FRACTIONATION AND STRUCTURAL MODIFICATION OF *PLANTAGO OVATA* (Ispaghula) SEED HUSK POLYSACCHARIDES

THESIS

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C E R T I F I C A T E

This is to certify that the work on "Fractionation and Structural Modification of Plantago ovata (Ispaghula) Seed Husk Polysaccharides" embodied in this thesis is original and has been carried out under my guidance and supervision by Shri Prakash C. Khasgiwal. All the work has been done in the laboratories of the Department of Pharmacy, Faculty of Science, Birla Institute of Technology and Science, Pilani.

(B.M. MITHAL)

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CONTENTS

CHAPTER I

INTRODUCTION

GUMS AND MUCILAGES

Plant gums and mucilages have been known to and used by the mankind since very early times. References to gums have been made in the Bible and they seem to have been commercial commodities in India, Asia, Africa, Australia and China for many years[1]. Gums in the form of naturally occurring polysaccharides or modified polysaccharides are consumed in tremendous volume by cosmetics, foods, adhesives, metallurgical, paper, textile and confectionaries[1]. In pharmaceutical industry gums are generally used as suspending, thickening, emulsifying, binding, disintegrating and gel forming agents[1,2].

Gums are hydrophilic macro-molecules, usually with colloidal properties, and in an appropriate solvent or swelling agent produce gels, highly viscous dispersions or solutions at low dry substance content[2]. The term gum as technically employed in industry refers to plant polysaccharides or their derivatives which are dispersible in either cold or hot water and produce viscous dispersions or solutions. The terms gum and mucilage have often been used interchangeably, although in pharmacy a mucilage is deemed to be a solution or dispersion of a gum. Gums are also sometimes referred as polysaccharidic hydrocolloids or polysacolloids. Sometimes the term gums are restricted

to those complex polysaccharides which are exuded from plants either spontaneously or after mechanical injury[3]. A large number of plant families such as Leguminoseae, Anacardiaceae, Combretaceae, Meliaceae, Rosaceae, Rutaceae, Sterculiaceae and Plantaginaceae, etc., are known to produce or secrete gums and mucilages[1,4].

CLASSIFICATION OF GUMS AND MUCILAGES

At a certain point of time gums were classified into two main groups[3]: (a) Real gums that is plant products which formed clear solutions in water, and (b) Vegetable mucilages which swelled but did not dissolve completely in water. This classification was partially useful and not very satisfactory since there were many exceptions. Thus gum Tragacanth, a tree exudate and a true plant gum, is only partially soluble in water[5], and exhibits characteristics normally attributed to mucilages.

Recently attempts have been made to classify gums according to their (a) origin[2,4], (b) physico-chemical properties[6] (such as solubilities, viscosity, feel and adhesiveness) or (c) chemical characteristics[1,2,7,8]. However, the most logical classification seems to be one based on their chemical composition and structure.

Origin wise[2] gums may be classified into the following categories:

(a) Exudate Gums: such as Acacia, Tragacanth, Mesquite, etc.

(b) Sea Weed Gums: e.g., Irishmoss, Alginates, Agar, Carrageenin, etc.

(c) Seed Gums: e.g., Guar, Locust bean, Psyllium gum, etc.

(d) Synthetic or Modified Gums: e.g. Derivatives of Starch and Cellulose, Dextrans, Dextrins, etc.

According to chemical properties following three categories have been suggested by Smith and Montgomery[1]:

Group I: Acidic gums containing

(i) Acid components: e.g. L-guluronic acid, D-glucuronic acid, D-galacturonic acid, Sulphate groups, phosphate groups, or ethers of any of these, and

(ii) Neutral components: such as hexoses, Pentoses, 6-Deoxyhexoses, Sugar alcohols, etc.

Group II: Neutral gums containing only neutral components such as Hexoses, 6-Deoxyhexose, Pentoses, Sugar alcohols, etc.

Group III: Basic gums containing (i) Basic components: such as Amino sugars, Amino alkyl ethers, Amino acids, Polypeptides, etc., and
(ii) Neutral components: such as Pentoses,

hexoses, 6-Deoxyhexoses, Sugar alcohols, or ethers of any of these.

This classification accommodates all the natural gums and mucilages. Although no basic gums and mucilages have been encountered so far in nature, synthetic gums containing basic group will most certainly be synthesized and hence the category of basic gums. Although the structure of individual gums is highly complex yet they may be fitted into the above classification, on the basis of the sugar residues which comprise the interior chain of the molecular structure. In this way polysaccharidic gums of diverse origin may be considered together, and the structural relationship of the exudate and seed gums with other groups of plant polysaccharides may be stressed[9].

MODIFIED GUMS

In general modification of the structure of polysaccharides brings about a significant change in their physical properties. Sometimes modification of a low priced polysaccharides introduces qualities that makes it a valuable substitute for a more expensive gum. Some gums such as gum Karaya, gum Tragacanth, Locust beam gum disperse slowly in water to give highly viscous dispersions. This is a particular disadvantage where an aqueous solution of such gums is desired at a short notice. It has been reported that carboxyl alkyl ethers and specially alkali metal salts of the carboxy alkyl ethers of these gums are

readily dispersible and form solutions of excellent clarity as compared to untreated gum[10]. Similarly, the properties of cellulose and starch molecules can be tailored to make them useful under a variety of circumstances[11-13]. Hence evolution of modified gums has become a stimulating challenge and indications are that gum derivatives are sometimes more useful than the parent materials. In general, the properties of neutral polysaccharides are altered to a remarkable degree by the introduction of small amounts of neutral or ionic substituent group.

Modified gum derivatives may be prepared[2] by the introduction of (a) neutral groups, e.g., methyl, ethyl, hydroxy ethyl, etc., (b) acidic groups, e.g. carboxyl, sulphate, phosphate, etc., or (c) basic groups e.g., amino groups.

Acetates[14], nitrates[15], sulphates and xanthates of polysaccharide gums have been prepared using acid anhydrides, acid chlorides, ketenes, isocyanates, betalactones etc. as esterifying agents. Acetate derivatives of guar[16], locust bean gum[17], alginic acid[18], laminarin[19], cellulose[20,21], and araoanogalactan[22] have been reported. Similarly sulphated derivatives of guar, locust bean gum, agar, laminarin, cellulose and starch have been reported to be blood anti-coagulants and possible substitutes for Heparin[23].

Gums may be oxidized, under controlled condition, with hydrogen peroxide in the presence of Sod. hydroxide, nitrogen dioxide, chromic acid or periodic acid[23]. Gum Karaya[24], Tragacanth[24], Agar[25], Carrageenan[25], Guar and locust bean gum[26] have been oxidized by these techniques.

Water soluble methyl cellulose can be prepared by a one step etherification provided that the ratio of alkali to water in the alkali cellulose is maintained at a high level[27]. Methyl derivatives of starch[28] and cellulose[20] have been reported. Hydroxy ethyl derivatives of locust bean[29], guar[29], laminarin[29], starch[30,31], cellulose[20,32] have been prepared using ethylene oxide or chlorohydrin as the etherifying agent. Similarly carboxy methyl derivatives of acacia[33], tragacanth[33], guar[33], locust bean gum[33], cellulose[25,34] and starch[36,37] have been reported.

PHYSICAL CHARACTERISTICS OF GUMS AND MUCILAGES

The solution of gums in water are colloidal in nature, exhibit swelling pressures and form gel structure at very low concentrations and over a wide range of concentrations. They have low surface tensions, do not crystallize, and act as protective colloids and solubilizing agents. In effect they prevent the agglomeration and settling of finely divided particles of precipitates. These properties of the gums make them valuable in so many manufacturing processes[1,5].

