



EXTRACTION OF PROTEIN FROM SKIM NATURAL RUBBER LATEX BY CONTINUOUS FLOW

Jirarat Srisatjang, Duangkamol Danwanichakul, Panu Danwanichakul*

Center of Excellence on Natural Rubber Technology, Department of Chemical Engineering,
Faculty of Engineering, Thammasat University, Rangsit Campus, Pathumthani 12120,
Thailand

*e-mail: dpanu@engr.tu.ac.th

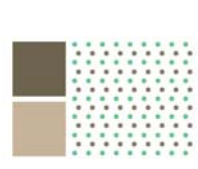
Abstract

The protein extraction from skim rubber particles in continuous flow using polyethylene glycol solution at a concentration of 6% w/v PEG with an average molecular weight of 6000 (PEG6000) was studied. The flow rate of skim natural rubber was varied to be 7.5, 11 and 14 ml/min at a constant PEG flow rate of 7.6 ml/min and vice versa. The extractable protein content was determined by Bradford assay. The result revealed that when using a tube with a diameter of 4 mm, the extractable protein content in serum was increased with increasing the skim latex flow rate. When increasing the PEG solution, the flow rate with the concentration of, the extractable protein content was decreased. In addition, increasing tube diameter from 4 mm to 5 mm and increasing the tube length from 45 cm to 90 cm could increase the extractable protein content in the flow extraction. The key factors influencing the efficiency of the extraction process were degree of turbulence and the residence time (contact time between rubber latex and extracting medium in the tube) of the solution. These two factors are dependent on the flow rate, the tube diameter and the tube length.

Keywords: protein extraction, natural rubber latex, continuous flow, polyethylene glycol, surfactant

Introduction

Natural rubber latex (NRL) has been used commercially for more than a century. It is composed of about 36% (w/v) of rubber fraction, 5% (w/v) of non-rubber components such as proteins, lipids and sugar, and 59% (w/v) of water (Bonner and Galston 1947). Field rubber latex is centrifuged to produce concentrated latex with 60% of rubber and skim rubber latex with 5-8% of rubber (George et al. 2009). Small factory may consider skim rubber as a waste while manufacturers use highly concentrate sulfuric acid to recover rubber from skim rubber latex. The natural rubber products, such as gloves, medical gloves and condoms can cause protein allergy in some people due to the remaining proteins in the natural rubber latex products. Several methods are used to reduce extractable proteins in the natural rubber latex or natural rubber film. Protein extraction from field natural rubber latex by using ^{60}Co with irradiation dose of 10 to 50 Gy/h was studied. The extractable protein (EP) could be obtained by Micro BSA Protein assay and the result showed that EP in serum increased from 8 mg/ml to 10 mg/ml (Rogerio et al. 2003). Using water-soluble polymer to extract protein from high ammonia concentrated natural rubber latex (HANR) was reported. The extractable protein could be reduced from 0.409 mg/g to 0.219 mg/g when measured by Modified Lowry method (Parra et al. 2005). The extractable protein measured by Modified Lowry method could be



reduced from 68 $\mu\text{g/g}$ to 10 $\mu\text{g/g}$ when using potassium hydroxide (KOH) solution (Maznah et al. 2008) while the extractable protein could be reduced from 1600 $\mu\text{g/g}$ to 200 $\mu\text{g/g}$ by treating with hydrochloric acid (HCl) solution (Maznah et al. 2008). Using sodium dodecyl sulfate (SDS) solution could reduce protein content in natural rubber chips more effectively than using phosphate buffered saline (PBS) solution (Kalapat et al. 2009).

In general, extraction of proteins could be operated either in batch or continuous flow. The batch extraction is popular and easy to operate by using the same centrifuge as in the process of concentrate rubber production. However, extraction in flow may reduce production cost and could be operated continuously with lower consumption of energy. Polyethylene glycol (PEG) was investigated as a polymer for protein extraction in the batch extraction (Abhilash et al. 2009) of concentrated latex. Moreover, using urea together with SDS for protein extraction from fresh rubber latex and HANR both in batch and continuous flow and measured protein content by Kjeldahl method was investigated (Yamamoto et al. 2008). In this study, we are interested in removing proteins from skim rubber latex to produce deproteinized rubber by studying many factors in the continuous flow and to determine extractable protein content by using Bradford micro-assay.

