EFFECT OF LEACHING NR LATEX FILM IN ENZYME SOLUTION FOR REDUCING EXTRACTABLE PROTEIN CONTENT

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CONTENTS

EXECUTIVE SUMMARY

CHAPTER 1 gives an account of the composition , preservation and properties of Natural Rubber (NR) latex ,preparation of latex compounds and manufacture of latex products by dipping methods . Type 1 allergic reactions such as contact urticaria and anaphylaxis associated with NR latex dipped medical products are reported to be due to the extractable protein (E P) content present in NR latex . This has necessitated the production of medical gloves with low EP content . The EP content can be reduced by the use of low protein latex , proper leaching , chlorination of products etc . Production of low protein latex by enzyme treatment using Anilozyme -P has been reported . But a process for producing low protein latex products by enzymatic leaching using Anilozyme - P has not yet been reported . Hence this work was undertaken with a view to studing the effect of wet gel and post cure leaching of latex film in aqueous Anilozyme - P solutions for reducing EP content .

CHAPTER 11 describes the raw materials and experimental procedure , used in this study . The following experiments were conducted to study the effect of leaching of latex films in Anilozyme solution for reducing E P content .

1) Wet gel leaching in Anilozyme - P solution : The effect of following factors on E P content were to be studied

a) Concentration of Anilozyme Solution

b)Time of Leaching

c)Temperature of Leaching

d) pH of Anilozyme Solutions

e) Addition of Ca^{++} ions in Anilozyme Solutions

2) Post cure leaching of NR latex films in Anilozyme - Solutions : The effect of the following factors on EP content were to be studied

a) Concentration of Anilozyme Solution

b) Temperature

3) Effect of Anilozyme - P solution for leaching of NR latex films on technological properties

CHAPTER 111 deals with ‘ Results and Discussion ’ part of this project and chapter 1V - the summary and conclusion . The results are summarised as follows :

Wet gel leaching in Anilozyme solutions on EP content of NR latex film

a) Wet gel leaching in Anilozyme solution has a marked effect in reducing E P content . As the concentrations of Anilozyme increases , EP content decreases to a minimum and thereafter it increases slightly. The optimum concentration of anilozyme is selected as 1% by weight .

b) The effect of increasing leaching time of wet gel in Anilozyme solution was studied using 1% Anilozyme solution at 50 ° C . It was seen that as the duration of leaching increases , the EP content decreases. The decrease is sharp during the first two minutes , and leaching time of two minute is selected as optimum .

c) The effect of increasing temperature of Anilozyme solution on wet gel leaching was investigated and seen that as temperature of leaching increases the EP content decreases .

d) The effect of pH of Anilozyme solution on wet gel leaching on reducing EP content of NR latex film was studied . It is seen that an aqueous solution of Anilozyme - P, (pH - 7) is most suitable for reducing EP content .

e)The effect of addition of Ca^{++} ions to enzyme solution in wet gel leaching of NR latex film was studied , and it was seen that the addition of calcium ions has no effect in increasing the deproteinisation efficiency of Anilozyme .

Effect of post cure leaching of NR latex films in Anilozyme solution on E P content

a) Effect of concentration of Anilozyme on post cure leaching of gloves was examined and it was seen that leaching of gloves in water at 50 ° C for five minutes helped to reduce EP content to a great extent. Leaching in Anilozyme solution helped to reduce the EP content further . There is not much effect on EP content , when Anilozyme concentration increased above 0 .1 %

b) As the temperature of leaching increases , the EP content of the film decreases

Effect of enzyme leaching on technological properties

Technological properties of NR latex films were unaffected by both wet gel and post cure leaching in Anilozyme solutions . The ageing resistance of the films were also not affected by enzyme leaching .

Wet gel leaching Vs Post cure leaching in Anilozyme solution

Compared the E P content of NR latex films prepared by Anilozyme leaching under wet gel and dry film conditions (the concentration of Anilozyme solution is 1% , the leaching time 2 minute at 50 ° C) and it was seen that the effect of enzyme leaching is more pronounced in wet gel leaching than in dry film leaching conditions . In the wet gel stage , the latex film contains water and thus has maximum porosity. This facilitates the penetration of the film in the leaching medium . This may help the enzyme molecules to diffuse to the surface of the interior rubber particles and react with the proteins .

Reduction of protein content in dry film leaching can be attributed to the fact that during drying and vulcanisation , the migration of moisture in the latex films to the outer surface takes the residual compounding ingredients and proteins along with it and is removed during leaching . The low effect of Anilozyme in dry film leaching may be due to the higher fusion and integration of rubber particles in the dry film .

The tensile properties and ageing resistance of NR latex films are not affected by wet leaching / dry film leaching in Anilozyme solution . The technological properties and the high efficiency of enzyme leaching in wet gel condition implies that it can easily be adopted for the on - line manufacture of latex products .

CHAPTER 1

INTERODUCTION

CHAPTER 1

1 .0 Introduction

Natural rubber latex products are facing serious challenges owing to the allergic effects from extractable protein . In this project the effect of wet gel and post cure leaching of natural rubber latex films in Anilozyme - P solution is evaluated in reducing extractable protein content .

1 . 1 Natural Rubber Latex

Natural rubber latex is an intracellular milky fluid produced by the laticifer cells of tropical rubber tree "Hevea Brasiliensis" and is obtained by the process of tapping. [1] Freshly tapped latex is a whitish fluid of density between 0.975 and 0.980 gm / cc , pH from 6.2 to 7.0 and surface energy from 40 to 45(ergs cm $^{-2}$). The rubber content of latex varies between 25 and 40 % by weight and this variation is owing to factors such as clone, tapping intensity , soil condition, climatic conditions etc. In addition to rubber hydrocarbon ,a number of non rubber constituents such as proteins, carbohydrates, lipids, inorganic salts etc are also present in latex .[2]The rubber hydrocarbon in latex is predominantly cis -1,4- poly isoprene and it occur as molecular aggregates in the form of discrete particles which are usually spherical with diameters ranging from . 02 to 3.0 microns .[3] The amount of total protein is approximately 1.7% ,60% of which is associated with the isoprene and 40% within the cytoplasmic serm.[4]

1 . 2 . Preservation and Concentration of Latex

Natural rubber latex in vessels of rubber tree is sterile, but as it comes out it gets contaminated by bacteria and yeasts[5] . The micro organisms metabolise the non - rubber constituents of the latex and produce volatile fatty acids such as formic ,acetic and propionic acids which lead to coagulation of latex .[6] Therefore , preservatives are added to latex immediately after collection . Among the preservatives ammonia is still widely used and it inhibit bacterial growth ; acts as a alkaline buffer , raises the pH and neutralises free acid formed in the latex .[7] But ammonia has the disadvantage that it is pungent smelling and prolonged exposure to the gas can cause discomfort to workers. Also high concentration of ammonia leads to processing problems. Therefore attempts have been made to develop low ammonia preserved systems.[8,9,10]

Preserved field latex is unsuitable for most latex application, as its rubber content is low . For the manufacture of most of the products , latex of 55% to 60% rubber content is preferred. The important method for the concentration of preserved field latex are evaporation , creaming , centrifuging and elecrodecantation . In India , only centrifuging and creaming are commercially used for the production of latex concentrate. [11] NR latex properties of significance are dry rubber content (drc) , non rubber solids , mechanical stability time ,volatile fatty acid number (VFA) ,Potassium hydroxide (KOH) number and alkalinity . The significance of these properties has been discussed by Blckely[12] and Cockbain. [13] Latex concentrate is a non Newtonian fluid and its viscosity decreases with increasing shear rate . [14]

1 . 3 Latex Compounding

Latex products are manufactured from concentrated latex by mixing it with various compounding ingredients. The compounding ingredients are solids or liquid and may be water soluble or water insoluble . They are added to latex as aqueous solutions , if they are water soluble solids or liquids, as dispersions if they are water insoluble solids and as emulsions , if they are water insoluble liquids . General principles to be considered while addition of solutions, dispersions and emulsions to latices are [15]

1) Particle size of dispersions and the droplet size of emulsions should be comparable to the particle size of the latex .

2) The colloid stability of dispersions and emulsion should be comparable to that of the latex.

3) The pH of solutions, dispersions and emulsions should be similar to that of latex.

4) It is desired that the ionic strength of the aqueous phase of the dispersion should be similar to that of the latex aqueous phase.

Dispersions of solid ingredients are prepared by first making a coarse slurry of the powdered ingredient with water which contains the required amounts of dispersing agents and colloid stabiliser. The slurry is then ground in a suitable mill such as colloid mill , ball mill or attrition mills for an appropriate length of time so as to produce the dispersion of the required small particle size. [15 - 20]

Emulsions are prepared by first making a coarse suspension of oil droplets in water in the presence of a suitable emulsifying agent using a high speed stirrer and then subjecting this to a refining process .

1 . 4 Compounding Ingredients

The different ingredients used in latex compound are classified as (i) surface active agents (ii) Vulcanising agents (iii) accelerators (iv) activators (v) antioxidants (vi) fillers (vii) viscosity modifiers and (viii) special additives .

1.4.1 Surface active agents

These are substances which can bring about marked modifications in the surface properties of the aqueous media, eventhough they are present in small amounts of the order of 1% m/v or less . They have got great importance in latex technology and in this respect latex technology differs significantly from that of dry polymer or polymer solutions . [16] Their principal effect is to reduce significantly the surface free energy of aqueous medium . One part of its molecule is strongly hydrophilic and the other part is strongly hydrophobic . As a result the molecule gets adsorbed at the interface between the rubber and the aqueous media . Depending on function , they are classified as stabilising agents , dispersing agents ,emulsifiers ,wetting agents ,viscosity modifiers and protective colloids .