CHEMICAL NATURE OF GUMS AND MUCILAGES

The plant gums are amorphous, contain carbon, hydrogen and oxygen, and are members of the carbohydrate group. In many cases small amounts of nitrogen are detectable[3], but it may be traced to proteinaceous impurities arising from the enzymes responsible for the formation of the gums or from other protein material of the tree.

The plant exudate gums are neutral salts of complex polysaccharidic acids, and are composed of hexose residues, uronic acid residues, pentose residues, and methyl pentose residues, which are joined together in the most diverse manner within the same molecule. With the exception of gum tragacanth, all exudate gums such as almond, arabic, cherry, cholla, mesquite, etc., are distinguished by the fact that D-glucuronic acid is the acid component present in them. Closely related to these plant exudate gums are the mucilages containing complex acidic polysaccherides, extractable from the endosperms of the seeds such as flax, quince, lucerne, guar, etc. The acidic reactions of these mucilages is due to the presence of D-galacturonic acid residues in the molecule[3]. The acidity of some marine gums is due to D-mannuronic acid, 2-keto-D-gluconic acid or sulphate ester residues.

The present state of knowledge of plant gums indicates that the uronic acid component (D-glucuronic acid) is present in the pyranose form. The hexoses encountered

in plant gums are D-galactose and D-mannose and they too have the pyranose form. Glucose has not been found in any of the plant gum examined so far[1]. The pentose arabinose is always found in the furanose form and is a member of the L-series of sugars, while xylose, which occurs in the pyranose modification, belongs to the D-series. The methyl pentose found in plant gums are L-rhamnose and L-fucose and these two assume the pyranose structure[1,3].

The determination of the structure of a gum involves the establishment of its homogeneity, equivalent weight, optical rotation, and uronic acid and Pentosan contents followed by determinations of the nature of the constituent sugars and the uronic acids (if present). Although the chemistry of gums has been reviewed[1,3,38-43] from time to time, very few gums have been tested for their homogeneity or examined from the view point of mode or order of union of the component sugars. In spite of the use of tremendous quantities of gums a clear insight into their chemistry is not available so far. It is, therefore, not surprising that even at the present time the use of gums is more of an art than a science, although certain empirical scientific controls have been employed in the industrial use of gums.

Gums and mucilages rarely consist of pure molecular species and one of the most important and difficult steps in their investigation is their fractionation into individual component polysaccharides. Many gums are mucilages such as gum tragacanth[44], barley gum[45], linseed mucilage[46],

carrageenan[47,48], alginates[49], tamarind gum[50], laminarin[51] and cereal gums[52] have been reported to be mixture of two or more polysaccharides.

GUM FROM SEEDS

Seeds are ancient sources of gums. These gums are often referred to as mucilages because they form a slimy mucilagenous mass upon addition of water[2]. The gum is found as a hard vitreous layer on the inside of the seed coat. Like other natural water soluble gums, the seed gums from quince, psyllium, flax seed, locust bean, guar seeds are meeting stiff competition from synthetic derivatives of starch and cellulose[1,2].

PLANTAGO OVATA (ISPAGHULA) SEED GUM

The genus Plantago belongs to the family Plantaginaceae, which comprises of over 200 minor species of acaulescent or short stemmed herbs, having very small greenish flowers in close bracketed spikes. Commonly used Plantago species are P. ovata, P. indica, P. arenaria, P. lanceolata, P. major, P. rugelli, P. rhodosperma, P. psyllium and P. amplexicaulis. Seeds from Plantago genus have attained considerable importance in the past few years as laxatives, emollients, demulcents, and astringents in medicine; and as sizing, printing and thickening agents in industrial processes[53-55]. Mithal and Khasgiwal[56] recently reviewed literature on P. ovata and

allied species. Plantago ovata Forsk - popularly known as 'Ispaghula' is an annual herb. It is mainly cultivated in India and is known by the following vernacular names[53,54].

Persian	:	Isabghul, Isparzah, Ispoghul
Arabic	:	Bazrekatima, Bazrequatuna
Sanskrit	:	Ishabgola, Snighabja
Hindi	:	Isapgol, Ispaghula, Isabghul
Bengali	:	Ishopgol, Isabghul, Ispaghul
Gujarati	:	Isafghol, Isapghol
Punjabi	:	Bartang, Isapghol
Tamil	:	Ishappukol, Iskol
Malayalam	:	Karkatasringi

The dried coats of the seeds of P. ovata Forsk, obtained by crushing the seeds and separating the husk by winnowing[57,58] are marketed as Ispaghula husk. According to B.P.C. (1968) the husk contains a greater proportion of mucilage than the seeds and is more effective in lower doses[59]. Crushing of the seeds is done with emery grinders or flat stone grinding mills which may be hand or power driven. The seeds after thorough cleaning are passed through these mills 6 or 7 times for complete removal of the husk. The husk obtained (Total yield - 20 to 27%) from different millings vary in quality. The husk of the seeds contain all the mucilaginous matter and is sometime prescribed instead of the whole seeds[58]. Husk is thin, boat shaped (2-3 mm × 0.5-1.0 mm) white transluscent, odorless, and having mucilagenous taste. According to

I.P.[57] it should contain Foreign organic matter ≯ 2%, Ash ≯ 2.9% and Acid insoluble ash ≯ 0.45%.

More than 800,000 Kg of Ispaghula seeds and more than 3 million Kg of husk are estimated to be exported annually[58] (Actual figures for 1966-67 : 884,053 Kg *P. ovata* seeds, and 3,580,389 Kg of Ispaghula husk[58]).

Physico-Chemical Properties of P. ovata Seeds

(A) Mucilage content and swelling properties:

P. ovata seeds have been reported[60] to contain 30.9% mucilage, while the mucilage content of *P. inflexa*, *P. rhodosperma* and *P. helleri* is reported[61] to be slightly less. Greenberg[62] studied the mucilage content of *P. ovata* and other species and described the method of its quantitative expression from the seeds. Since the laxative properties of the mucilage are related to its swelling capacity, appreciable amount of work has been done on its swelling characteristics[61-64]. Swelling factor of *P. ovata* seed husk[65] has been reported to be 90.

(B) Rheology:

Baveja et al.[66-68] reported the effect of concentrations, storage condition, effect of electrolytes, non-aqueous solvents and stirring on the flow properties of *P. ovata* seed husk mucilage. A fresh dispersion of *P. ovata* seed husk in water has been reported to possess negative thixotropy.

Chemistry of P. ovata Seeds and Husk

P. ovata seeds contain proteins, a fixed oil, mucilage, some cellulose and traces of starch. A glycoside named aucubin ($C_{13}H_{19}O_8H_2O$) was isolated from the plant and reported to be pharmacologically inactive. A non-reducing trisaccharide plantiose was isolated by French et al.[69] which on hydrolysis gave glucose, fructose and galactose. Fixed oil is present to the extent of 11.42% and contains saturated and unsaturated fatty acids. Atal et al.[70] while studying the oil of P. ovata emoryo reported that the same may be used as common source of linoleic acid. The acid equivalent[71] of P. ovata seed gum acid obtained by direct titration with 0.1N Sodium hydroxide has been found to be about 10,000.

The seeds of the P. ovata upon extraction with hot and cold water yield polysaccharide which differ in chemical composition[2,3]. Extraction of the seeds with cold water gives a product with a uronic acid content of 20% and a pentose content of 52%, whereas polysaccharides extracted with hot water at 90-95° give a product with a uronic acid content of 3% and pentose content of 90%.

The seed mucilages of Plantago genus have been studied widely but so far no account of their chemical structure is available.

Pharmaceutical Applications of P. ovata Seed Husk

Mithal and Kasid[72] studied the emulsifying capacity of Ispaghula husk and its cold water soluble mucilage (CWSM) and reported that 1.0% w/v CWSM of Ispaghula husk produces stable emulsion with Light Liquid Paraffin and Arachis oil. The HLB values of Ispaghula husk and its 1.0% w/v CWSM have been reported to be 9.34 and 9.54 respectively[73].