Methodology

Materials

Skim rubber latex was obtained from Rubber Estate Organization with a total solid content of 7.67%, a dry rubber content of 4.229%, initial protein content in serum of 0.66 mg/ml, total protein content on the surface of the rubber chip of 11.17 mg/g rubber and a pH value of 9.03. Polyethylene glycol with M.W. 6000 (PEG-6000) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co.LLC. (Germany). Acetic acid was purchased from Merck. Sodium dodecyl sulfate (SDS) was purchased from Ajax Finechem Pty Ltd. Triton X-100 and Toluene were purchased from Panreac Quimica S.L.U. Dye reagent concentrate was purchased from Bio-Rad.

Protein Extraction and Coagulation Skim Rubber Latex by Continuous Flow

In the study of the effect of protein extraction by using continuous flow, PEG with M.W. of 6000 was chosen as an extraction agent. The experiments were divided into two parts. In the first part, the flow rate of natural rubber latex was fixed at 7.5 ml/min while the flow rate of PEG was varied to be 7.6, 11 and 14 ml/min. In another experiment, the flow rate of polyethylene glycol was fixed at 7.6 ml/min while the flow rate of natural rubber latex was varied to be 7.6, 11 and 14 ml/min. The diameters 4 and 5 mm were chosen while the tube length was varied to be 45 and 90 cm. The mixture of skim natural rubber latex and PEG solution with concentration of 6%w/v was collected at the end of the tube until the volume total reached 60 ml and collecting time was also recorded. In the coagulation of skim rubber latex, the mixture were stirred at 150 rpm for 9 min, and then 12 ml of 2%w/w acetic acid solution were added and stirred at 150 rpm for 12 min. Finally, the coagulated skim rubber was filtered to separate serum from skim natural rubber (skim NR). The extracted skim NR was then dried in an oven at 60°C for 24 hr before extracting proteins again with different extracting mediums including 2%w/v sodium dodecyl sulfate (SDS), 2%w/v Triton X-100 and 2%w/v NaOH solutions. The serum and extracting mediums were then checked for the extractable protein content immediately. The results are the average from three samples.

Extraction of Dry Skim Rubber with different extracting mediums

In the study of the effect of extracting medium on extractable protein content from rubber film, 2% w/v SDS, 2% w/v Triton X-100 and 2% w/v NaOH solutions were chosen and compared with pure water. Initially, the dry skim NR was dissolved in toluene and then the solution was cast into a film on a glass plate and then dried at room temperature for 2 days. The thickness of the cast films was 0.34 ± 0.05 mm. To extract proteins from the film, the natural rubber films were cut into 0.5×0.5 cm² pieces, each of which was put in centrifuge tube and 2 ml of each extracting medium was added and centrifuged at 1000 rpm for 5 min. The protein content in each extracting medium was measured by diluting 10 μ l of extracting medium with 1990 μ l of distilled water. Extractable protein content (EP) can be calculated according to equation (1)

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

Determination of Extractable Protein Content by Bradford Micro-Assay

Determination of water-soluble protein content was done by Bradford micro-assay as the same to Kalapat et al. (2009). BSA was used as a standard protein. In this method, the color of protein solution was converted into blue after adding a Bio-Rad dye (reagent). The calibration curve of BSA was prepared from the BSA solution with the concentration of 1.25, 2.5, 5, 7.5 and 10 μ g/ml. UV-VIS spectrophotometer was applied at a wavelength of 595 nm for measuring the protein content. The amount of protein can be determined by comparison with the calibration curve of the standard BSA.

Results

The effect of the various flow rates of skim latex and PEG solution.