Stabilisers are first added into latex during compounding to maintain the latex in the colloidal condition during compounding and further processing . The dispersing agents prevent the dispersed particles in dispersions from re-aggregating and alkyl sulphonates are used for this .The emulsifying agents are soaps, usually olates formed insitu . Wetting agents are used to reduce the interfacial ; tension between two surfaces. Proteins, alginates, polyvinyl alcohols and cellulose derivatives are used as protective agents and viscosity modifiers in the processing of latex compound [16,21, 22]

Surface active substances are also classified broadly into ionic and non-ionic types. The ionic types are sub classified into anionic, cationic and amphoteric according as the surface active part of the molecule is anionic, cationic or amphoteric . According to the nature of ionic groups , the anionic types are classified as carboxylates , sulphonates , and sulphates . (e.g. Potassium Oleates ,Sodium naphthalene formaldehyde sulphates etc) Non - ionic surface active substance do not ionise in aqueous media . Important types used in latex technology are ethoxylates. They are less soluble in hot water than in cold water .

1.4.2 Vulcanising agents.

The normal vulcanising agent for NR latex is sulphur. Depending upon the particular property required for the product , sulphur is added at different levels ranging from 0.3 - 2.5 phr.Thiuram polysulfides (TMTD) along with Thiourea is used as vulcanising agent for heat resistant products .[23] Dunn [24] reported that butyl xanthogen disulphide in conjunction with a dithiocarbamate may be used to vulcanise latex film in the absence of sulphur. It has been reported that organic peroxide and hydroperoxide may also be used to vulcanise latex films .[16]

1 . 4 . 3 Accelerators

Accelerators are chemicals used in the latex compound to reduce the vulcanisation time and also to improve the technological properties of the vulcanised product . Their dosages vary from 0.5 to 3phr depending on whether the vulcanisation system is high sulphur or low sulphur . Generally fast accelerators are used in latex technology because the vulcanisation temperatures are relatively low . The important classes used in latex compounding are metallic and amine dialkyl dithiocarbamates .[16]

The thiazoles and to a lesser extent ,thiurams are of importance as secondary accelerators in conjuction with dithiocarbamates . Limited use has also been made of "Xanthates" for room temperature vulcanisation of latex .

1 . 4 . 4 Activators

Zinc oxide is used as activator in sulphur vulcanisation .It boosts up the cure time and also imparts additional heat resistance to latex products . Its dosage is 0.25 to- 1 phr for latex foam products .

1 . 4 . 5 Antioxidants

The antioxidants protect rubber from deterioration by oxygen , heat , light , ozone etc. BHT, Styrenated phenol and polymerised 1 ,2 -dihydro 2 ,2 , 4 - trimethyl quinoline is in small dosages (0.25 phr) are typical antioxidants used in latex products .

1 . 4 . 6 Fillers & Pigments

Fillers are added to latex compound to modify the mechanical properties, and to cheapen the product . Usually used fillers are Calcium Carbonate , Barium sulphate, Titanium dioxide, Chinaclay, Silica ect. It is seen that no effect analogous to the reinforcement of dry rubber , occurs when fillers are added to the latex .

1 . 4 . 7 Softners

These are used to reduce the modulus of the product . one example is mineral oil which is used in the manufacture of balloons

1 . 5 Preparation of Latex Compound [18]

Preparation of latex compound involves mixing of concentrated latex with solutions/ dispersions / emulsion of different compounding ingredients. High ammonia or low ammonia preserved latices can be used for compounding . In the case of high ammonia latex ,for mixes containing higher amounts of zinc oxide ,deammoniation is done prior to compounding to avoid Zinc oxide thickening .

After the addition of each dispersion / emulsion / solution , the latex compound is stirred slowly but thoroughly to get even distribution of the chemical in the latex . It is important to avoid contact between the stirrer and the container since latex may get coagulated by friction . After the addition of all the compounding ingredients, the compound is matured for about 24 - 48 hrs and used for dipping .

1 . 6 Coagulants for Latices

Colloidal destabilisation of latices can be brought about by the addition of Chemicals . These substances often react chemically with bound surface groups which confer colloidal stability to the latex there by reducing the potential energy barrier conferred by those groups. These substances are mainly classified under three broad heading .

1) Direct or contact coagulants

2) Delayed action coagulants

3) Heat sensitizing coagulants

1 . 6 . 1 Direct or Contact Coagulants are substances which are immediate in their effect , provided that an adequate quantity of the chemical is present , brings colloidal destabilisation rapidly. A large number of substances are able to function as direct coacervants for NR latex . They are conveniently classified under the headings.

1) Acidic substances e.g.: acetic acid

2) Water - soluble electrolyte especially salts of di and equivalent cations e.g. Calcium chloride, Barium Chloride, Salts of Lithium, Sodium, Potassium etc.

3) Hydrophobic Organic liquids.

4) Hydrophilic organic liquids e.g. : ethyl alcohol ,acetone to NR latex

5) Cationic Surface active substances e.g. : n-hexadecyltrimethyl pyridinium bromide

6) Water - soluble hydrophilic polymers.

1 .6.2 Delayed action -Coagulants

Delayed- action coacervants are substances which have little apparent destabilizative effects when first added to the latex but bring about obvious colloidal destabilisation after a certain time has elapsed . The most important delayed - action coacervants for carboxylate stabilized latices are the alkali metal salts of hydroflurosilicic acid, H_2SiF_6 commonly known as silicoflurides eg:Na_2 SiF_6, K_2 SiF_6. They are added to latex as dispersions . the usual dosage of addition 0.5 phr to 1 phr. further information concerning the behaviour of Silicofluorides as delayed-action cocervants for Carboxylate stabilized rubber latices , especially for NR latex has been given by Murphy [25] by Madge and Pounder [26] and by Madge [27]

1 . 7 Latex Dipping Process

The latex [28] dipping is a process in which thin walled rubber products are produced by first immersing a former into a latex compound and then slowly with drawing the former from the latex so as to obtain a uniform deposit on the former. Thickness of the deposit is increased by repetition of the process . The process involves steps as dipping, leaching, drying ,beading vulcanising, dusting and stripping off the deposit from the former. Different types of gloves, condoms, balloons etc are produced by dipping technique.

1 . 8 Principal Dipping Methods

Latex dipping processes are classified according to whether or not any colloid destabilisers are used to assist in the formation rubber deposit.

1 . 8 . 1 Simple Dipping or Straight dipping :

In this process, no destabilising agent is used .The process consists of immersing the former in latex compound, withdrawing and drying of the deposit adhering to the surface of the former. The deposit formed on the former is by virtue of the viscosity of the latex and of its tendency to wet out , the former.

1 . 8 . 2 Wet coagulant Dipping

In this method a 5- 10 % solution of acetic acid or formic acid in water is used to assist the build up of a deposit. It can be worked in two ways , according as the former is dipped first into the coagulant or first into the latex compound.

1 . 8 . 3 Dry Coagulant Dipping :

In this , the coagulant is dry such as calcium nitrate, cyclohexyl ammonium acetate and other salts. The former is first immersed in a solution of the coacervants in a volatile solvent. After withdrawal from the solution the solvent is allowed to evaporate leaving either a dry deposit or one which is in the form of very viscous, concentrated solution. The former with its coacervant deposit is then immersed in the latex compound and is allowed to dwell for a predetermined time . The thickness of the deposit depends largely upon the time of dwell and upon the stability of the latex compound towards the particular coacervant which is used.

1 . 9 Sequence of latex - dipping operations:

A typical sequence of operations primarily for the production of dipped goods by dry coacervant dipping is discussed below .

1 . 9 . 1 A suitable latex compound is prepared and allowed to mature for about 24 - 48 hrs .

1 . 9 . 2 Pre - treatment of formers

The formers are cleaned ,washed and heated , the presence of dirt grease, oil etc. on the former may lead to uneven thickness of the deposit.

1 . 9 . 3 Immersion of formers in latex compound

The hot former is then slowly immersed into the latex compound. The speed of immersion should be sufficiently low to prevent air from being drawn into the latex bath along with the formers, but sufficiently high to maintain the meniscus surrounding the former in a convex condition to air . In manual dipping ,the operator may find it advantageous to vary the angle and mode of immersion ,as well as speed of immersion .

1 . 9 . 4 Dwell : Dwell time is the period for which the former is kept in the latex compound during dipping .In dipping ,the duration of the dwell period is determined by the thickness of deposit required in relation to the colloid stability of latex and the potency and quantity of coacervant held by the former . As dwell time increases , the thickness increases reaches a maximum and thereafter remains the same . The time for getting maximum thickness is known as the optimum dwell time .

1 . 9 . 5 Withdrawal of former from latex bath

The former is withdrawn at such a rate so that drainage of the latex does not lead to unacceptable variations in the wall thickness of the dried deposit .

1 . 9 . 6 Drying , leaching , beading and, Vulcanisation

The order in which the operation are effected can vary . Drying may be effected by passage through hot air which is heated directly by gas , infra -red rays , electricity or steam etc . Whatever be the method of heating , it is desirable that the film should set as quickly as possible to prevent further flow of ungelled latex . The drying step may be combined with the final vulcanisation or these two stages may be separated . Temperatures are typically within the range 100 - 140 °C in hot air continuous automatic vulcanisation . It is advantageous that the temperature increases progressively as drying proceeds .

The leaching medium is invariably water , which may be hot or cold . The purpose of leaching is to remove as far as possible all the water soluble substances present in the latex film . It has been noted in recent years that the mechanical properties of dipped films from Sulphur - Prevulcanized natural rubber latex can be significantly improved by leaching with water . This is due to the removal of hydrophilic substances and improved inter particle coalescence . Leaching also improves the clarity of the latex film .

The process of beading involves rolling down the thin film of rubber , which has been left for this purpose , while the article is still on the former . The beading should be done before vulcanisation to get good adhesion . It can be done manually or automatically .

1 . 9 . 7 Dusting & Stripping - The article may be dusted to prevent adhesion to itself and to other articles after removal from the former . This adhesion is only seen in the case of unvulcanised products . a wide range of dusting powders are available . The removal of article from the former commonly known as stripping . Generally , it is done manually . After stripping the products are subjected to visual examinations and tested for physical properties . They are then packed and ready for despatch .