Mithal and Gupta[74,75] observed that a 0.8% w/v dispersions of the husk is good suspending media for different materials and reported the effect of homogenization, heat, pH, aging and added substances on the viscosity of 0.8% w/v Ispaghula husk mucilage. Baveja et al.[76] reported the rheology of various suspensions prepared with P. ovata seed husk and made an attempt to correlate rheological parameters to the sedimentation rates. Mithal and Gupta[77] reported the compatibility of P. ovata seed husk with a selected range of inorganic and organic materials. Out of the total of 31 substances tested only Lead subacetate was found to be incompatible with husk mucilage.

The possibility of the use of husk as a disintegrant for compressed tablets was studied by Mithal and Bhutiani[78]. They reported that the husk powder when included to the extent of 1.0 to 2.0% w/w of the total weight of the tabletting material disintegrates tablets

rapidly. Kamath and Srivastava[77] reported the possibility of use of husk powder as a sustained release factor in tablet formulation.

Mithal and Bhutiani[80] reported a 2.0% w/v mucilage of husk in cold water to compare favourably with starch mucilage (10% w/v) in its binding properties.

Pabrai et al.[81] indicated that a mixture of 1.25 to 1.5% Ispaghula husk with 0.25-0.5% Agar (mixture known as Isphagar) could be substituted for agar in preparing culture media for microbiological manipulations. Gel forming properties of Ispaghula husk have been studied by Zacharias and Mithal[82]. A 2.0% w/w husk powder with 30 parts of glycerol and 68% parts of water has been reported to be an ideal water soluble ointment base. Addition of surfactant (HLB-8.0) was found to decrease its yield value and antithixotropy[83].

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CHAPTER II

STUDY - ENVISAGED

Plantago ovata seed husk is now a commercial article of some repute. Large quantities of this commodity are being consumed at home and sizeable quantities exported every year. But in spite of the medicinal attributes and pharmaceutical potentialities of Ispaghula husk the chemical nature of this material is very poorly understood. Generally gums are known to be mixtures of different species of polysaccharides. Hence as a first step towards the delineation of its chemical composition the separation of the naturally occurring material into its component polysaccharides and their characterisation was undertaken.

The substitution of some groups on the polysaccharide molecules is known to impart changed physical characteristics to them. This manipulation has yielded many commercially important materials. For instance derivatives of cellulose and starch find wide applications in a range of industries. Hence it was considered worthwhile preparing certain derivatives of the Ispaghula husk and studying their physico-chemical characteristics and pharmaceutical applications in comparison to those of the naturally occurring husk.

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CHAPTER III

FRACTIONATION OF PLANTAGO OVATA SEED HUSK POLYSACCHARIDES

THE MATERIAL

Twenty five packets (100 g. each) of Plantago ovata (Ispaghula) seed husk (B.G. Telephone Brand, Sidhpur, Sat-Isabgol Factory, Sidhpur - Gujarat, India) were procured and combined into a single lot by proper mixing. The material consisted of white, translucent, thin boat shaped structures, having no odour but possessing a mucilaginous taste. The mixed lot of Ispaghula husk was dried in a hot air oven at 50° for 24 hours and then powdered. The fraction passing through sieve No. 100 mesh (aperture 0.152 mm) was collected and stored in tightly closed container. This material henceforth referred as 'the husk' was used for the following work.

PURIFICATION OF THE HUSK POLYSACCHARIDES

The naturally occurring and commercially processed gums often contain variable amounts of foreign matter. Hence for any scientific work it is essential that they are obtained in a pure form. Most often the purification of water soluble gums is achieved by precipitating them from their aqueous solutions/dispersions by alcohol or acetone. The Ispaghula husk gum was purified by precipitating it from its aqueous dispersion with alcohol as discussed below.

The husk (10 g.) was extracted with sodium hydroxide solution (1.0% w/v, 1 lit.) for 24 hours by stirring with

mechanical stirrer (4,000 r.p.m.) at room temperature (30°), since alkaline solutions generally give oetter extraction of gum polysaccharides[1]. After passing through a muslin cloth, the filtrate was centrifuged at 2,000 r.p.m. for 20 mts. The supernatant liquid was dialysed against running water for two days and then ethanol containing 1% v/v Hydrochloric acid was added to it. The precipitate, thus obtained, was recovered by centrifugation. It was again dispersed in small amount of distilled water and reprecipitated with acidified ethanol. This process was repeated twice, the precipitate being repeatedly washed with absolute alcohol-methanol mixture, till the filtrate was free from chloride ions. The precipitate was finally dried at 40° under vacuum, powdered and stored in a vacuum desicator. The final product (7.5 g.) was a white powder corresponding to 75% w/w yield (Sample-I). 10 g. and 50 g. portions of the husk were again processed in a similar manner giving 7.8 g. (78% w/w - Sample-II) and 37.5 g. (75% w/w - Sample-III) of the purified product respectively. Hence average yield of the purified product (purified husk) was reckoned to be 76.0% w/w.

CHARACTERISATION OF PURIFIED HUSK

The purified husk was characterised in comparison with the husk in the following respects.

(i) Infrared Spectra

Infrared spectroscopy is a rapid means of the identification of specific polysaccharides[84]. It can also be used as a criterion of the purity, and in the quantitative determination and identification of functional groups in chemical compounds[85,86].

Experimental

Small amounts of Samples I, II and III were thoroughly mixed with Potassium bromide (Analar grade) previously dried under vacuum. The mixture was placed in a die and a very thin film (pressed disc) was prepared by applying pressure with the help of a mechanical press (Fabricated in B.I.T.S. Workshop). The IR spectra of this disc was recorded (Fig. 1), in 2.5-15.5μ (4000-650 cm^{-1}) region, using Perkin-Elmer Infrared Spectrophotometer (Scan speed : slow, Slit : normal). The IR spectra of the husk was also recorded (Fig. 2).

Results and Discussion

It was found that Infrared spectra (Fig. 1) of purified husk, sample I, II and III, were identical (superimposable) indicating that above mentioned procedure for purification was suitable and reproducible giving purified product of same chemical composition.

