EXTRACTABLE PROTEINS FROM ELECTRON BEAM (EB) IRRADIATED NATURAL RUBBER (NR) LATEX

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ABSTRACT

The protein assay of natural rubber latex (NRL) vulcanized by low energy electron beam (EB, 300 keV, 30 mA) has been carried out using Bicinchoninic acid (BCA) reagent. Extractable protein in irradiated latex film was determined by measuring the absorption of colored solution at 562 nm using UV spectrometer. The effect of various radiation doses on the extractable protein content of NRL was investigated. It was found that the quantity of extractable protein increases with radiation dose. When compared with gamma-ray irradiated samples the same trend was observed. EB irradiated latex films are leached in 1% ammonia water for various lengths of time. From the results it was established that within 2 hours of leaching in ammonia water most of the extractable protein (96%) were removed from rubber film.

INTRODUCTION

Radiation vulcanization of natural rubber latex is an alternative to conventional sulfur vulcanization for the production of goods being in contact with the human body. Vulcanization by gamma radiation does not need chemicals such as dithiocarbamates, thiozoles, amines and thurems and therefore should be a suitable basic substance for nitrosamine free products. Recently low energy electron beam has been successfully tried to induce vulcanization of NRL (Makuuchi 1995; Makuuchi et al. 1995). The use of low energy electron beam is more economical compared to high energy electron beam or gamma rays as because bioshielding is not necessary. Moreover during vulcanization by gamma rays monomer n-butyl acrylate (n-BA) is used as a radiation sensitizer, but electron beam does not need n-butyl acrylate to initiate the reaction of radiation crosslinking.

Fresh natural rubber latex contains proteins. The composition of these proteins changes on processing the fresh latex to high ammoniated latex concentrate and also on vulcanization of high ammonia latex concentrate. Natural rubber products contain two kinds of proteins - strongly bound to the rubber particles in the latex and soluble serum derived protein. The latter generally gives rise to most of the extractable protein. Allergy to latex containing articles is becoming more important because it can result in unexpected life-threatening anaphylactic reactions in sensitized individuals. Serum of latex contains proteins, most of them are in the acidic zone of the pH gradient, display the ability to bind human IgE indicating their allergenic character (Pendle 1993; Palosuo 1993; Soili 1993).

The present study investigates the extractable protein content of latex films with various radiation doses. The effects of various leaching agents and extraction time on extractable protein content has been studied.

PROCEDURE

To vulcanize natural rubber latex by radiation 50% concentrated latex was prepared by diluting with 1% ammonia water. Potassium hydroxide at a quantity of 0.02% was added to stabilize the latex .

Electron beam irradiation of the latex was carried out at beam current 30 mA using self-shielded accelerator model 330-100-60 of Nissan High voltage company. During irradiation the latex was constantly stirred (150 r.p.m.) to ensure homogeneous irradiation of the latex. Defoamer BYK-022 at 0.24% was added to latex to destroy the ability of latex to form foam on the surface of latex when it was stirred.

Natural rubber latex was irradiated by gamma radiation at doses 10, 15, 20, 30 kGy using 5 phr n-BA as a sensitizer. The irradiated latex was cast on glass plate and air dried till transparent and then heated at 80° C for 1 hour.

Extractable protein in natural rubber was determined using Bicinchoninic acid (BCA) assay method (Yagami et al. 1993). Two grams of latex specimen were extracted with 10 mL portions of phosphate buffered saline pH 7.4 (PBS) at room temperature for two hours and then the resulting solution was assayed by BCA method. The BCA method utilize the color change resulting from the strong complex formed between Cu^{2+} and BCA. PBS solution containing rubber protein was reacted with BCA working reagent using a standard protocol (Smith et al. 1985). The assay was conducted by adding 250 µL aliquot of the sample to 5mL of the BCA protein working reagent and incubating the resulting solution at 37°C for 1 hour. After the sample had been cooled to room temperature, its absorbence at 562 nm was determined using Shimadzu UV-visible spectrometer. A standard curve was obtained using concentrations of BSA (bovine serum albumin) from 0.2 to 1.0 mg/mL in aqueous solution against water blank.

RESULTS AND DISCUSSION

Electron beam irradiation of latex shows that the increase of irradiation time gives the increase in extractable protein content (Fig.1). An increase of 213% was observed when the length of radiation time was 35 min with beam current 30 mA. Proteins in natural rubber latex can be decomposed by radiation which gives rise to increase the amount of extractable protein in irradiated latex film.

