution, as well as the gravitational effect leads to axial mixing or residence time distribution, yielding the non-uniform mixing during flow in pipe.[35,36] The effect of axial dispersion may be interpreted from axial dispersion coefficient (K) which is defined[35] as

$$K = \frac{t_R^{0.082} R^{1.836} v^2}{48 D_{AB}^{0.918}} \quad (11)$$

Where R is the tube radius equal to $D/2$, v is the average velocity, t_R is mean residence time, and D_{AB} is diffusivity as described earlier. For both schemes, the product of Rv (or Dv) were maintained, thus, the axial dispersion coefficient increased with mean residence time. This effect will be more pronounced when t_R is large or it could be considered that the effect of mixing time and axial dispersion are competitive. The one that dominates will control the process.

The Effect of Reynolds Number

In this part, mean residence time was fixed in order to study the effect of Reynolds number. According to eqs. (9) and (10), the increase of *Re* could be obtained in two schemes by either keeping the ratio Q/L and D constant and increasing the flow rate (Q) or keeping the product LD^2 and Q constant and increasing the inverse of tube diameter ($1/D$).

In the first scheme, the ratio of total flow rate of PEG and skim natural rubber latex to tube length was fixed at 0.41 mL/min-cm. The tube with a diameter of 4 mm was chosen while total flow rate was varied to be 18.6, 27.7, 37.2, 46.5, 55.8, and 65.1 mL/min. In the second scheme, flow rates of PEG and skim latex were fixed at 7.6 and 11 mL/min, respectively and the product LD^2 was fixed at 7.2 cm^3. The tube diameter was varied to be 0.4, 0.5, 0.6, and 0.7 cm. The results of the first and second schemes are displayed in Figures 9 and 10, respectively.

As seen in Figures 9 and 10 for both schemes, EP content increased with an increase in *Re* at first and then it reached a maximum and dropped. This again is attributable to the axial dispersion effect. In the first scheme, while Q was increased, v had to be increased as well, which resulted in higher degree of axial dispersion as shown in eq. (11). In the second scheme, when keeping LD^2 constant, an increase of $1/D$ resulted in an increase in L, implying an increase in the average velocity since the mean residence time was fixed so the axial dispersion coefficient was increased. This clearly shows that the axial dispersion could dominate the effect of *Re* for the case of laminar flow where degree of turbulence is usually not important.[35] The evidence of competition between the effect of *Re* and the effect of axial dispersion was also reported in the study of mass transfer in the flow reactor containing some foams, where the results were scattered when varying *Re*,[37] and in that of the mass transport in the oscillatory flow electrochemical reactor, where some sets of results showed increasing trend of mass transfer coefficient when *Re* increased and some showed uncorrelated data.[38]

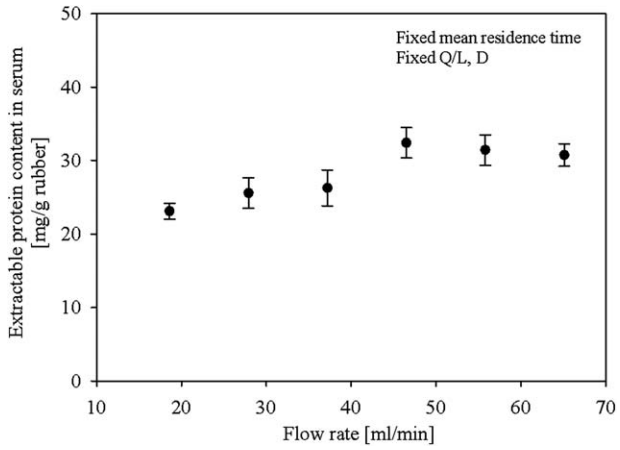

Figure 9. The effect of Reynolds number on EP content in serum in continuous flow extraction by fixing mean residence time in Scheme 1 (keeping Q/L and D constant and varying Q).

Comparison Between Centrifugal and Continuous Flow Extractions

It is interesting to compare the extraction efficiencies of both systems. The percentage of an increase of EP content (mg proteins/g rubber) in serum can be calculated by eq. (12), which is defined as the difference of EP contents in serum before and after extraction compared with initial EP content before extraction.