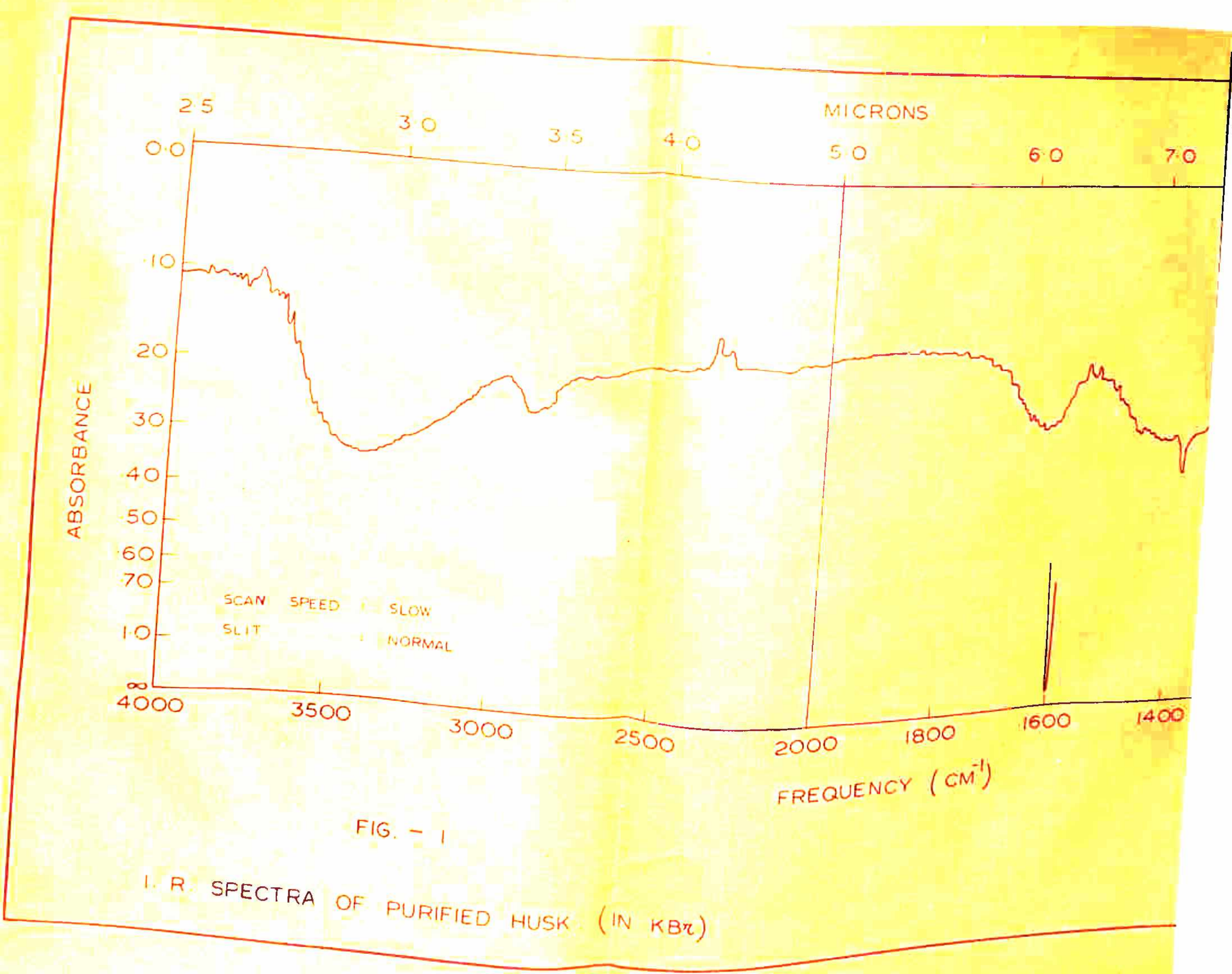


FIG. – 1

I. R. SPECTRA OF PURIFIED HUSK (IN KBr)

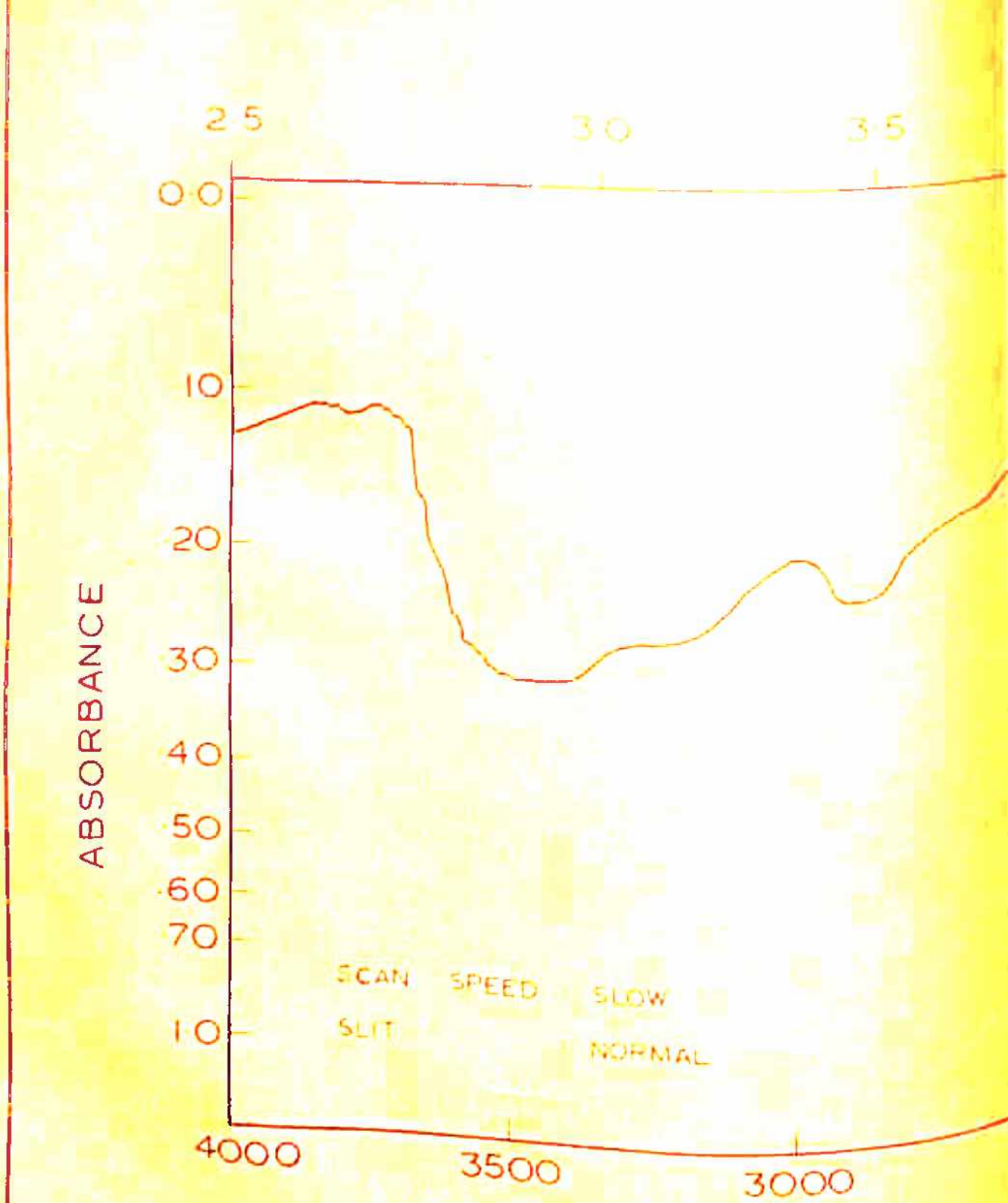
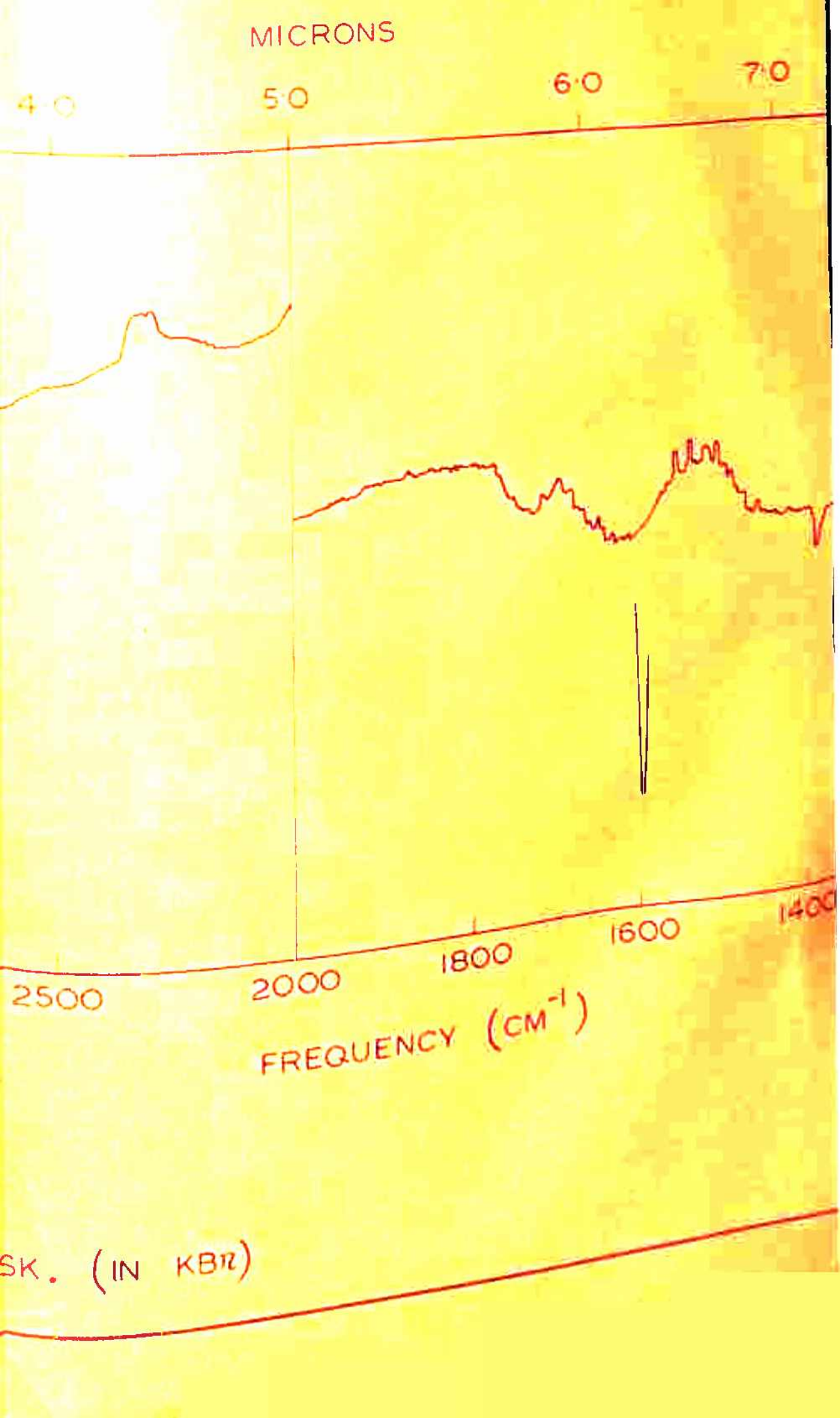