Results of irradiated latex film prepared by gamma radiation are shown in Fig.2. The latex is irradiated at radiation doses 10, 15, 20 and 30 kGy and for the latex films extractable protein contents are 2.06, 2.10, 2.57 and 2.94 mg/g respectively. From the results it can be seen that with the increase of radiation dose extractable protein content also increases. This may also be due to degradation of proteins in natural rubber latex by gamma radiation to water soluble form.

For radiation vulcanized latex film leaching is a important step (Feroza et al. 1995; Shukri et al. 1990) which gives rise to increase tensile properties. It also improves the appearance of product. Natural rubber field latex contains less than 5% protein (Feroza et al, 1995). Washing removes mainly water soluble proteins. Fig. 3 shows the results of leaching of EB irradiated latex film in 1% ammonia solution at various lengths of leaching time. From the figure it is evident that most of the soluble protein (96%) is removed from latex film within 2 hours of leaching.

As the quantity of extractable protein and also the nature of soluble proteins is not unique, so to get reproducible result is very difficult. Latex films, irradiated by gamma rays are leached with water and 1% ammonia solution (Fig. 4 & 5). The variability of results of extractable protein content indicates that 1% ammonia solution can extracts more protein from the film than water.

Alcohol is used as leaching agent and it effects on tensile properties of latex (Feroza Akhtar, et al, 1995, Wahab S.B.H.A et al, 1990). As a leaching agent four concentrations of ethanol were tried and it was observed that extractable protein content was higher when alcohol concentration was low (Fig.6). Dilute alcohol solutions can remove more protein than concentrated alcohol solution. The results indicate that at a extraction period of 24 hours 25% ethanol can remove as much protein as with 1% ammonia solution at extraction period of 1 hour. Extractable protein content of latex films after alcohol and ammonia treatments is shown in Table 1 where methanol, ethanol and i-propanol are used as leaching gents. The electron beam irradiated cast films are leached in each alcohol for 1 and 24 hours. Then immediately these films are subjected to ammonia leaching for 24 hours. Results in Table 2 shows that values of extractable protein always higher (16%) when leached with alcohol for 24 hours.

Table 1: Extractable protein content of latex film after alcohol and ammonia treatment of radiation vulcanized natural rubber latex film.

Sl.no.	Treatment condition	Protein content, (mg/g)
1.	Control (not treated)	2.104
2.	24 hrs in 1% ammonia water	0.0483
3.	1hr. in methanol followed by 24 hrs in 1% ammonia water	0.0543
4.	24hrs. in methanol followed by 24 hrs. in 1% ammonia water.	0.0632
5.	1 hr in ethanol followed by 24 hrs. in ammonia water.	0.050
6..	24 hours in ethanol followed by 24 hours in 1% ammonia water	0.0576
7.	1 hr in i-propanol followed by 24 hrs. in ammonia water.	0.0497
8.	24 hours in i-propanol followed by 24 hours in 1% ammonia water.	0.0588

Table 2: Extractable protein content of latex film before and after irradiation

	Before processing		Processed by		
	Without n-BA	With n-BA	Electron beam	Gamma ray	Conventional
Extractable protein (mg/g)	2.104	1.632	2.807	2.104	1.562

For irradiation of latex by gamma rays n-BA was added to latex as a sensitizer to reduce the optimum radiation dose of vulcanization. Electron beam vulcanization of natural rubber latex does not need sensitizer. Table 2 shows the results of cast films prepared from latexes before and after radiation by gamma rays, electron beam and conventional sulfur vulcanized latex film. Optimum radiation dose for gamma ray was taken 20 kGy and that of electron beam (EB) was 15 minutes of exposure time of 300 keV (low energy) accelerator with beam current 30 mA. From the results stated in Table 2 it can be seen that before irradiation cast latex films without and with n-butyl acrylate contain different quantities of extractable protein and they are 2.104 and 1.632 mg/g. respectively. The addition of n-BA suppresses the value of extractable protein content. After irradiation the electron beam irradiated cast latex film has higher protein content than gamma ray irradiated latex film and for sulfur vulcanized latex film the value is yet less.