1 . 10 Vulcanisation Of Natural Rubber Dipped Products

Vulcanisation is the process by which the mainly viscous rubber is converted into elastic rubber through the crosslinking of the macromolecle at their reactive sites . It is an intermolecular reaction which increases the retractive force and reduces the amount of permanent deformation remaining after removal of the deforming force . According to the theory of rubber elasticity , the retractive force resulting from a deformation is proportional to the number of network supporting polymer chain per unit volume of elastomer .[29] Vulcanisation usually produces network junctures by insertion of chemical cross links between polymer chains . The cross links may be formed through chain of sulphur atoms ,single sulphur atom or Carbon - carbon bonds . The vulcanite properties are affected by crosslink density , type of crosslink , nature of polymer type and amount of filler etc . Vulcanisation was first discovered by Charles Good Year and by Thomas Hancock (1841) by heating rubber with sulphur .[30] The introduction of organic accelerators then enabled to reduce the vulcanisation time and sulphur dose required for optimum physical properties , improve the ageing resistance of rubber goods and prevent blooming of sulphur .[31]

Vulcanisation of latex dipped goods may be effected by two methods .

1) The latex may be vulcanised before the product is being shaped (Pre - vulcanisation)

2) The vulcanisation is carried out after the product has been shaped (Post vulcanisation)

The first method involves vulcanisation of latex in the fluid state so that it deposit elastic films of vulcanised rubber on drying . The second method is carried out by heating the shaped compound for optimum cure time to get the balanced technological properties .

1 . 11 Challenges Facing Natural Rubber Latex In The Manufacture Of Dipped Medical Products

Centrifuged N R latex is presently used in the manufacture of a variety of dipped products used in the medical field viz. ,examination gloves ,surgical gloves ,feeding bottle teats ,catheters , medical tubing etc . The demand for examination and surgical gloves has boosted during the last ten years , particularly due to the spread of AIDS in USA & Western countries. Disposable gloves are being used by doctors for examining each patient . It is reported that the demand for dipped latex products in medical field is steadily increasing and will continue to do so in the years to come .[32]

However ,various problems are also reported which could inhibit this potential growth . Some of the problems are nitrosamine regulations for NR latex products ,demand for low levels of cytotoxic activity and allergy , biodegradability , free from pinholes . [33] The western population is becoming more sensitive to allergic effects of products made from NR latex products .

1 . 12 N R Latex Protein Allergy

There are two types of allergies associated with natural rubber latex products, described as allergic contact eczema (a Type 1V response) and contact urticaria (a Type 1 response). The characteristics of these two types are given in the table .

CHARACTERISTICS OF ALLERGIES

Allergy	Characteristics
Type 1V	Delayed reaction - may be hours after contact . Reddened skin. eczema .blisters Non - life threatening.
Type 1 Urticaria	Immediate reaction - within minutes of contact , Development of wheals flares , hives
Anaphylaxis	Increased heart rate , lowered blood pressure

Allergic contact eczema (Type 1V allergy)

Type 1V response has been known for almost as long as vulcanised latex rubber has been used and is attributed to the presence of accelerator residues in the product . Some of the additives in latex goods reported to cause Type 1V allergy are

Thiurams ,

Mercptobenzthiazole / dibezthiazyl disulphide ,

Zinc di- alkyldithiocarbamates ,

Diphenylguanidine ,

Sym di ß naphthyl - P - phenylene diamine

The skin reaction is not serious and rapidly clears when contact with rubber is stopped ; however, it is very unpleasant and irritating to suffer .

Type 1 allergy is proved to be caused by human allergenic reactions to certain proteinacous substances which are present in films obtained from natural rubber latex . The most serious reaction attributed to contact with latex proteins is known as anaphylatic shock . It is characterised by a severe reduction in blood pressure , breathing difficulties , increased heart beat rate and unconsciousness . It can result in death . Other reactions include contact urticaria ,which is a temporary eczematous reaction in which wheals or flares form at the contact site ,with itching or stinging .

Nutter [34] first provided evidence that contact urticaria could be caused by latex itself and additives normally found in rubber gloves were not involved . In this work and that detailed below ,the main lines of evidence were standard dermatological tests (e.g. ; skin prick tests, patch tests) to establish that rubber articles were involved and the use of the invitro RAST procedure [35] radioallergosorbent test to show that latex proteins were the allergens . The first report of anaphylatic shock , in this case caused by surgical gloves , came from Finland in 1984[36] . In out line , anaphylatic shock can be described as follows : exposure to protein allergens in certain individuals lead to a response in their immune system which brings about a condition of hypersensitivity known as anaphylaxis. On subsequent exposure , the interaction of the allergens with antibody formed in the sensitising phase , culminates through a complex mechanism in a systematic release of histamine and other amines . The main effect of histamine in the circulation is to dilate the peripheral vessel , with a consequent severe fall in blood pressure and anaphylatic shock . Among the effects seen are quickening of the heart - beat rate , breathing difficulty , urticaria , fluid release in the tissues and unconsciousness . The actual frequency of protein allergy among the general population of Western countries may be of the oder of one person in a million but in certain areas or among certain limited sections of people , it may be more marginally .

Between 1979 & 1986 Morales et [37] al studied six patients with anaphylatic shock caused by glove and balloon contact and published strong evidence that proteins in the article were responsible . They also made the interesting observation that simple washing of gloves allowed the patients to wear them without any problems . In 1987 , a report appeared showing that 7.5 % of doctors and 5.6 % nurses in certain operating departments were allergic to latex .[38]

The amounts of extractable protein remaining in a product depends almost enirely on the details of the production process through which the product has passed . The formulation used to make the product has little effect , because the protein is naturally present and is not an additive and because the amount of protein is virtually constant . If double centrifuged latex was used the amount of protein present would be about half that of single centrifuged .

Natural rubber latex protein has been reported as the cause of Type 1 allergy in individuals who have been exposed to natural rubber latex devices (Refs. 2 to 8) Repeated exposure to latex protein is considered to increase the probability that an individual will become sensitised . The total water extractable protein is considered an indirect measure of the potential allergenicity . The total water extractable protein level measured using the standard is found to correlate well with the currently used allergen measurement methods . Most importantly ,a total water extractable protein correlates also with the skin pick test , which is a direct measure of allergic response in a sensitised individual. [39] (Ref.32)

1 . 13 Proteins In Natural Rubber Latex

Being a natural product , the composition of fresh natural rubber latex varies between wide limits. The amount of proteinacious substances vary between 2 -3 % m/m on the whole latex . They are distributed between the following three phases viz , the rubber phase , the aqueous phase and the lutoids phase . Of the total content of proteinacious substances , about half is disolved in the aqueous phase , quarter is adsorbed on the surface of the rubber particles and remaining quarter is associated with the lutoids . The protein in the rubber fraction are mainly associated with the interfacial layer surrounding the rubber particle . They are mostly water insoluble and are extractable by detergents or organic solvents such as SDS and chloroform - methanol . A small fraction of rubber bound proteins can be extracted by ammonia .

Aqueous phase of fresh natural rubber latex contains several proteins of which the most important ones are - globulin and hevein . On processing fresh latex to HA latex concentrate , some of the proteins are removed in the serum , reducing the protein concentration to about 1 - 1.5 % on the whole of latex .

1 . 14 Proteins Extractable from Latex Films

The amount of water extractable proteins from latex film is very much lower than the actual amount of protein present in the rubber . Extractable protein content of clonal HA latex is 20 -26 mg / gm rubber . Compounding the latex with surface active agents and vulcanising ingredients increases the amount of extractable protein . During heating , the high molecular proteins degrade to low molecular weight proteins . The extractability of proteins from latex film could thus depend on the presence of soap , potassium hydroxide , ionic species in the system and also on the processing temperature ..[40]

1 . 15 Methods For Reducing Extractable Protein Content

Type 1 allergy problems attributed to the water soluble proteinacious substances in the natural rubber latex have necessiated the production of latex products with low EP content . There are several methods reported for reducing the EP content . There are three approaches to [41] to decrease the EP content in latex products .

1. 15 . 1 Pre-processing

Ammoniation and preservation of latex degrades the NR latex proteins . During normal centrifuging of field latex , approximately 2/3rd of the serum proteins are removed thus reducing the proteins to rubber ratio to 1/3rd of original . In a typical double centrifuged latex , there is an over all reduction of serum protein of about 90 % .

Use of de - proteinised latex (DPNR) by enzyme treatment and centrifuging or by pre vulcanisation and centrifuging for product manufacturing can reduce the EP content . The use of low protein latex will reduce [42, 43] the level of the EP content from latex products .

1 . 15 . 2 Processing

Leaching process is critical . It is an essential process for the manufacture of dipped latex products. The removal of excess of the coagulant and the added compounding ingredients , water soluble non -rubbers including proteins etc . results in the improvement of physical properties such as Tensile strength , film clarity , prevention of surface blooms and reduction in water absorption of the dipped products . The effectiveness of leaching process is critical in the determination of the overall quality of the product .

There are basically two methods of leaching , wet gel leaching and dry film [44, 45] leaching . The former involves the washing of the gel , i.e. the gelled deposit on the former , prior to drying and vulcanisation . It is usually carried out on -line . Dry film washing is an off -line process and consists of washing of dried vulcanised latex product after removal from the former .

In the production of latex examination gloves , [46] wet gel leaching is often carried out for a period of several minutes , 1 5 minutes in a continuous chain dipping line . The various leaching parameters like temperature and flow rate of leaching water , time of leaching , TDS (total dissolved solids) and hardness of leaching water etc can affect the residual protein content in the latex gloves . [47]

1 . 15 . 3 Post processing

Leaching and chlorination are both very effective . Coating of NR latex products with non permeable substances is possible . Lubricants like silicone to reduce the migration of proteins . Autoclaving degrades the allergens but also decreases the stability of the gloves

1 . 15 . 3 a. Chlorination

It is an off - line process and is very effective approach for reducing soluble proteins . It is believed that chlorine renders the proteins insoluble (denaturing) or it forms an impermeable barrier that prevents proteins migrating to the surface . It is also used where the usage of powder is not suitable , as it enhances the smoothness of the surface . However the process changes the properties and appearances of the products .