FIG. - 2

I. R. SPECTRA OF ISPAGHULA HU


MICRONS
4·0
5·0
6·0
7·0
2500
2000
1800
1600
1400
FREQUENCY (CM⁻¹)
SK. (IN KBr)

(ii) Ash Value[87]

Weighed amounts of the husk and the purified husk (Sample I, II and III) were taken in tared silica crucibles and incinerated by gradually increasing the heat. The heating was continued until samples were free from carbon. Crucibles were cooled and weighed to constant weights. The percentage of ash was calculated with reference to air dried husk[87]. Results are recorded in Table-1.

TABLE-1

Ash Content of the Husk and Purified Husk

Sample	Weight taken (g.)	Weight of ash (g.)	Ash content (%)	Average value (%)
Husk	1.0300	0.0220	2.13	
	1.0266	0.0208	2.04	
	1.0200	0.0212	2.07	2.08
Sample-I	1.0425	0.0066	0.63	
	1.0450	0.0070	0.67	0.65
Sample-II	1.0580	0.0068	0.64	
	1.0490	0.0064	0.61	0.625
Sample-III	1.0280	0.0062	0.60	
	1.0220	0.0061	0.60	0.60

(iii) Chemical Tests

Reactions of the purified husk (Sample I, II and III) with different chemical reagents were carried out as recommended by Smith et al.[1] and Jacob[88]. Similar tests were also carried out simultaneously on unpurified husk.

Experimental

0.5 g. samples of purified husk were dispersed in water (100 ml) and the resulting mucilage centrifuged at 2,000 r.p.m. for 20 minutes. To 5.0 ml of this test solution, 5.0 ml of chemical reagents (listed in Table-2) were added. Observations are recorded in Table-2.

(iv) Test for Halogens, Sulphate and Nitrogen

Tests for chloride, sulphate and nitrogen were carried out on the purified husk as below[89,90].

Experimental

A small amount of purified husk (Sample I, II and III) was fused separately with metallic sodium in a fusion tube, which was plunged, while still hot, in a china dish containing water. The admixture was heated and filtered, and the filtrate tested for the presence of chlorine, sulphur and nitrogen. The natural husk was worked up similarly.

For Chloride. 1.0 ml of the filtrate was acidified with dil. sulphuric acid, diluted with distilled water

TABLE - 2

S. No.	Chemical Reagents	Unpurified husk	Purified husk		
			Sample I	Sample II	Sample III
1.	Stokes Acid Mercuric Nitrate Reagent	No ppt.	No ppt.	No ppt.	No ppt.
2.	Neutral Lead acetate (20% w/v) Sol.	Flocculent pale yellow ppt.	Flocculent pale yellow ppt.	Flocculent pale yellow ppt.	Flocculent pale yellow ppt.
3.	Basic lead acetate (AOAC)	Curdy ppt.	Curdy ppt.	Curdy ppt.	Curdy ppt.
4.	Neutral Ferric Chloride	No ppt.	No ppt.	No ppt.	No ppt.
5.	Pot. Hydroxide (10% w/v)	No ppt.	No ppt.	No ppt.	No ppt.
6.	Millon's Reagent	No ppt.	No ppt.	No ppt.	No ppt.
7.	Borax (4% w/v)	No ppt.	No ppt.	No ppt.	No ppt.
8.	Iodine Sol.	No reaction	No reaction	No reaction	No reaction
9.	Absolute alcohol	White fibrous ppt.	White fibrous ppt.	White fibrous ppt.	White fibrous ppt.

(30 ml) and then boiled down to 4 ml in a dish. After cooling, 1.0 ml of dil. nitric acid and 1.0 ml of silver nitrate solution was added. Since no ppt. was obtained in any case absence of chlorides was indicated.

For Sulphate. To 1.0 ml of the filtrate, freshly prepared solution of sodium nitroprusside was added. Absence of purple color in any of the tested samples, indicated absence of sulphur (sulphate) in all of them.

For Nitrogen. To 1.0 ml of the filtrate, few crystals of ferrous sulphate were added, and after shaking 3 drops of Sod. hydroxide solution (5.0% w/v) was incorporated. Boiled the solution for half a minute and cooled. Acidified with dil. hydrochloric acid. Absence of blue ppt. or greenish blue color in all of the samples, indicated absence of nitrogen.

Result

Chlorine, sulphate and nitrogen were found to be absent in the husk and in all the three samples (I, II and III) of purified husk.

(v) Test for Carbohydrates[89]

Aqueous dispersions (0.5% w/v) of husk and purified husk (Sample I, II and III) were prepared and tested as below.

For Carbohydrates. To 2.0 ml of aqueous dispersion 2.0 ml of Molisch's reagent was added[89] and 2.0 ml of conc. sulphuric acid added along the side of the tube to form a layer under the aqueous solution. A red-violet ring developed at the junction of the two layers and on shaking gave dull violet precipitate, indicating presence of carbohydrates in all the samples.

For Free Reducing Sugars. Equal amounts of Fehling solutions A and B (B.D.H.) were mixed. To 1.0 ml each of the dispersions Fehling solution was added and the mixture heated. Since there was no reduction, free reducing sugars were assumed to be absent.

FRACTIONATION

In the study of polysaccharide gums one of the first requirements is that the material be chemically homogeneous i.e. it should consist of only one type of molecules[41]. To establish homogeneity or otherwise various methods of fractionation have been used from time to time which were reviewed recently by Mithal and Khasgiwal[91]. Methods commonly used are Fractional precipitation with organic solvents, Fractional precipitation with complexing agents, Graded extraction method, Electrophoresis, Column chromatography, Ultrafiltration, Gel filtration and Ultra centrifugation. These techniques ultimately depend on assessment of the homogeneity of the products by measurements of the

specific rotation, viscosity, molecular weight, electrophoresis and on the component sugars in each fraction. Additional proof of the homogeneity of a polysaccharide fraction may be obtained by I.R. studies[85,92,93], differential thermal analysis[94,95] and X-Ray crystallographic examinations[96,97].