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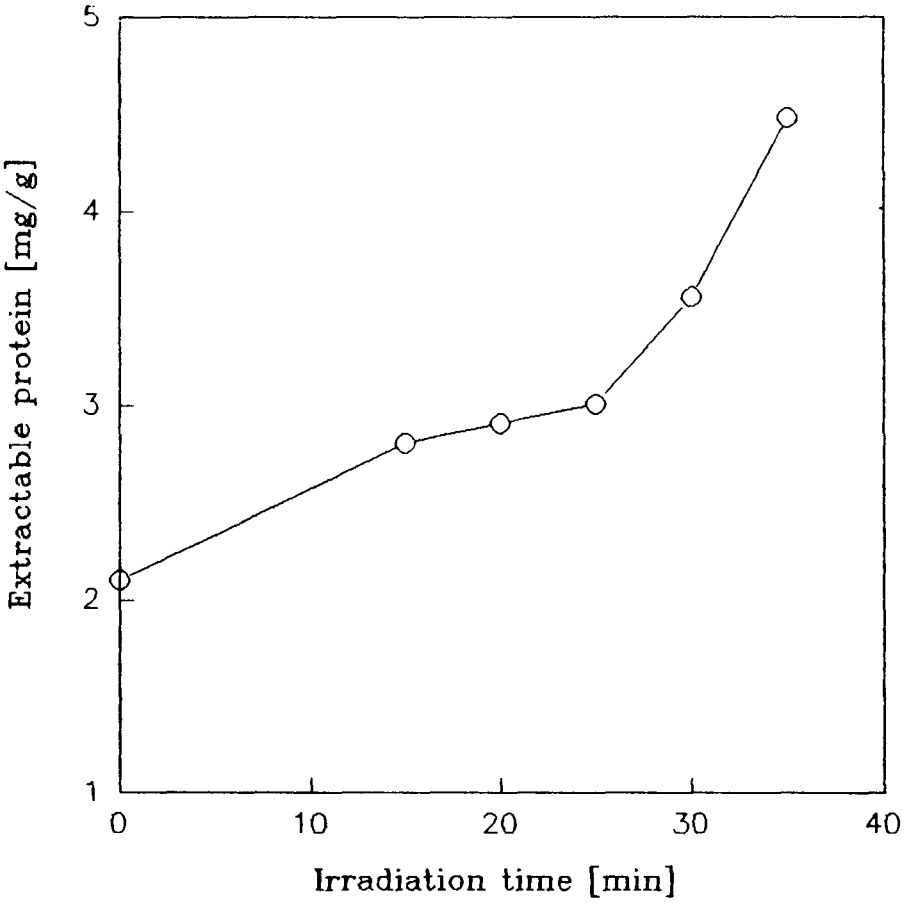

Fig. 1. Protein content of latex irradiated for various lengths of time.
(Electron energy= 300 keV, beam current= 30 mA)