Chlorination of gloves and other latex products involves only a surface reaction in the initial stage . Depending on the thickness of the glove , the time of chlorination and concentration of the chlorine solution , the reaction can proceed to the inside .

After chlorination , neutralisation and washing , the products would have to be heated or tumble dried . Extreme care should be taken to ensure that no over heating occurs . Over heating of chlorinated gloves will result in colour changes and poor properties .

1 . 15 . 3b Dry gel leaching

Since heating increases the EP content , latex films leached after drying have lower EP content than wet gel leached films for short leaching times . However , on prolonged leaching , the difference between wet gel leaching and dry gel leaching become narrower . During leaching , the extraction of soluble proteins is rapid initially , but the rate of extraction decreases quickly and levels off after some time . Slow leaching of proteins can continue for several hours , but further reduction in EP content is small .

1 . 16 Aim of the Project

Natural rubber latex products are facing serious challenges owing to the allergic effects of extractable proteins . [33] Several techniques are being adopted for manufacturing products with very low extractable protein content so that allergic effects can be minimised . Use of low protein centrifuged latex , judicious choice of comrpounding ingredients , proper leaching of the product and chlorination are some of the techniques being generally practised . Normal NR latex contains approximately 1.7% proteins and during centrifuging process a part of soluble protiens are removed The adsorbed proteins on the surface of the particles cannot be removed by this process . [12] Various methods are being reported to reduce the extractable protein content in latex products .These include the use of an enzyme deproteinised natural rubber latex , leaching & Chlorination of latex products[48] and on line and off line enzyme treatment of gloves[49].

Anilozyme P is a proteolytic enzyme which is reported to be used in the deprotenisation of NR [50] . Studies on enzymatic deproteinisation of natural rubber latex using Anilozyme P has been carried out [51]. But a process for producing low protein latex products by enzymatic leaching using Anilozyme -P has not yet being reported . Hence in this work , it is proposed to study the effect of wet gel leaching and post cure leaching of latex films in aqueous Anilozyme -P solutions for reducing extractable protein content . The study will be conducted in three parts

1)WET GEL LEACHING IN ANILOZYME - P SOLUTION:

The effecf of following factors on EP content are to be studied

Concentration of Anilozyme solutions

Time of leaching

Temperature of leaching

pH of Anilozyme solutions

Addition of Ca $^{++}$ ions in Anilozyme solution

2) POST CURE LEACHING OF NR LATEX GLOVES IN ANILOZYME - - SOLUTIONS

The effect of following factors on EP content are to be studied

Concentration of Anilozyme solution

Temperature of Leaching

3)EFFECT OF ENZYME LEACHING ON TECHNOLOGICAL PROPERTIES

CHAPTER 2

EXPERIMENTAL

CHAPTER 11

2 . 0 Experimental Techniques

2 . 1 Raw Materials

The raw materials used are

2 . 1 . 1 60 % Centrifuged Natural Rubber Latex (High Ammonia)

60 % Centrifuged Natural Rubber latex HA type , collected from the Pilot Latex Processing Centre , Chathackal , Rubber Board was used for this study . Properties of the latex ,given in Table 2.1 conformed to BIS specification 5430 - 1981

Table 2.1

Properties of 60 % Centrifuged NR Latex (HA Type)

Properties	Value	Requirements (BIS 5430 - 1981)
1. Dry Rubber content %	60.2	60 % (min)
2. Non Rubber solids % by mass	1.5	2 (Max)
3. Sludge Content % by mass	0. 05	0.10 (Max)
4. Ammonia content % by mass	0.7	0. 6 (Min)
5.KOH No	0.5	1 (Max)
6. MST (sec)	1300	475 (Min)
7.VFA No	0 .05	0.15 (Max)
8.Coagulam Content % by mass	0.03	0.05 (Max)
9. Cu ppm	1	8 (Max)
10. Mn ppm	Trace	8 (Max)

2 . 1 . 2 Anilozyme- P, is a proteolytic enzyme preparation , manufactured by M/s Anil Starch Products Ltd, Ahmedabad ,was used for deproteinisation . It is a neutral protease derived from species of the germs " Bacillus" . The active portion is soluble in water . The insoluble fraction can be removed by filtration

2.1.3 Sulphur (Rhombic type)

Laboratory grade manufactured by E . Merk Ltd , Bombay was used in this study . Yellow crystals stable at room temperature , specific gravity 2. 06 .

2.1.4 Zinc Oxide

Laboratory grade manufactured by E . Merk Ltd , Bombay was used in this study . It is a coarse white powder , specific gravity 5.47

2.1.5 Zinc dithiocarbamate

Laboratory grade manufactured by E . Merk Ltd. , Bombay was used in this study .

2.1. 6 Dispersol F - Dispersing agent manufactured by M /s ICI Ltd . , Calcutta .

2.1.7 Potassium hydroxide , calcium nitrate , etc . were laboratory grade chemicals .

2 . 2 Preparation of Dispersions .

Fifty percent dispersion of Sulphur , ZnO & ZDC were prepared separately by grinding in a ball mill using Dispersol F as the dispersing agent . The recipes used for making dispersions are given in Table 2.2

Table 2.2

Formulation for Dispersion

Ingredients	Parts by weight	
A. 50% Sulphur dispersion		
Sulphur	100	Ball milled for 72 hrs
Dispersol - F	3	
Water	97	
B . 50% ZDC dispersion		
ZDC	100	Ball milled for 48 hrs
Dispersol F	2	
Water	98	
C. 50 % ZnO dispersion		
ZnO	100	
Dispersol F	2	Ball milled for 48 hrs
Water	98	

2 . 3 Preparation of Latex Compounds

For studying the effect of leaching in anilozyme solutions for reducing extractable protein content in NR latex films , a base latex compound was first prepared . Formulation of this compound is given in Table 2.3 .

Table 2.3

Formulations

Ingredients	Parts by weight Dry	Parts by weight Wet
60% Centrifuged NR latex (HA type)	100	167
20 % Potassium hydroxide (Solution)	0.1	0.5
50% Sulphur dispersion	1 .25	2.5
50% ZDC dispersion	1	2
50 % ZnO dispersion	0.25	0.5

NR latex was mixed with stabilzer , dispersions of S , ZDC & ZnO under constant stirring at a speed of 50 -60 rpm . It was then kept for maturation for 24 hrs . The TSC of the compound was adjusted to 55 % by the addition of 1% ammonia water .

2.4 Preparation Of Latex films

Latex compound was prepared as per the formulations given in table 2.3 . Latex films having thickness in the range of 0 . 2 +/_ .02 mm were prepared by dipping using glass formers . The glass plate was first cleaned and dried . It was then dipped into the coagulant (10 % Calcium nitrate solution) partially dried , immersed into the latex compound , allowed to dwell for 30 seconds and then slowly withdrawn . The wet gels were then leached in Anilozyme - P solutions , and then leached in hot water for 2 minutes in water . It was then dried and cured by keeping in an oven at 100 ° C for 60 minutes . Cooled , dusted with talc and the film was stripped off from the mould . A flow chart of the dipping process is given below .

Former ------------> Former Drying --------->Coagulant--------------->Latex Dip
Cleaning Oven Dip

Wet- gel leaching ----> Leaching in----->-Curing Oven----->-Powdering------>Stripping
enzyme solutions water

The latex films were then tested for extractable protein content and tensile properties as per ASTM standards

2 . 5 . 1 Determination of extractable protein content of latex films

For the determination of extractable protein content in NR latex films , ASTM D 5712 -95 is used . This colorimetric test method is used for the determination of protein levels in NR and its products. The test method involves the extraction of residual aqueous soluable proteins from NR followed by the precipitation of these proteins to remove interfering , water - soluble substances . The protein content is then determined by the Lowry method of protein analysis using a protein standard for quantitation. Spectrophotometric measurements is made at a wavelength range in the range 600 to 750 Hz (nm).

In this process 2 gm of the NR film was accurately weighed , cut it into small pieces and extracted with 20 ml distilled water at 37 ° C in a polypropylene container for 120 +/_ 5 min with all the surfaces of the test specimen evenly exposed to water . During the extraction process , the vessels were shaken for 15 s each at the beginning , the middle and the end of the extraction period . The extract was then centrifuged for 20 min at 2000 rpm to remove powder in the extract .

Protein precipitation & colour development ; 1ml of the extract was taken in a 1.5 ml polypropylene tube and added .1 ml Sodium Deoxycholate (0. 15 % w/v) solution . Mixed it and allowed to stand for 10 min at room temperature . Added .1 ml of Trichloroacetic acid (72 %) and mixed and then added 0 .1 ml Phosphotungstic acid (72 %) and then mixed thoroughly and allowed to stand for 20 min at room temperature . The extract was then centrifuged at 7500 rpm for 30 min to get a firm protein precipitation . Decanted the supernatant liquid . The protein precipitate was redissolved in 0 .25 ml of 0.1N NaOH at room temperature by stirrring for 30 sec until dissolved completely .

Added 0 . 1 ml alkaline Copper tartarate into each 1.5 ml tube . Added 0. 2 ml of redissolved protein solution in to test specimen tubes . Added 0 . 8 ml of reagent B to each tube and mixed thoroughly . The absorbance was measured after 15 to 30 min in a spectro photo meter at a wave length in the range of 600 - 750 nm and the concentration of EP content in the sample was computed from a calibration graph plotted with standard solutions of Ovalbumin . The EP content is expressed in micro gram per gram of the sample .

2. 5 . 1 Determination of physical properties using Tensile Tester

For determining the tensile properties of the film , the latex film vulcanizates obtained in the experiments were used . Tensile properties such as Modulus Tensile strength and Elongation at break were tested using Tensile tester (Instron model 4411) as per ASTM D -3188 . The machine consists of a load frame an electronic control unit and a monitor . Load measuring device is a load cell fixed at the top of the load frame . Below the load cell is a moving platform driven by a continuously variable speed motor .

Grips suitable for the dumbell specimens were fixed on to the load and the moving platform . During testing the specimen was subjected to stretching at constant speed . Force and elongation are continuously monitored by the electronic circuit . As soon as the test specimens breaks , test results are displayed in the electronic circuit and printed as a table .