Various complexing agents such as metallic salts[98], borax and quaternary ammonium compounds[99] have been widely used for fractionation of polysaccharide mixtures. Frequent use of Fehling solution[100,101,102], cupriethylene diamine[103,104,105], cupric chloride[106], cupric sulphate[107] and cupric acetate[108-110] is on record. The insoluble polysaccharide-copper complex formed by treatment of polysaccharides with cupric ions are decomposed by acidic-alcohol (e.g. ethanol containing 5.0% v/v conc. Hydrochloric acid) and the free polysaccharides washed until washing gives negative test for chloride ions.

ELECTROPHORETIC SEPARATION

Electrophoresis has been used for establishing the homogeneity of polysaccharide materials[111,112] and for assessing minimum number of constituent polysaccharides in a given material[113-116]. Acidic polysaccharides can be readily separated from the neutral ones by electrophoresis in non-complexing buffers such as acetate, phosphate or veronal buffers[11]. Borate buffer forms complexes with neutral polyhydroxy compounds of certain structural

configuration. The complex thus formed has a negative charge and migrates towards the anode, but on strip support and on the glass column at pH 9.3, electro endosmosis is great and may result in a net movement towards the cathode[117]. Use of other complexing ions such as sulphonated benzeneboronic acid, germanates, stannates, arsenite, vanadate, tellurate, etc. have also been reported[118]. Electrophoresis using glass powder columns has been reported for fractionation of polysaccharide mixtures on marcoscale[119,120]. Theoretical aspects and general procedure of zone electrophoresis have been reviewed[111,112,121]. The electrophoretic behaviour of purified Ispaghula husk was studied using borate buffer (complex forming) and acetate buffer (non-complexing) to assess its homogeneity.

(A) Zone Electrophoresis using Borate Buffer (pH 9.2)

Zone electrophoretic studies of the purified husk were carried out using Thomas Electrophoresis Cabinet (Model-20, Cat. No. 4937-V-20) and Thomas Electrophoresis power supply unit (Model-20, Cat. No. 4937-V-30). Whatman chromatographic paper (No. 1) was used as paper strip support for the material. The positions of the polysaccharides were detected by the method of elution and the colorimetric estimation using α-naphthol in conc. sulphuric acid[111,122].

Experimental

1.0 litre Borate buffer (0.05 M, pH 9.2) was

prepared[123] and its pH determined using Beckmann pH meter (Model H-2). Whatman No. 1 chromatography paper strip (28 cm length) was used as support. Paper strip was dipped in borate buffer, dried and the sample (0.1% w/v purified husk in water) was introduced by using micropipette onto the strip as compact zone along the origin line (which was at right angle to the length of the paper). The strip support was suspended horizontally in the electrophoresis chamber. Voltage (from anode to cathode was 300 mV, 40 mA) was applied for 4 hours. The paper was removed thereafter and dried. It was then cut into 2 cm segments which were numbered consecutively from the anode end and the polysaccharides on each segment were eluted, by distilled water, into correspondingly numbered tubes. The eluates were made up to 5.0 ml in each case and 3.0 ml of α-naphthol-conc. sulphuric acid reagent[124] added (The reagent was stored for at least 8 hours in the dark before use[124]). The tubes were then heated in a water bath for ten minutes, their contents cooled and absorptions determined at 550 mμ using spectronic-20 (Bausch and Lomb). A blank was carried out simultaneously using 2.0 cm segment from the same paper strip. Results are recorded in Table-3 and graphically represented in Fig.-3.

(B) Electrophoresis using Acetate Buffer

The zone electrophoresis was conducted in the manner described above, using Acetate buffer (pH 4.0, ionic strength $\mu = 0.1$) of the following composition:

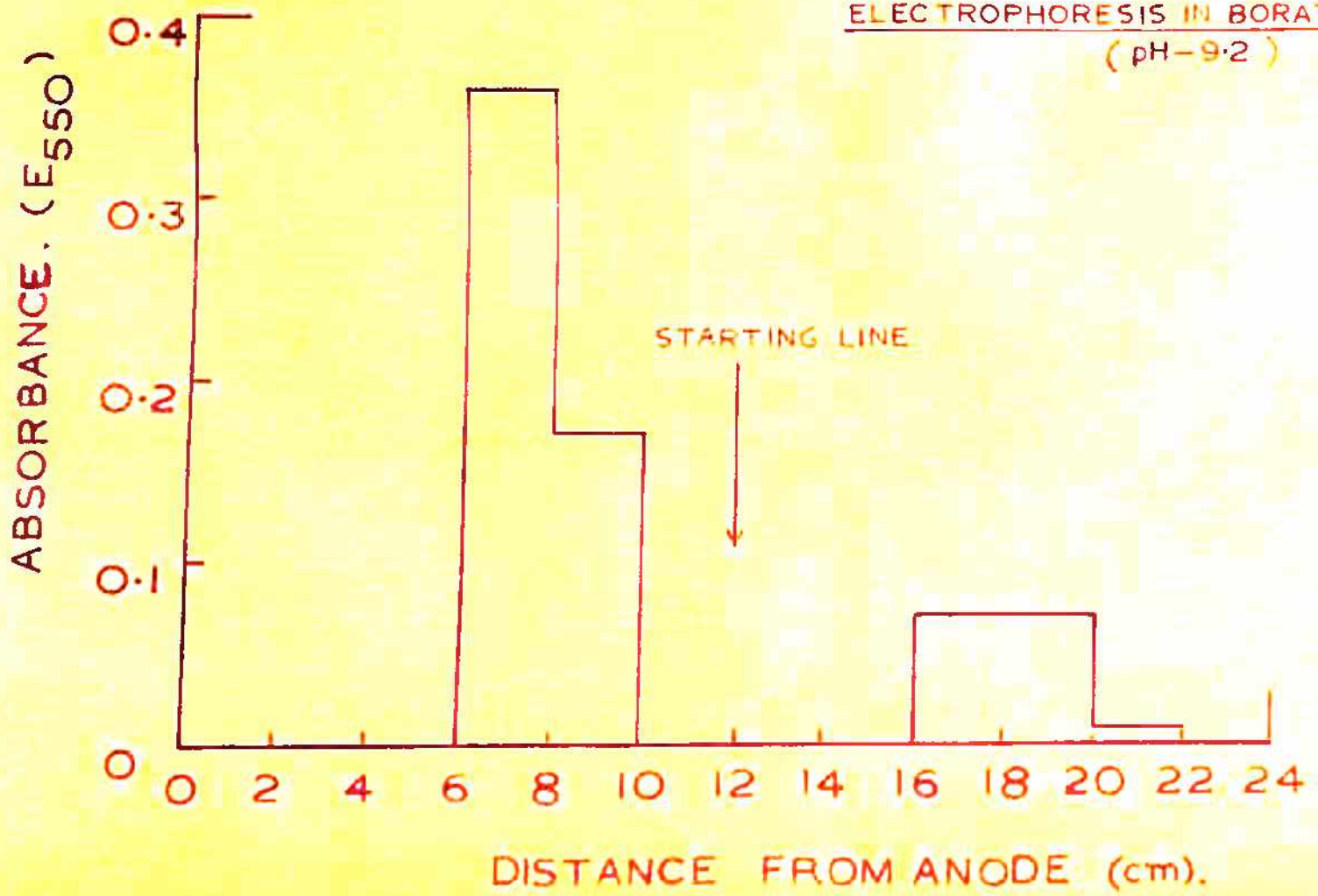
TABLE - 3

Electrophoresis of purified husk in Borate buffer (pH 9.2)

Tube No.	Distance from anode (cm)	Absorbance at 550 mμ		
		I	II	Average
1	Blank	0.0	0.0	0.0
2	0.0 to 2.0	0.0	0.0	0.0
3	2.0 to 4.0	0.0	0.0	0.0
4	4.0 to 6.0	0.0	0.0	0.0
5	6.0 to 8.0	0.36	0.36	0.36
6	8.0 to 10.0	0.17	0.17	0.17
7	10.0 to 12.0	0.0	0.0	0.0
8	12.0 to 14.0	0.0	0.0	0.0
9	14.0 to 16.0	0.0	0.0	0.0
10	16.0 to 18.0	0.07	0.07	0.07
11	18.0 to 20.0	0.07	0.07	0.07
12	18.0 to 22.0	0.01	0.01	0.01
13	22.0 to 24.0	0.0	0.0	0.0

FIG-3

ELECTROPHORESIS IN BORATE BUFFER
(pH-9·2)



Glacial acetic acid	.....	25.89 ml
Sodium acetate	.....	13.61 g.
Distilled water - ad	... q.s.	1.0 lit.