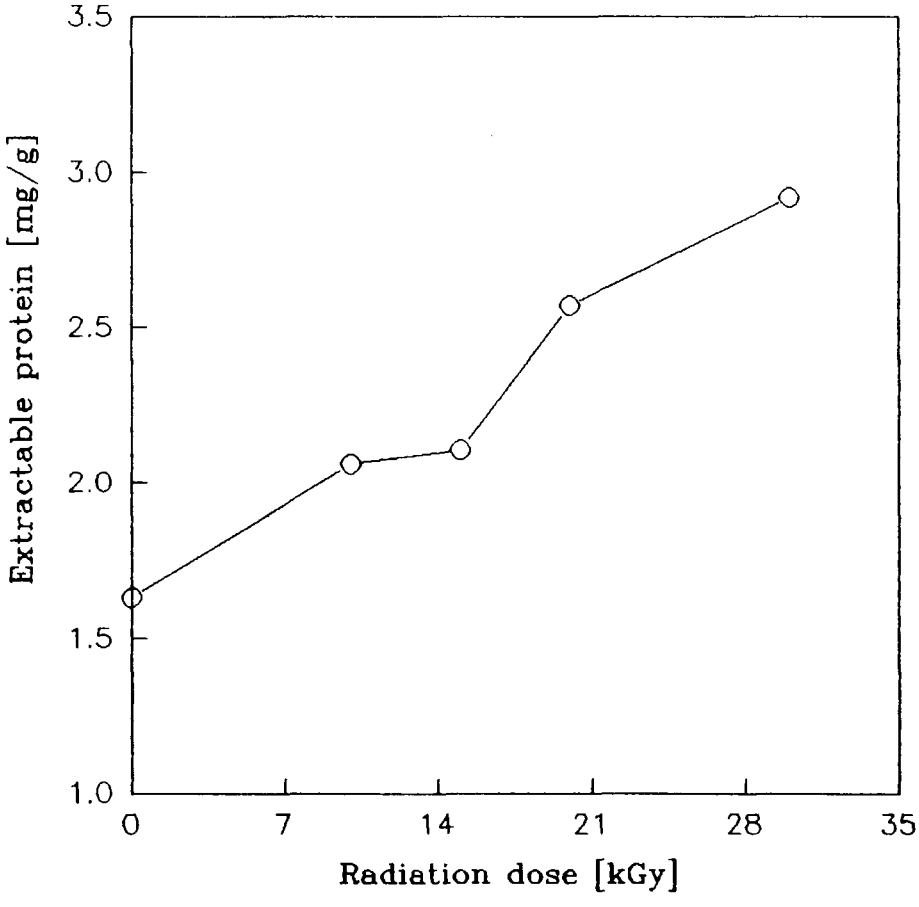

Fig. 2. Extractable protein content in latex film at various gamma radiation doses.

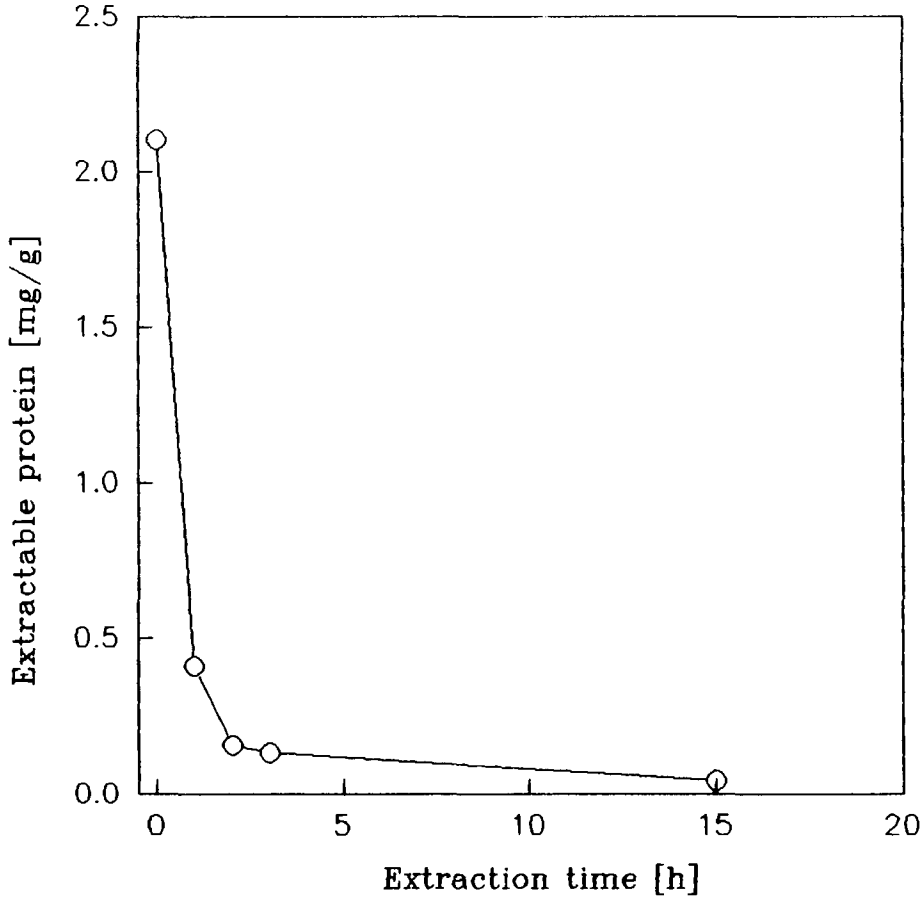

Fig. 3. Extractable protein content of EB irradiated latex film leached in 1% aqueous ammonia for various lengths of time.