In this experiment, a tube, which is 45 cm long and 5 mm in diameter, was used. The plots of flow rates of the skim natural rubber latex and the PEG solution versus extractable protein content in serum are shown in Figure 1. Considering the extractable protein content in serum, it was found that when the flow rate of skim natural rubber latex equaled to 7.5 ml/min and the flow rate of PEG equaled to 7.6 ml/min, the extractable protein content in serum was higher than that obtained for the un-extracted skim natural rubber latex (blank sample). While increasing the flow rate of skim natural rubber latex from 7.5 ml/min to 11 ml/min with the PEG flow rate of 7.6 ml/min, it was found that the extractable protein content in serum was increased and higher than that for the un-extracted one (blank sample) because with a higher flow rate of the skim natural rubber latex, a larger total amount of rubber in the mixture was obtained, resulting in more extractable protein content. However, when the flow rate was increased from 11 ml/min to 14 ml/min, the extractable protein content was decreased due to the lower ratio of the PEG solution to the natural rubber latex and the shorter extraction period as shown in Table 1. On the other hand, when increasing the flow rate of PEG solution and the flow rate of skim natural rubber latex was fixed at 7.5 ml/min, it was found that the extractable protein content in serum tended to decrease due to higher viscosity of the extracting medium in the system. Since PEG solution has higher viscosity than skim natural rubber latex, when the ratio of PEG solution to skim natural rubber latex flow rates increased, the degree of turbulence of extraction process will be decreased. Therefore, extractable protein content tends to decrease.

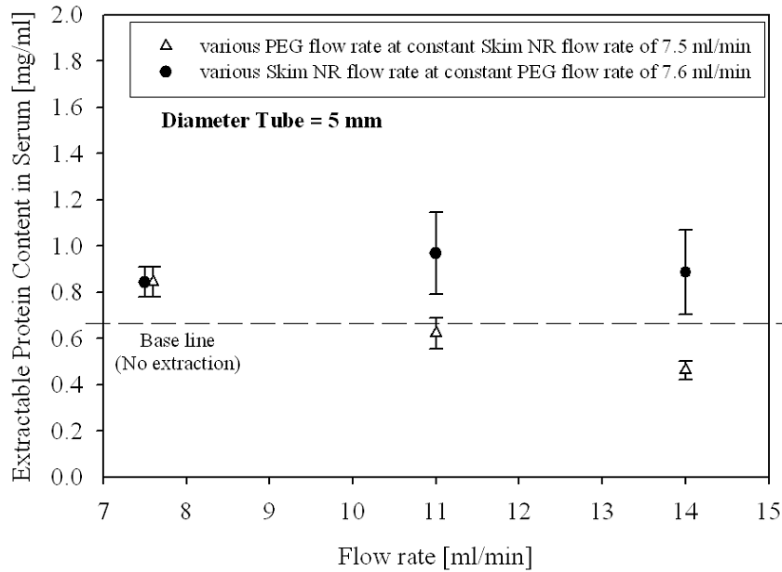


Figure 1 Flow rate of skim natural rubber latex and PEG vs. extractable protein content in serum (mg/ml)

Table 1 The Time taken for each flow rate of Skim NR latex and PEG to obtain the total volume of solution equal to 60 ml when using the tube with a diameter of 5 mm

Flow rate of skim natural rubber latex (ml/min)	Flow rate of PEG (ml/min)	Time (sec)
7.5	7.6	255
11	7.6	215
14	7.6	183

The effect of tube diameter.

It is important for the extraction process that good contact between extracting polymer solution and natural rubber latex could increase the efficiency of extraction. Increasing the degree of turbulence would obtain better contact between those for a flow in a circular tube. The degree of turbulence could be explained via the Reynolds number, which is defined as

$$Re = \frac{\rho D v}{\mu} \quad (2)$$

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

$$Q = v A = V / t \quad (3)$$

Where v is the velocity, A is the cross-sectional area of the flow ($A = \frac{\pi D^2}{4}$), V is the volume

of the solution and t is the time. Therefore, $Re = \frac{4 \rho Q}{\pi \mu D}$. For all cases, the Reynolds numbers

are small so all the flows are in laminar flow region. In addition, the contact time or residence time of the mixture flowing in a tube could be defined as

$$t = \left(\frac{\pi D^2}{4} \right) \frac{L}{Q} \quad (4)$$

Where Q is the flow rate, t is contact time or residence time in tube, D is the diameter of the tube and L is the length of the tube

In this experiment, the flow rate of 6% w/v PEG6000 solution was fixed at 7.6 ml/min and a tube length was fixed at 45 cm while the diameter was varied to be 4 and 5 mm and the flow rates of skim natural rubber latex were varied to be 7.5, 11 and 14 ml/min.