Ooservations are recorded in Table-4 and the histogram is shown in Fig. 4.

TABLE - 4

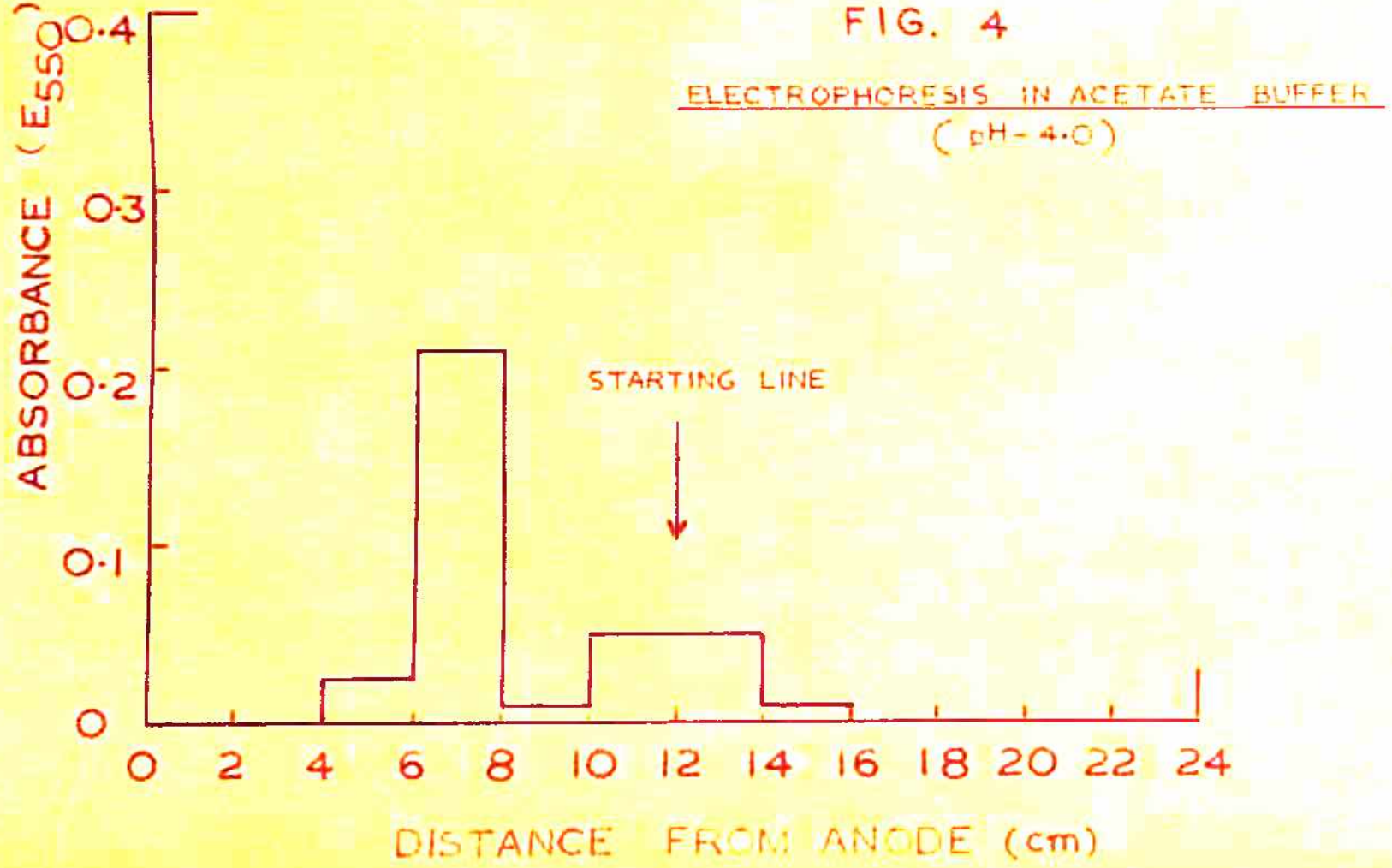
Electrophoresis of Purified husk in Acetate buffer (pH 4.0)

Tube No.	Distance from anode (cm)	Absorbance* at 550 mμ ($E_{550\ m\mu}$)
1	Blank	0.0
2	0.0 to 2.0	0.0
3	2.0 to 4.0	0.0
4	4.0 to 6.0	0.025
5	6.0 to 8.0	0.21
6	8.0 to 10.0	0.01
7	10.0 to 12.0	0.05
8	12.0 to 14.0	0.05
9	14.0 to 16.0	0.01
10	16.0 to 18.0	0.0
11	18.0 to 20.0	0.0
12	20.0 to 22.0	0.0
13	22.0 to 24.0	0.0

*Average of two readings

FIG. 4

ELECTROPHORESIS IN ACETATE BUFFER
(pH - 4·0)



Results and Discussion

It is evident from Figs. 3 and 4 that purified Ispaghula husk is heterogeneous in nature and consists of at least two polysaccharide components. With Borate buffer (complexing agent) it gives two major peaks, one on the anode and the other on the cathode side. With acetate buffer (pH 4.0) it gives one major peak on anode side and another on the starting line itself, indicating non-migration in an electrical field. Since acetate buffer is non-complexing the stationery peak may possibly correspond to neutral polysaccharide. Hence it was concluded that P. ovata seed husk consists of at least two polysaccharidic components one acidic and one neutral.

FRACTIONATION WITH COPPER(II) ACETATE

Fractionation of purified husk polysaccharides was attempted at using cupric acetate - ethanol as the precipitant, according to the general procedure recommended by Jones et al.[125]. The parameters used to assess whether fractionation has been effected were: optical rotation, equivalent weights, IR, and paper chromatographic examinations of the sugar components produced on complete hydrolysis of the fractions.

Experimental

Purified husk (1.0 g.) was dissolved in 200 ml of sodium hydroxide solution[125] (0.5% w/v) and filtered. 10 ml

each of the filtrate was transferred to serially numbered tubes (1 to 10) and copper acetate solution (10% w/v) added to each tube in increasing volumes (Table-5). The blue gelatinous precipitate obtained in each case was removed by centrifugation at 10,000 r.p.m. for 15 mts.

TABLE - 5

Weight percentage of Copper Complex obtained with different volumes of copper acetate solutions

S. No.	Mucilage 0.5% w/v (ml)	Copper acetate (10% w/v) solution (ml)	Weight fraction after decomposition of copper complex (g.)	Yield w/w (%)
1	10.0	0.1	No ppt.	-
2	10.0	0.2	No ppt.	-
3	10.0	0.3	No ppt.	-
4	10.0	0.4	No ppt.	-
5	10.0	0.5	No ppt.	-
6	10.0	1.0	0.020	40%
7	10.0	2.0	0.025	50%
8	10.0	3.0	0.035	70%
9	10.0	4.0	0.035	70%
10	10.0	5.0	0.035	70%

(JANETZKI Laboratory Centrifuge, Model T-24). To regenerate the polysaccharide, the insoluble copper complex was mixed with absolute alcohol containing 5% v/v of conc. hydrochloric acid and filtered. The polysaccharide was washed with

ethanol until the washings gave a negative test for chlorides. Copper (ic) chloride dissolves in acetone to give a intense yellow color, hence small amount of acetone was added during washings to ensure the complete removal of copper (ic) chloride. The precipitate was initially dried under suction pump and finally under vacuum at 40°. The dried material was powdered and weighed. The process was repeated twice with similar results. The weight percentage obtained from each tube is shown in Table-5.