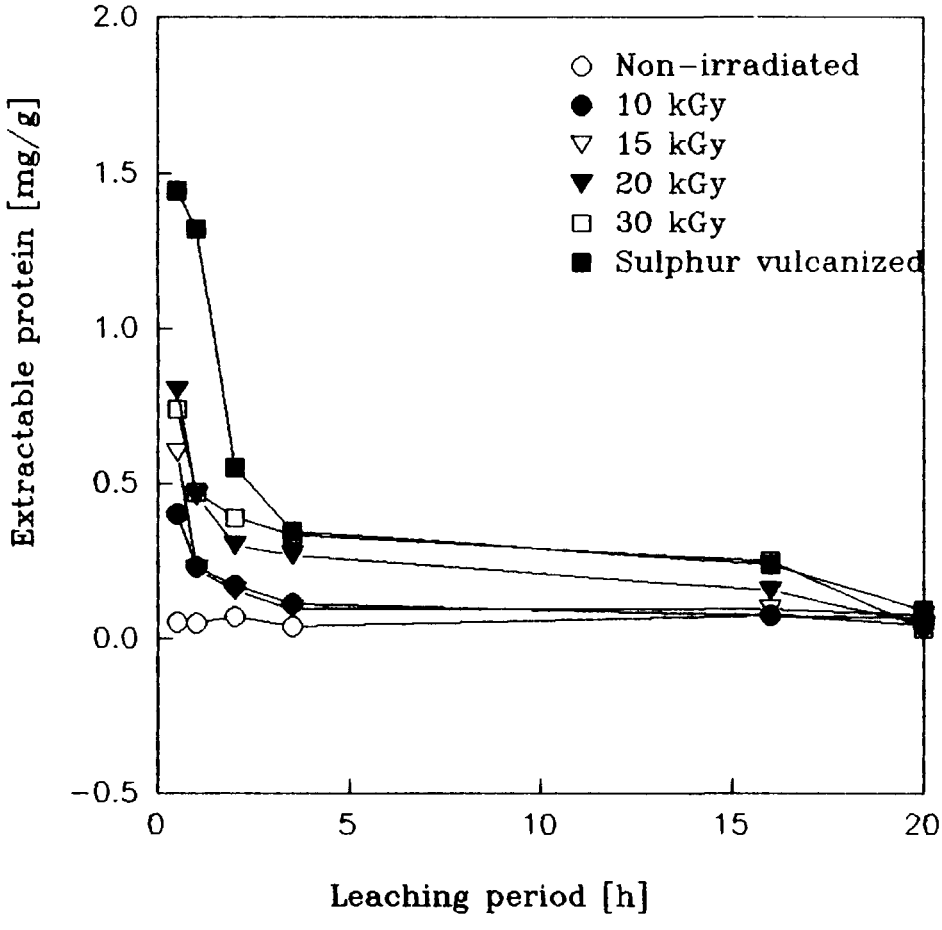

Fig. 4. Extractable protein content of latex films at various lengths of leaching time in water.

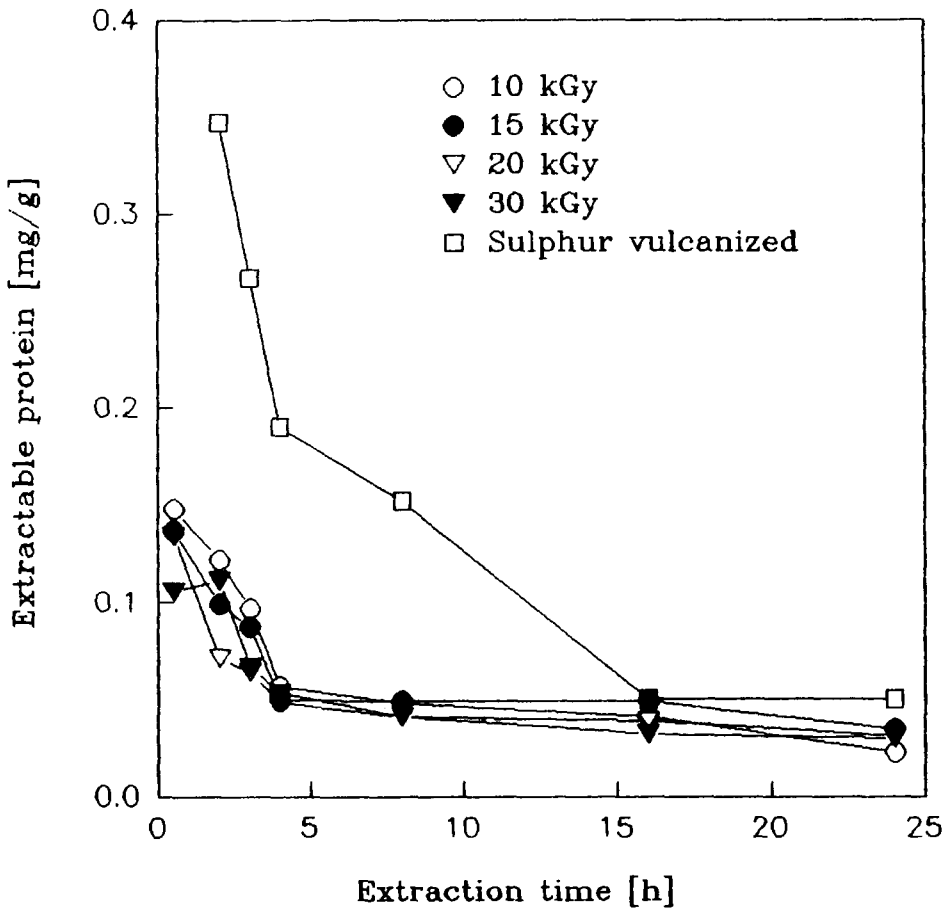

Fig. 5. Extractable protein content of latex films leached with 1% aqueous ammonia containing 50 ppm $CaCO_3$.