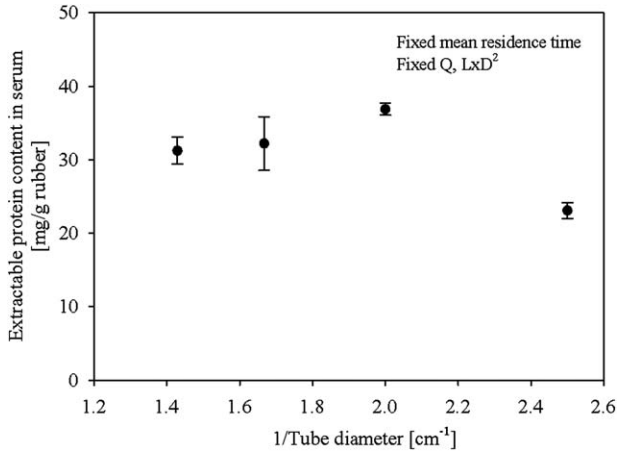

Figure 10. The effect of Reynolds number on EP content in serum in continuous flow extraction by fixing mean residence time in Scheme 2 (keeping LD^2 and Q constant and varying $1/D$).

Table I. The Comparison of the Increase of EP in Serum Between Extractions in Centrifugation and Continuous Processes

Extraction process	Extraction time (min)	EP content in serum (mg/g rubber)	% increase of EP content in serum	% increase of EP/g PEG
No extraction	-	30.59	-	-
Centrifugal extraction (30 mL latex, 30 mL PEG sol., speed = 3000 rpm)	5.00	46.57	52.2	29.0
Continuous Flow (Latex flow rate =7.5 mL/min, PEG flow rate = 7.6 mL/min, L = 45 cm, D = 0.5 cm.)	4.83	39.12	27.9	15.5
Continuous Flow (Latex flow rate = 11 mL/min, PEG flow rate = 7.6 mL/min, L = 90 cm, D = 0.5 cm)	4.06	40.92	33.8	23.0

$$\%\ \text{increase of EP} = \frac{\text{EP}_{\text{after}} - \text{EP}_{\text{initial}}}{\text{EP}_{\text{initial}}} \times 100 \quad (12)$$

In addition, if % increase of EP content is divided by PEG weight, the efficiency of PEG as the extracting agent can be expressed in eq. (13).

$$\text{Efficiency of extracting agent} = \frac{\%\ \text{increasing of EP}}{\text{weight of extracting agent}} \quad (13)$$

The percentage of increase of EP content and the efficiency of extracting agent are shown in Table I. To compare both extraction processes, the ratio of rubber amount to PEG amount was prepared almost the same. In the centrifugal extraction, 30 mL of latex was mixed with 30 mL of PEG solution while the flow rates of latex and PEG solution were 7.5 and 7.6 mL/min, respectively. In Table I, it was seen that % increase of EP content and the efficiency of PEG by using centrifugal extraction at 2000 rpm was 52.2 and 29.0% per gram of PEG, respectively, which were better than those in continuous flow extraction.

However, % increase of EP content by the flow process could be boosted by adjusting parameters as also shown in Table I. The best result of the flow system was that the increase of EP content and efficiency of PEG were 33.8 and 23.0% per gram of PEG, respectively. Considering this efficiency and processing time required by flow extraction, the continuous flow system is a promising method in deproteinized rubber production.

CONCLUSIONS

The study of protein extraction in skim natural rubber latex by using PEG solution in low speed centrifugations and continuous flow systems was performed and the results were compared. It was found that smaller PEG molecules were more efficient, which should be dependent on the system condition and that increasing PEG concentration could increase EP content in serum. In addition, EP content increased with centrifugal speed and time when the speed was quite low. However, at a higher speed and a longer time, the opposite was seen. When the rubber chips from this process were leached with 2 %w/v SDS solution in a centrifuge, the trend of EP content in the leachate was similar to that in serum, implying that PEG plays an important role in protein extraction both in the latex and from the chips. In the continuous flow extraction, when increasing Reynolds number or mean residence time, EP content reached the maximum and then dropped. These results reflect the effect of axial dispersion or residence time distribution which needs to be investigated in detail. The extraction efficiencies expressed in % increase of EP per gram PEG of centrifugal and flow processes were comparable, showing the potential application of flow processes.

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