Tests were carried out at ambient temperature (30 ° C) . Dumbbell specimens were cut from the dipped film for measuring tensile properties . The thicknesswas measured by bench thickness gauge . The moving cross head is adjusted by operating the switch . The velocity of the moving cross head was adjusted to 500 mm /min . The specimen was elongated and the values corresponding to ultimate tensile strength , elongation , modulus 300 % and 500 % were measured .

EXPERIMENTS

2 . 6 Effect of Wet Gel Leaching in Anilozyme Solution on Extractable Protein Content

Trial 1 Experiments

To study the effect of wet gel leaching in Anilozyme solution, some preliminary trials were carried out as explained in 2. 4 . The treatment details and extractable protein content of the films are given in table .

S . No .	Treatment details	E .P content (μ gm /gm)
1	No leaching	1100
2	Leaching in water at 50 ° C for 5 min .	440
3	Leaching in 2% Anilozyme -P solution. at 50 ° C for 3 min. & 2 min. water leaching at 50 °C	90

It is seen that leaching in Anilozyme solution followed by water leaching has much significant effect in reducing the extractable protein content than corresponding water leaching . Hence in the present study enzyme leaching involves "leaching in Anilozyme solution followed by water leaching" .

To standardise the conditions of wet gel Anilozyme leaching to reduce E P content , the effect of varying the following factors were studied .

2 . 6 . 1 Concentration Of Anilozyme - P

Anilozyme solutions of various concentrations , say , 0.5% , 1 % , 2% , and 4 % were prepared and used for leaching at 50 ° C for two minutes on wet gel films as in 2 .4 . The EP content of the films were determined as per ASTM standards The results are given graphically in fig 3. 1. 1.

2 . 6 . 2 Leaching time

1% Anilozyme solution was prepared and films were leached for different intervals say , 2 , 10 , 15 and 30 minutes at 50 °C as shown in 2.4 . Then the EP content of the films were determined as per ASTM standards . The results are given graphically in fig 3 . 1 . 2

2. 6 . 3 Temperature of leaching

1 % Anilozyme solution was prepared and wet gel NR films were leached with this solutions at say , 20 ° C, 30 ° C , 40 ° C, 50 ° C, 60 ° C as discribed in 2 . 4 Then the EP content of the films were determined as per ASTM . The results are given graphically in figure 3 .1 .3.

ere leached

2.6.4 pH of Anilozyme solution

The pH of 1 % anilozyme solution was found to be 7 . It was varied from 5 to 9 and used for wet gel leaching of NR latex films . The EP content was determined as per ASTM . The results are given in figure 3 . 1 . 4.

2.6.5 Addition of Ca^{++} ions in Anilozyme solution

NR latex films were prepared with formic acid as coagulant . 1 % anilozyme solution containing varying amounts of Ca^{++} ions (Calcium nitrate 0 .5 -1 %) were used for wet gel leaching as shown in 2 .4 . The EP content of NR latex films were determined as per ASTM . The results are given in table 111

2.7 Effect of Post cure Leaching of NR latex films in Anilozyme Solution on Extractable Protein Content

NR Surgical gloves collected from M/s Kanam latex products Ltd . were used to study the effect of post cure leaching of NR latex films in Anilozyme - P solutions on EP content . Treatment details and the E P content obtained in a preliminary study are given below .

S . No	Treatment details	E . P Content (µ gm /gm)
1 .	Unleached	805
2 .	Leached with hot water at 50 ° C for 5 min.	161
3 .	Leached with 0 .5 % Anilozyme Soln. for 3 min. & water leaching for 2 min.	88

It is seen that post cure leaching in Anilozyme solution has some effect in reducing the E P content . To standardise the conditions of post cure leaching , gloves of medium size and weighing about 8.5g ,were leached under different conditions in anilozyme solution (400 ml) ml . After leaching, samples were taken out , washed in deionised water ,dried and the remaining EP content was estimated . The estimations were done at room temperature . The effect of varying the following factors were studied

2 . 7 . 1 Concentration of Anilozyme solutions .

Gloves were leached in Anilozyme solutions of varying concentrations say , 0.1 , 0.5 , 1 , 2% respectively at 50 ° C for 2 minutes as described in 2 . 7 . The results are shown graphically in figure 3 . 2 .1

2.7.2 Temperature of Anilozyme solutions .

Gloves were leached in 0. 5 % anilozyme solution at different temperatures and follow the process as in 2.7 . The E P content of the films were determined as per ASTM . The results are shown graphically in figure 3.2.2

2 . 8 Effect of Leaching in Anilozyme Solutions on Technological properties in NR latex films

NR latex films were prepared by wet gel leaching in 1% & 0 . 5 % Anilozyme solutions at 50 ° C as in process flow chart 2.4 were used for this study . The tensile properties of the films were determined following ASTM D 3577 - 1977 (reapproved , 1982) .

Films were aged at 100 ° C for 22 hrs and tensile properties were again determined following ASTM D 3577 - 1977 (reapproved) . Tensile properties of control sample were also evaluated . The results are given in Table 1 .

Gloves obtained from experiment 2 . 7. 1 were tested for tensile properties before and after ageing at 100 ° C for 22 hrs following ASTM D 3577 - 1977 (reapproved , 1982). The results are given in Table 11 .

CHAPTER 3

RESULT AND DISCUSSION

CHAPTER 111

RESULTS AND DISCUSSION

3 . 1 Wet Gel Leaching of NR Latex Films in Anilozyme solutions on EP content

3 . 1. 1 Effect of Anilozyme concentration

From figure 3.1.1 , it is seen that wet gel leaching in Anilozyme solution has a marked effect in reducing the E P content at all concentrations studied (viz . zero to 4% by weight) . As the concentration of Anilozyme increases , the EP content decreases from 1100 μ gm/gm to 88 μ gm / gm and there after , it increases slightly . Hence , a concentration of 1 % can be selected as optimum .

The principle of leaching in Anilozyme solution is that Anilozyme - P being a proteolytic enzyme , may hydrolyse the proteins on the surface of rubber particles to water soluble peptides and amino acids and these would be removed during the next leaching process in water . So as the concentration of anilozyme increases , the E P content decreases . But the increase in E P content after reaching a minimum (at 1% Anilozyme concentration) may be due to the presence of residual Anilozyme present in the latex films .

3 . 1 . 2 Effect of leaching time

The effect of increasing leaching time in Anilozyme solution is given in figure 3 . 1 . 2 . It is seen that as the duration of leaching increases from 0 to 60 min. the E P content decreases from 1100 to 60 μ gm / gm . The decrease is more sharp during the first two min. , hence leaching time of two min . can be selected as the optimum .

3 . 1 . 3 Effect of Temperature

The effect of varying temperature during wet gel leaching in Anilozyme solution is depicted in figure 3 .1 . 3 . As the temperature of leaching increases from 20 ° C to 60 ° C the EP content of the film decreases steadily from 306 μ gm /gm to 60 μ gm / gm .

3 . 1 . 4 . Effect of pH of anilozyme solution

The effect of pH of Anilozyme solutions on deproteinisation is given in figure 3 . 1 . 4 . It is seen that the E P content is low at a pH of 7 , showing that an aqueous solution of Anilozyme - P is most suitable for deproteinisation .

3 . 1 . 5 . Effect of addition of Ca $^{++}$ ions

The effect of addition of Calcium nitrate solution in the Anilozyme solution during the wet gel leaching is given in table 111 . It can be seen that the addition of calcium ions has no effect in increasing the deproteinisation efficiency of anilozyme .

3 . 2 . Effect of Post cure Leaching of Gloves in Anilozyme solution On EP Content

3 . 2 . 1 Concentration of Anilozyme solution

From figure 3 . 2.1 , it is seen that leaching of the gloves in water at 50 ° C for five min. helps to reduce the E P content to a great extent that is from 805 μ gm/gm to 160 μ gm /gm . Leaching in anilozyme solution helps to reduce the E P content further . There is no much effect on EP content , when the concentration of Anilozyme solution increases above 0 . 1 % .

3 . 2 . 2 Temperature of Anilozyme solution

It is seen from the figure 3. 2 .2 that as the temperature of leaching increases from 20 ° C to 60 ° C the E P content of the film decreases from 805 to 40 μ gm /gm .

3 . 3 Effect of Leaching on Technological Properties

The technological properties of NR films after wet gel leaching and post cure leaching in Anilozyme solution are given in table 1 & 11 respectively . The ageing resistance of the films are also not affected by the enzyme leaching , in both wet gel and dry film conditions .

3 . 4 Wet gel leaching Vs Post cure Leaching in Anilozyme solutions

A comparison of the E P content of NR latex films prepared by Anilozyme leaching under wet gel and dry film conditions is given in figure 3 . 4 , the concentration of Anilozyme solution was 1% , the leaching time 2 minutes at 50 ° C. It is seen that the effect of enzyme leaching is more pronounced in wet gel leaching than dry film leaching conditions . In the wet gel stage , the latex film contains water and thus has maximum porosity. This facilitates the penetration of the film in the leaching medium . This may help the enzyme molecules to diffuse to the surface of the interior rubber particles and react with the proteins .

Reduction of protein content in dry film leaching can be attributed to the fact that during drying and vulcanisation , the migration of moisture in the latex films in the outer surface takes the residual compounding ingredients and proteins along with it and is removed during leaching . The low effect of film Anilozyme in dry film leaching may be due to the higher fusion and integration of rubber particles in the dry film .

The tensile properties and ageing resistance of NR latex films are not affected by wet leaching / dry film leaching in Anilozyme solution .. The technological properties and the high efficiency of enzyme leaching in wet gel condition implies that it can easily be adopted for the on - line manufacture of latex products .