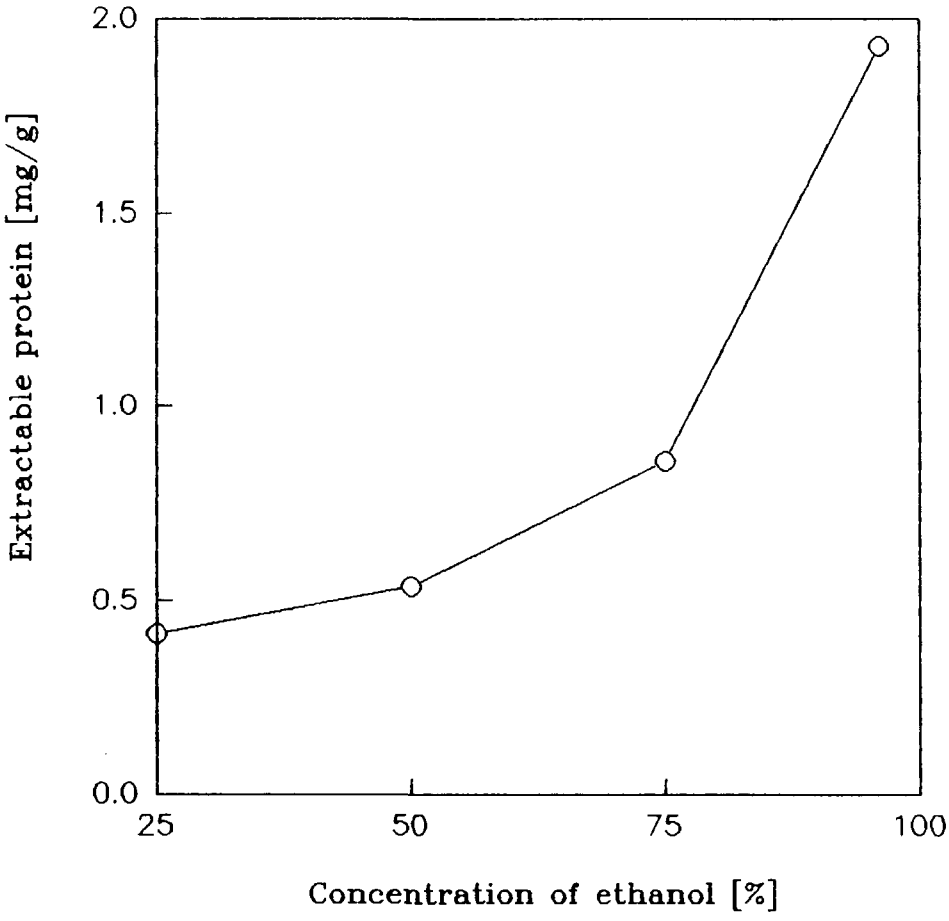

Fig. 6. Extractable protein content of electron beam irradiated latex film at various concentrations of ethanol.

QUESTIONS & ANSWERS, and COMMENTS

Session II

C1.	Machi	It would be useful to determine the effects of irradiation upon the molecular weight of latex proteins.
	Geertsma	Differences in the composition of latex proteins before and after irradiation of latex were not observed.
Q2	Geertsma	Has Dr. Bez carried out similar studies using SPVL?
A2	**Bez**	**Not yet.**
C3		Protein issues seem to create more problems for RVNRL.
	J. Anand	The leaching process will determine the extractable proteins of RVNRL films.
	Geertsma	The advantage of RVNRL in Type IV allergy is more significant than the protein problem in RVNRL.
C4	Thomas	The specification for tensile strength of RVNRL film should be at least 20 MPa.
	Bez	We reduced the specifications for RVNRL in order to avoid problems of selling the products.
	Desai	The ASTM specification for tensile strength of examination gloves has been lowered to 14 MPa now.