As can be seen in Figure 2, when increasing the tube diameter from 4 to 5 mm, the extractable protein content in serum was higher because increasing tube diameter would increase the contact time (Eq. (4)) but decrease the Re (Eq. (3)). It seemed that contact time in this case played a more important role in extraction than the Re in this low range. However, the competing effect would be less when the flow rate of the rubber was higher. When the tube diameter was fixed, increasing the flow rate seemed to increase the extraction efficiency due to the increase in degree of turbulence while the contact time decreased. This again shows the competitive effect between degree of turbulence and contact time. It is coupling and need to investigate in more detail.

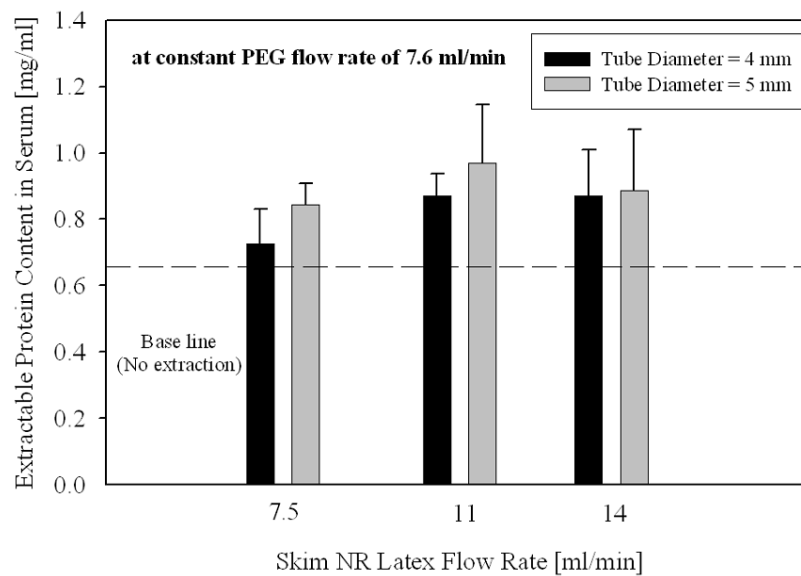


Figure 2 The flow rate of PEG (ml/min) vs. extractable protein content in serum (mg/ml)

The effect tube length.

The experiment was done using a tube with a diameter of 5 mm, the flow rate of skim natural rubber latex of 11 ml/min, and the flow rate of PEG solution of 7.6 ml/min. The tube length was varied to be 45 and 90 cm and the results are compared in Figure 3. The extractable protein in serum when using the tube length of 90 cm was higher than that when using the tube length of 45 cm due to longer contact time in extraction process.

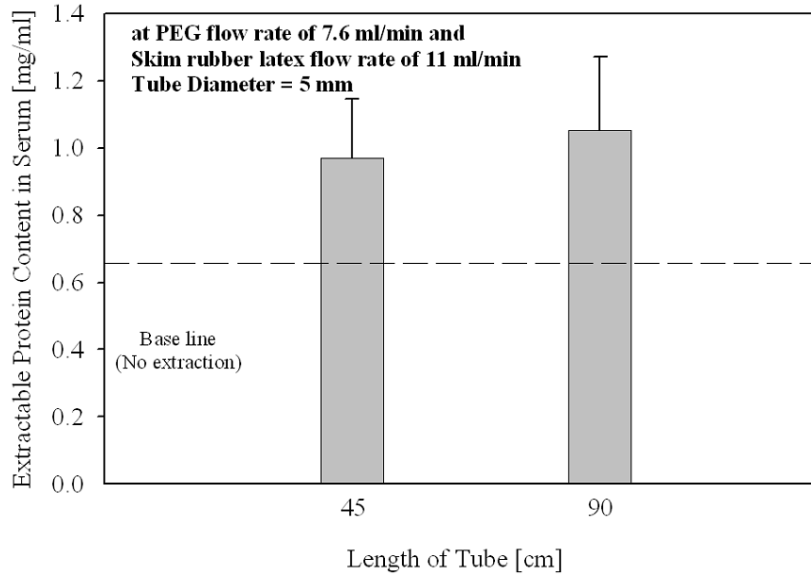


Figure 3 Length of tube (cm) vs. extractable protein content in Serum (mg/ml)