Figure 3.1.1 Wet-gel Leaching

Effect of Concentration of Anilozyme Solution on EP Content

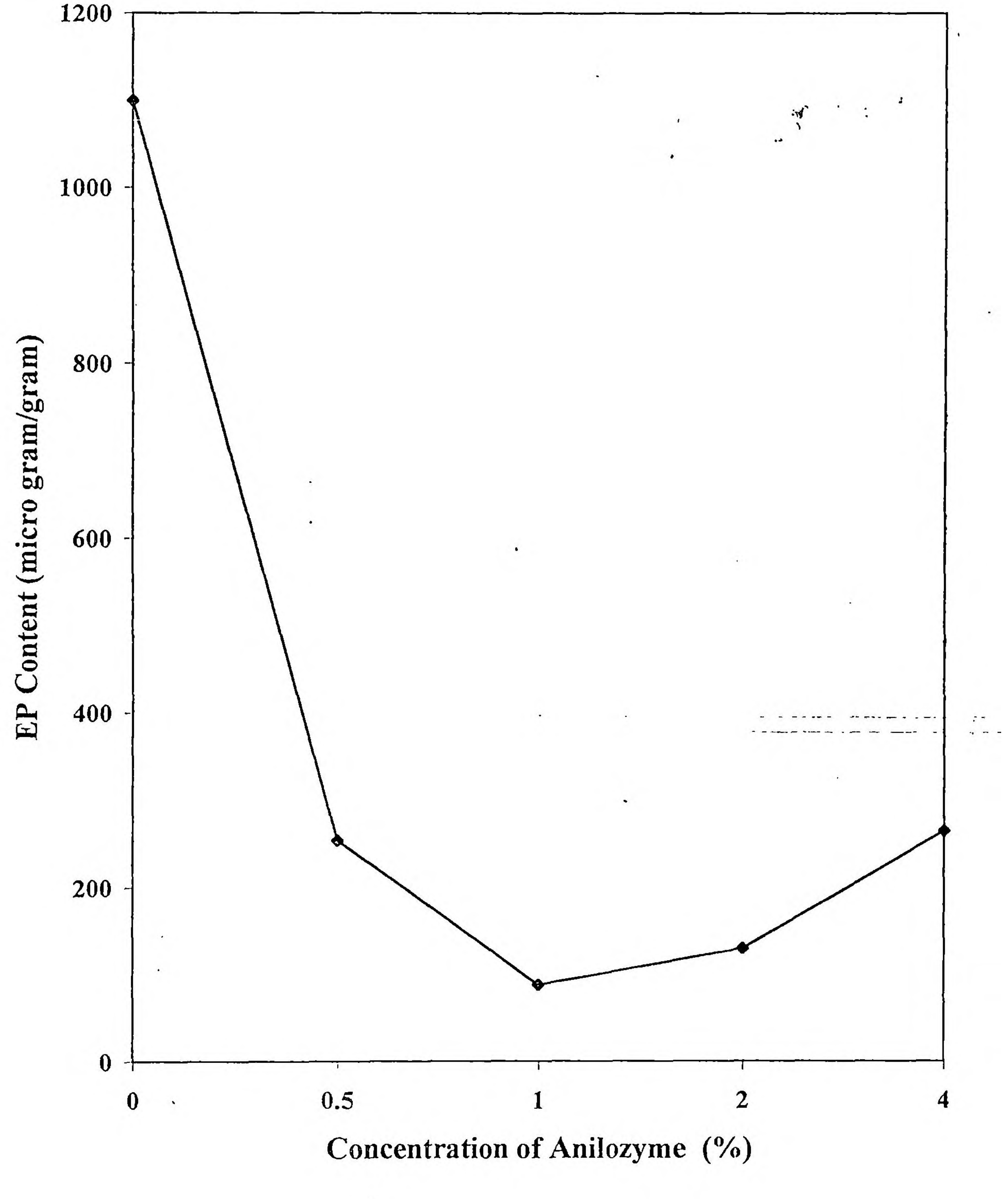


Figure 3.1.2. Wet-gel Leaching
Effect of Time on EP Content

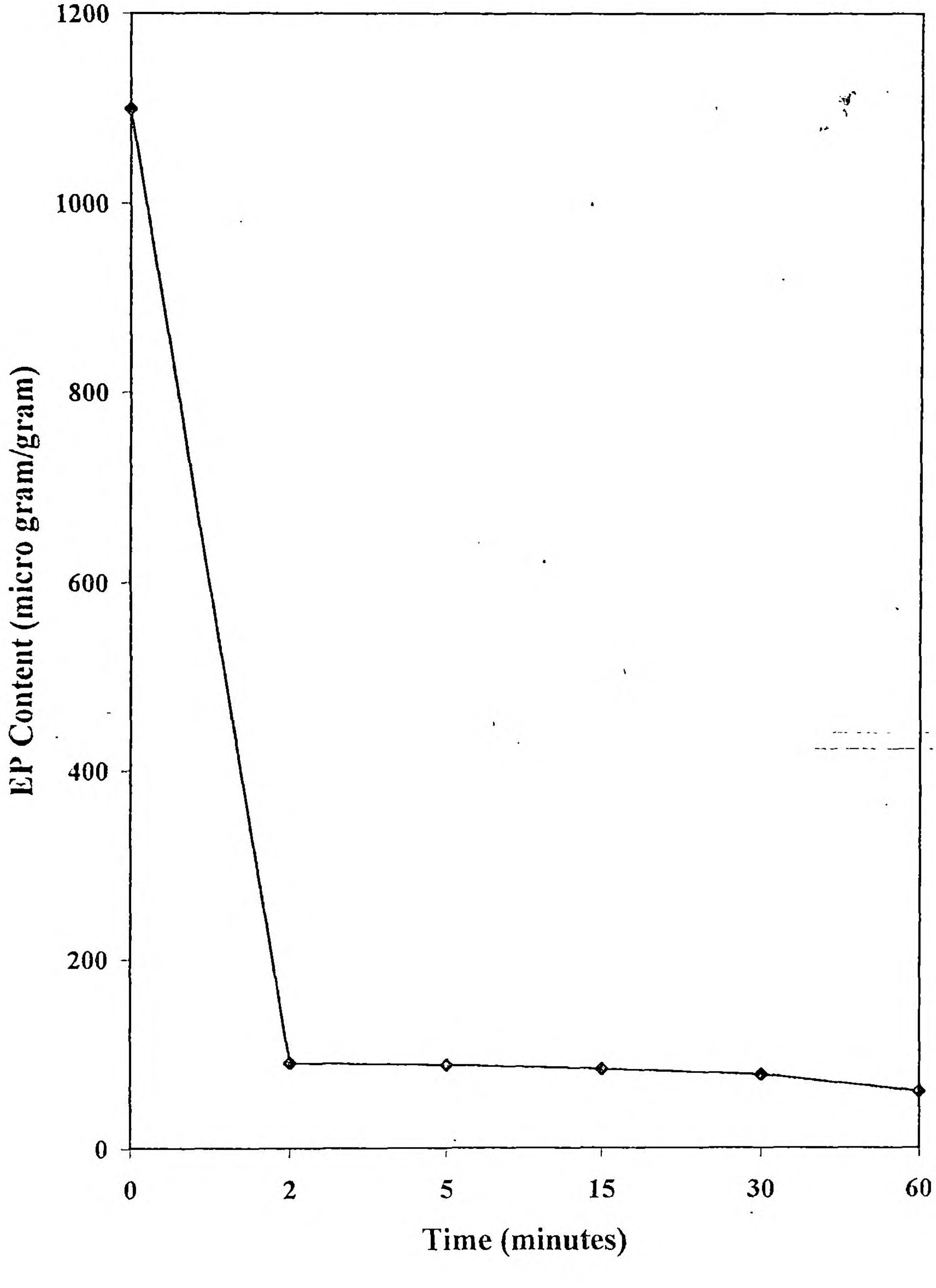
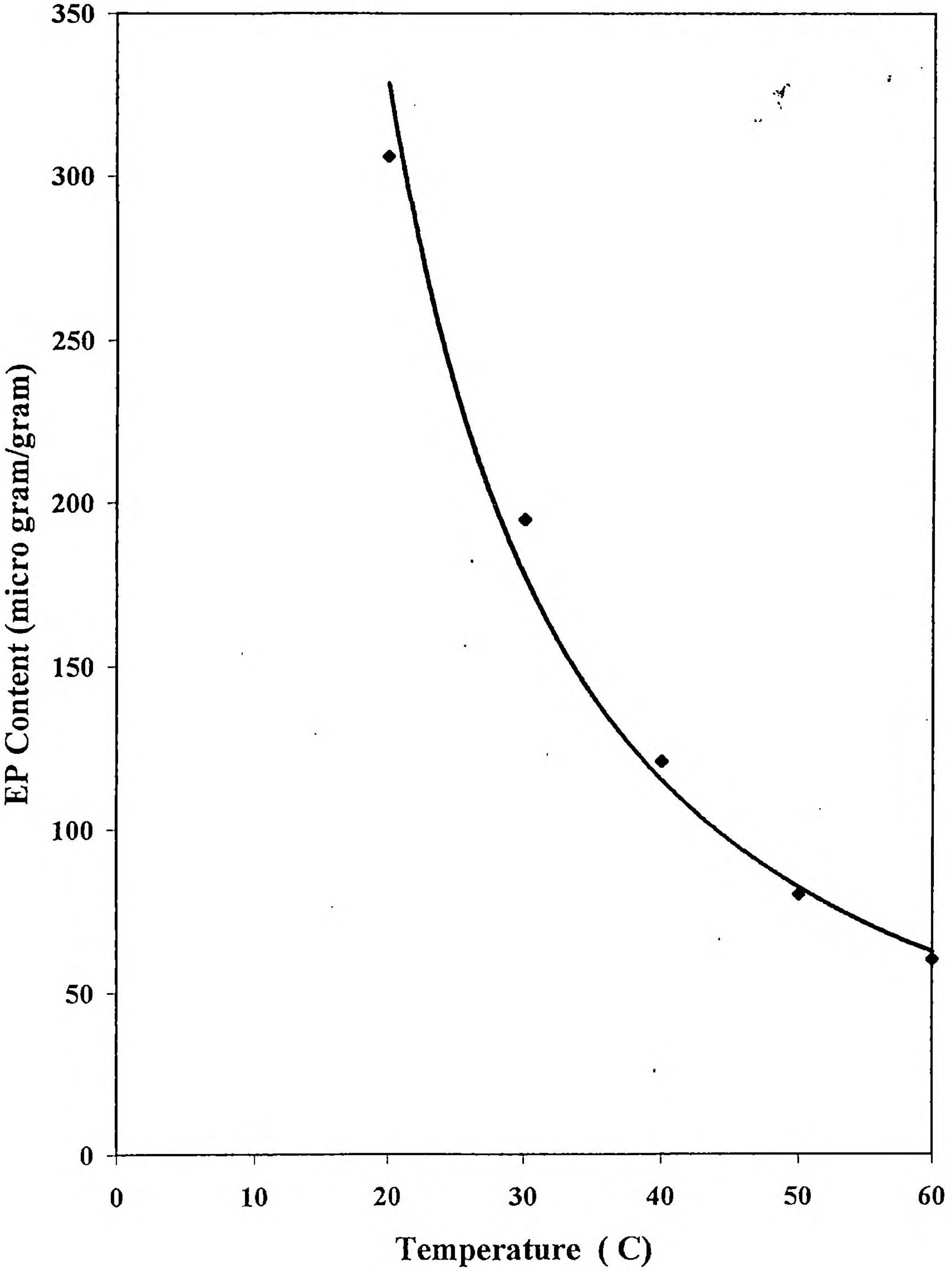


Figure 3.1.3. Wet Gel Leaching

Effect of Temperature on EP Content



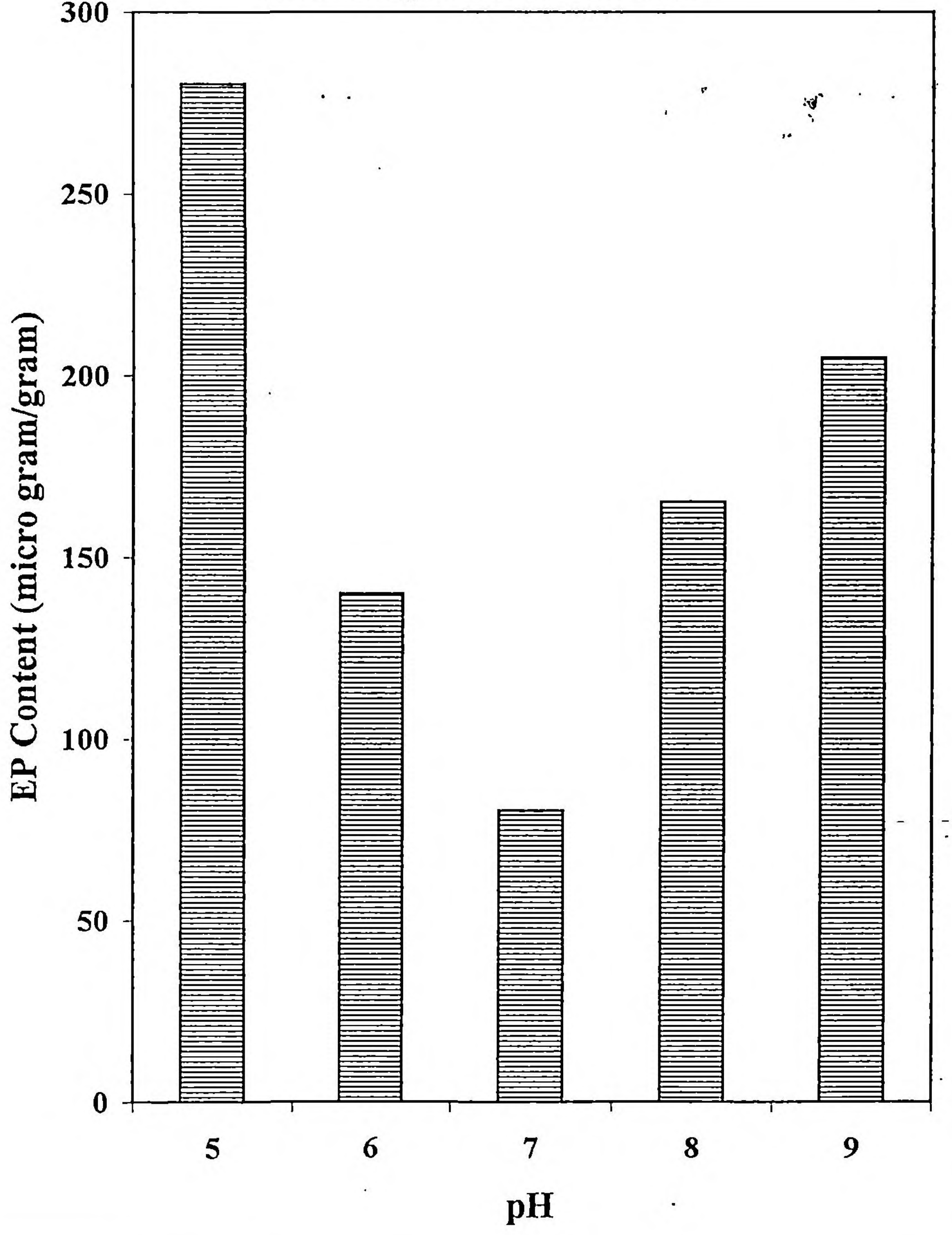


Figure 3.1.4. Wet Gel Leaching
Effect of pH on EP Content

Table III

Effect of addition of Ca^{++} ions in Anilozyme solution

Sl. No.	Treatment	EP content (Micro gram/gram)
1	Wet-gel leached in 1% Anilozyme solution	82
2	Wet-gel leached in 1% Anilozyme + 0.5% Calcium nitrate solution	84
3	Wet-gel leached in 1% Anilozyme + 1% Calcium nitrate solution	82