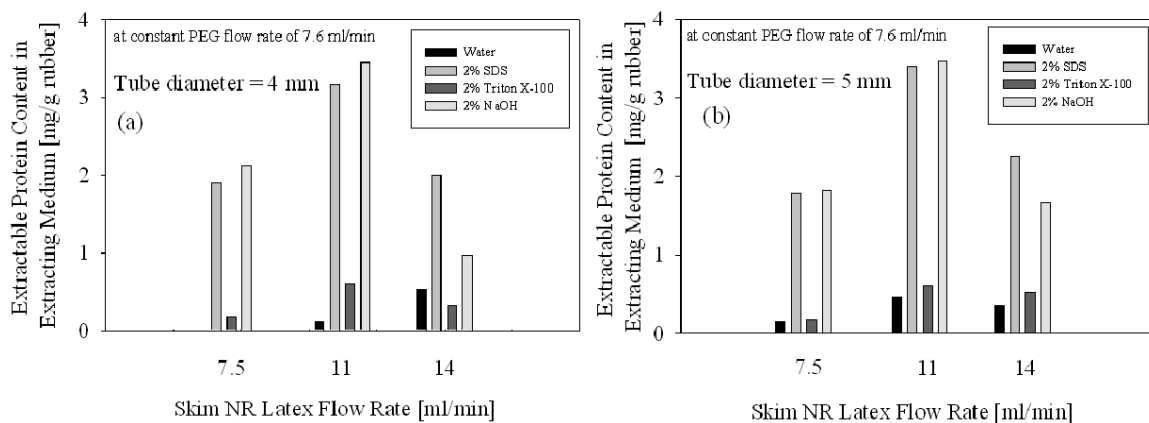


Figure 4 Length of tube (cm) vs. extractable protein content in extracting mediums (mg/g rubber)

Extraction of skim rubber film with different extracting mediums.

The experiment was done with various extracting mediums including water, surfactant solution and a basic solution. Figure 4 shows the results of the rubber samples obtained from experiments using a tube length of 4 mm and 5 mm while the flow rates of PEG solution was set at 7.6 and the flow rate of skim rubber was varied to be 7.5, 11, and 14 ml/min, respectively. It could be seen that SDS solution could extract proteins from the film as well as NaOH solution, which is better than Triton X-100 solution and distilled water, respectively. The overall trend showed that the sample from the experiment using the flow rate of skim rubber latex of 11 ml/min had higher extractable protein content than that of 7.5 ml/min and 14 ml/min. It seemed that there was the optimum condition for extracting protein from the rubber particles in order to be easily extracted further from the rubber film.



Discussion and Conclusions

To our knowledge, there is only one work (Yamamoto et al. 2008) involving the flow of fresh latex together with urea and SDS solution in a very long open pipe. They only varied the concentration of urea and found that denaturation with urea could reduce protein content in rubber efficiently. However, there has been no work studying the factors related with the flow including tube diameter, tube length and the flow rate. The flow rate of skim latex and that of 6%wt PEG solution were ranged between 7.5 and 14 ml/min. It was observed that the viscosity of PEG solution prevented the good mixing when increasing the ratio of polymer to latex phases. On the contrary, increasing the ratio of latex to polymer phases could increase the degree of turbulence in mixing, thereby, increasing the extraction efficiency. For the range of the flow rate studied, increasing the tube diameter from 4 mm to 5 mm could increase the extraction efficiency due to higher degree of turbulence. However, the results showed the competition between an increase in the degree of turbulence and an increase in contact time. Since an acid is used in coagulation of rubber from the latex phase, the extractable proteins may denatured and it may not be worth recovering from the serum. In addition, it seemed that the rubber films obtained with conditions that were good for extracting proteins in latex phase could yield more protein content in extracting medium when extracting with SDS solution.

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