Figure 3.2.1. Post Cure Leaching

Effect of Concentration on EP Content

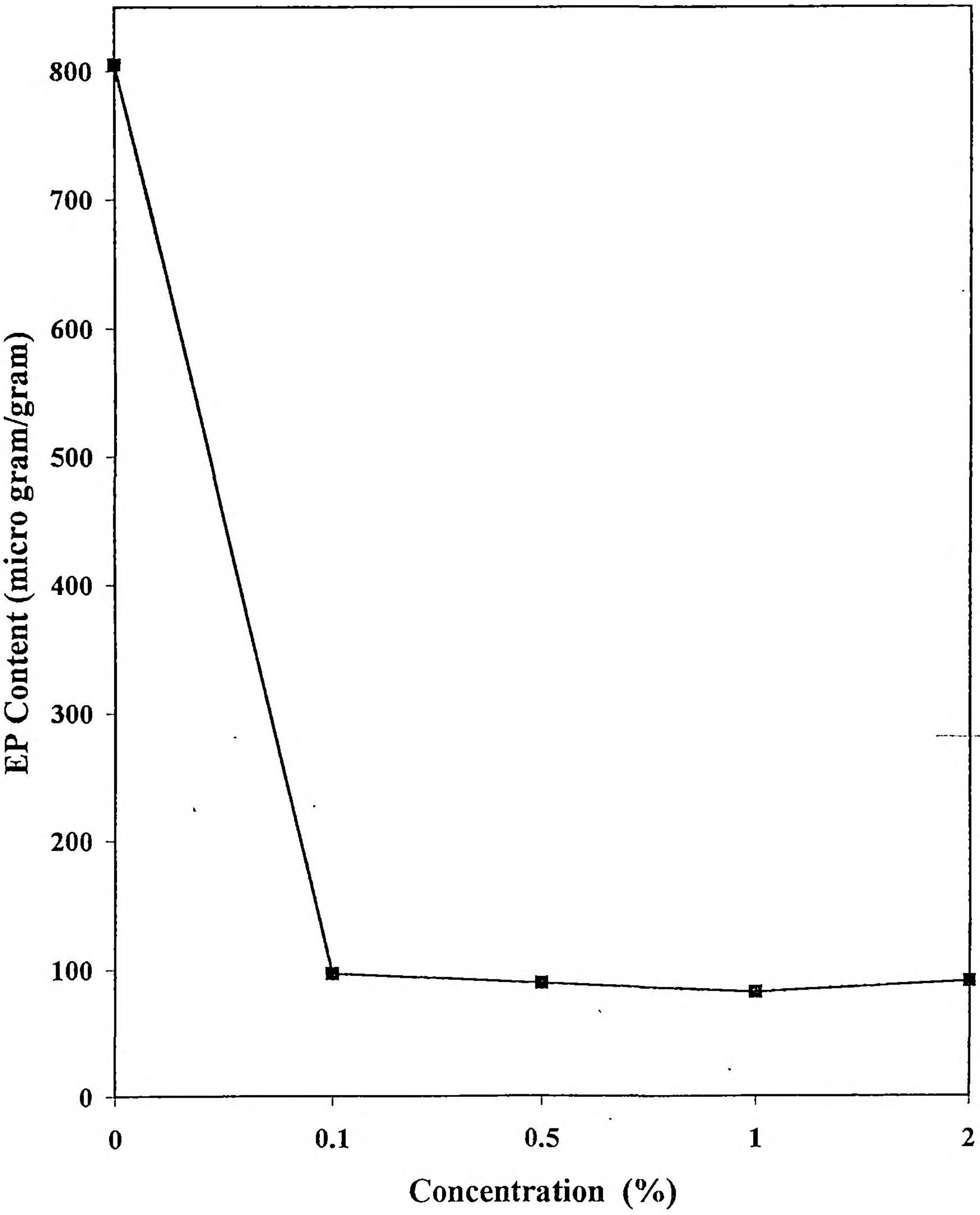


Figure 3.2.2. Post-cure Leaching

Effect of Temperature on EP Content

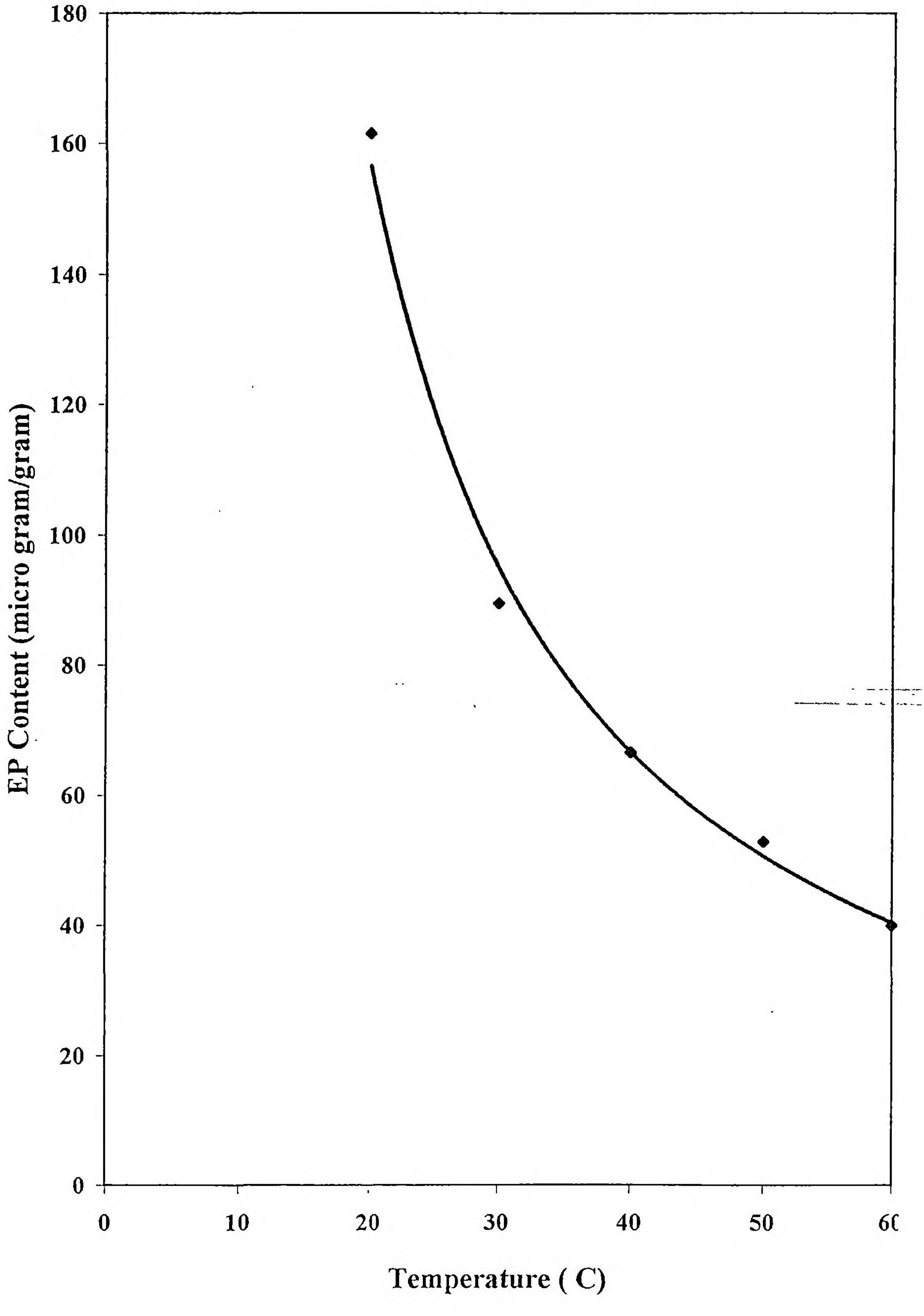


Figure 3.4.
Comparison of Wet-gel Vs Post Cure Leaching

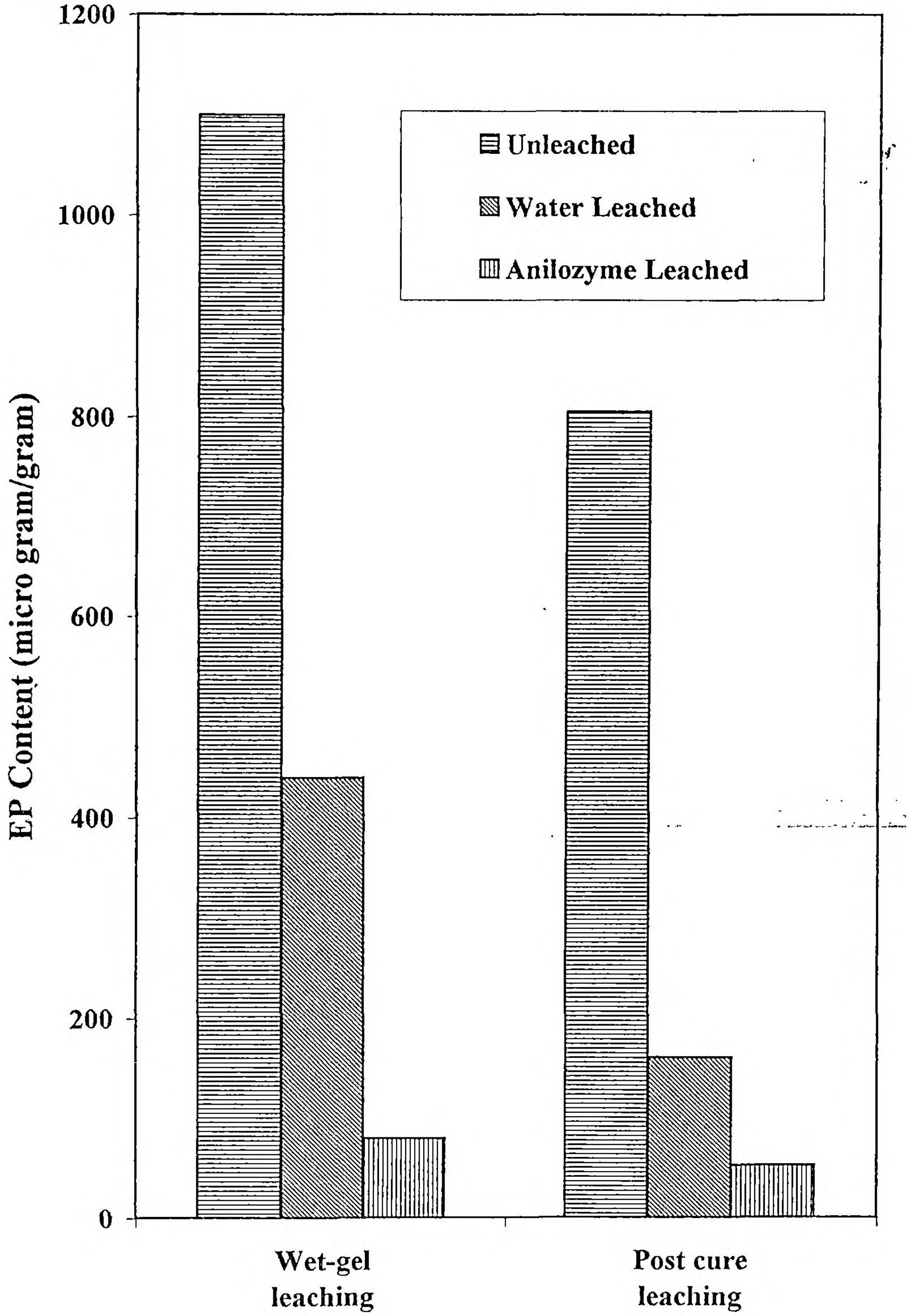


Table 1

Effect of Wet-gel Leaching on physical properties

Treatment	Physical properties					
	Before ageing			After ageing		
	T.S. (Mpa)	E B %	Modulus at 500 % Mpa	T.S Mpa	E B %	Modulus 500% Mpa
Leaching in water for 4′ at 50^{0}C	35	818	6.8	25	840	4.4
Leaching in 0.5% Anilozyme for 2′ followed by water leach for 2′	36	180	6.2	24	855	3.9
Leaching in 1% Anilozyme for 2′ followed by water leach for 2′	36	855	5.9	23	824	4.0

Table II

Effect of post cure leaching on physical properties

Treatment	Physical Properties					
	Before Ageing			After Ageing at 100^{0}C for 22hrs		
	T S (Mpa)	EB %	Modulus at 500 % Mpa	T. S (Mpa)	EB %	Modulus at 500% Mpa
Gloves leached for 5′ in water at 50^{0}C	35	763	7.99	31	723	7.5
Gloves leached in 0.1% Anilozyme for 3′ at 50^{0}C followed by water leaching	34.5	753	8.36	32	704	7.0
Gloves leached in 0.5% Anilozyme for 3′ at 50 °C followed by water leaching	34	750	8.8	32	720	7.3
Gloves leached in 1% Anilozyme for 3′ at50 ° C followed by water leaching	36	753	8.5	31	729	7.5
Gloves leached in 2% Anilozyme for 3′at 50 °C followed by water leaching	36	757	8.2	32	715	7.3

CHAPTER 4

SUMMARY AND CONCLUSION

CHAPTER 1V

SUMMARY & CONCLUSION

4 . 1 Summary

In the present work the effect of leaching of NR latex films in Anilozyme - P solution for reducing EP content is investigated . The effect of both wet gel and post cure leaching have been examined and compared . The technological properties of films have also been studied . From the results obtained , the following conclusions are drawn.

4 . 2 Conclusion

In wet gel leaching at a concentration of 1 % anilozyme solution at 50 ° C for 2 minutes , the extractable protein content is reduced from 1100 to 80 µ gm / gm . Whereas in water leaching the EP content reduced from 1100 to 440 µ gm / gm only .

In post cure leaching at the concentration of 1% Anilozyme at 50 ° C for 2 minutes , the EP content is reduced from 805 to 82 µ gm / gm . Whereas in water leaching the EP content reduced from 805 to 161 µ gm / gm .

Technological properties of the enzyme leached films are comparable to water leached films . The technological properties and the high efficiency of enzyme leaching in wet gel condition implies that it can easily be adopted for the on - line manufacture of latex products .

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