Results and Discussion

It is evident from above observations that a 100% recovery of purified husk polysaccharide was not obtained when copper acetate sol. is used as a precipitating agent. Part of polysaccharide either gets decomposed or does not precipitate out with copper acetate, probably forming a water soluble copper-polysaccharide complex.

FRACTIONATION USING COPPER ACETATE AND ETHYL ALCOHOL AS PRECIPITANTS

Fractionation was tried using copper(II) acetate solution and ethyl alcohol as precipitants, as below:

Experimental

Purified husk (1.0 g.) was dissolved in sodium hydroxide solution[125] (0.5% w/v, 200 ml) and filtered. 10.0 ml each of the filtrate was then transferred to serially numbered tubes (1 to 10) and copper(II) acetate solution

(10% w/v) added in increasing amounts to each tube as shown in Table-6. The precipitate was removed by centrifugation at 10,000 r.p.m. for 15 mts and filtrate removed. To the filtrate, double its volume of ethanol was added. The precipitate formed was again recovered by centrifugation. The precipitates obtained in each case were then regenerated as described in the above section, dried and weighed. Observations are shown in Table-6.

TABLE -6

Weight percentage (yield) of precipitate after fractionation with copper acetate and ethyl alcohol

S. No.	Mucilage (0.5% w/v) (ml)	Copper acetate (10% w/v) solution (ml)	Weight fraction after decomposition of copper complex		Weight fraction after adding alcohol to supernatant		Total yield (w/w)
			Weight (g.)	Yield (%)	Weight (g.)	Yield (%)	%
1	10.0	0.1	-	-	-	-	-
2	10.0	0.2	-	-	-	-	-
3	10.0	0.3	-	-	-	-	-
4	10.0	0.4	-	-	-	-	-
5	10.0	0.5	-	-	-	-	-
6	10.0	1.0	0.020	40%	0.013	26%	66%
7	10.0	2.0	0.025	50%	0.013	26%	76%
8	10.0	3.0	0.035	70%	0.013	26%	96%
9	10.0	4.0	0.035	70%	0.013	26%	96%
10	10.0	5.0	0.035	70%	0.013	26%	96%

(-) indicates no precipitation

Results and Discussion

From the above observations it may be concluded that copper acetate forms two types of complexes with Ispaghula husk polysaccharides, one being water insoluble and the other water soluble which is ultimately recovered by precipitation with ethyl alcohol. The yield obtained by this method is 30%. It may be presumed from these observations that P. ovata husk consists of at least two polysaccharidic components, one forming water insoluble polysaccharide-copper complex and the other water soluble polysaccharide-copper complex.

Graded Fractionation with copper acetate and ethyl alcohol as precipitants

In order to confirm whether fractions obtained in the previous experiment (from Sl. No. 6 to 10, Table-6) were of similar nature or not, graded fractionation using copper acetate and ethyl alcohol was carried out. Various fractions recovered were subjected to determinations of optical rotation, equivalent weight, IR spectra and chromatographic separation of component sugars after hydrolysis.

Experimental

Purified husk (5.0 g.) was dissolved in sodium hydroxide solution (0.5% w/v) and precipitation carried out with copper acetate solution (10% w/v). The resultant ppt. was recovered by centrifugation (10,000 r.p.m. for 15 mts) and is referred as Fraction-A. To the filtrate further

quantity of copper acetate solution was added and the ppt (Fraction-B) recovered in the same manner. Like this with repeated addition of copper acetate solution and recovery of resultant ppt, in all four fractions (A, B, C and D) were obtained (Table-7). Further addition of copper acetate solution did not result in any precipitation. To this clear liquid ethyl-alcohol (three times the total volume) was added, a further precipitate was obtained (Fraction-E) which was also recovered by centrifugation. All the fractions (A to E) were decomposed as discussed before. The weight of each fraction and the percentage of the total is shown in Table-7.

TABLE - 7

Graded fractionation with copper acetate-ethanol

Fraction	Weight of fraction after decomposition			Average yield of total amounts %
	I	II	Mean	
A	0.5 g.	0.5 g.	0.5 g.	10%
B	0.8 g.	0.8 g.	0.8 g.	16%
C	0.8 g.	0.8 g.	0.8 g.	16%
D	1.2 g.	1.2 g.	1.2 g.	24%
E	1.6 g.	1.6 g.	1.6 g.	32%
			Total yield	98% w/w

CHARACTERISATION OF FRACTIONS A TO E

(i) Optical Rotation

The optical rotation of fractions (A to E) were determined following the procedure recommended by Findley[126] and using sodium lamp (D-line) as the light source. Specific rotations were calculated using the following formula:

$$\text{Specific rotation} = [\alpha]_D^{30} = \frac{a \times 100}{l \times c}$$

$[\alpha]_D^{30}$ = specific rotation using sodium light at 30°C

a = corrected observed rotation in degrees

l = length of the polarimeter tube in decimeter (dm.)

c = concentration of the solution expressed as the number of g. of the substance in 100 ml of solution

Experimental

The polarimeter tube (2 dm.) was filled with sodium hydroxide (0.1N) and the analyzer rotated so that two halves of eye piece were matching[126]. Suitable amounts (0.5% w/v) of different fractions (A to E) were dissolved in 0.1N sodium hydroxide (solvent), filtered, transferred to polarimeter tube and rotation determined. Specific rotations of different fractions are shown in Table-8.

TABLE - 8

Specific optical rotations of Fractions (0.5% w/v) in 0.1N sodium hydroxide

Fractions	Specific optical rotation* $[\alpha]_D^{30}$ (C, 0.5%)
A	-60.0
B	-59.0
C	-60.0
D	-60.0
E	+38.0

*Average values of three readings (in 0.1N NaOH)

(ii) Equivalent weights

Equivalent weights of different fractions were determined by direct titration with sodium hydroxide (0.01N) solution[71,127].

Experimental

Weight amounts of fractions A to E were titrated with 0.01N sodium hydroxide solution (Normality 0.0122N) using phenolphthalein as the indicator. The equivalent weights are shown in Taole-9.

(iii) I.R.

I.R. spectra of fractions A to E were recorded in 2.5 μ to 15.5 μ regions using Potassium bromide according to the procedure discussed earlier. The IR spectra of fractions A to D were found to be superimposable

TABLE - 9

Equivalent weights of different fractions

Fraction	Weight taken (g.)	Vol. of NaOH (ml)	Equivalent weight
A	0.1120	6.0	1530
B	0.1028	5.8	1453
C	0.1200	6.2	1586
D	0.1110	6.0	1517
E	0.1250	2.1	4767

(representative spectra shown in Fig.-5) whereas IR spectra of Fraction-E was entirely different (Fig.-6). Since the IR of A to D were similar it was assumed that all the fractions are chemically similar in nature and constitute a single component, whereas Fraction-E was a different material.

(iv) Hydrolysis and Chromatographic Separations of Sugar Components

The sugar components of the sulphuric acid (2N) hydrolysate of fractions A to E (after neutralising with barium carbonate) were identified by descending paper chromatographic technique[128-130] using Whetman (No. 1) chromatographic paper as support.

Experimental

(A) Acid hydrolysis of the fractions:

Fractions (A to E) were hydrolysed with sulphuric

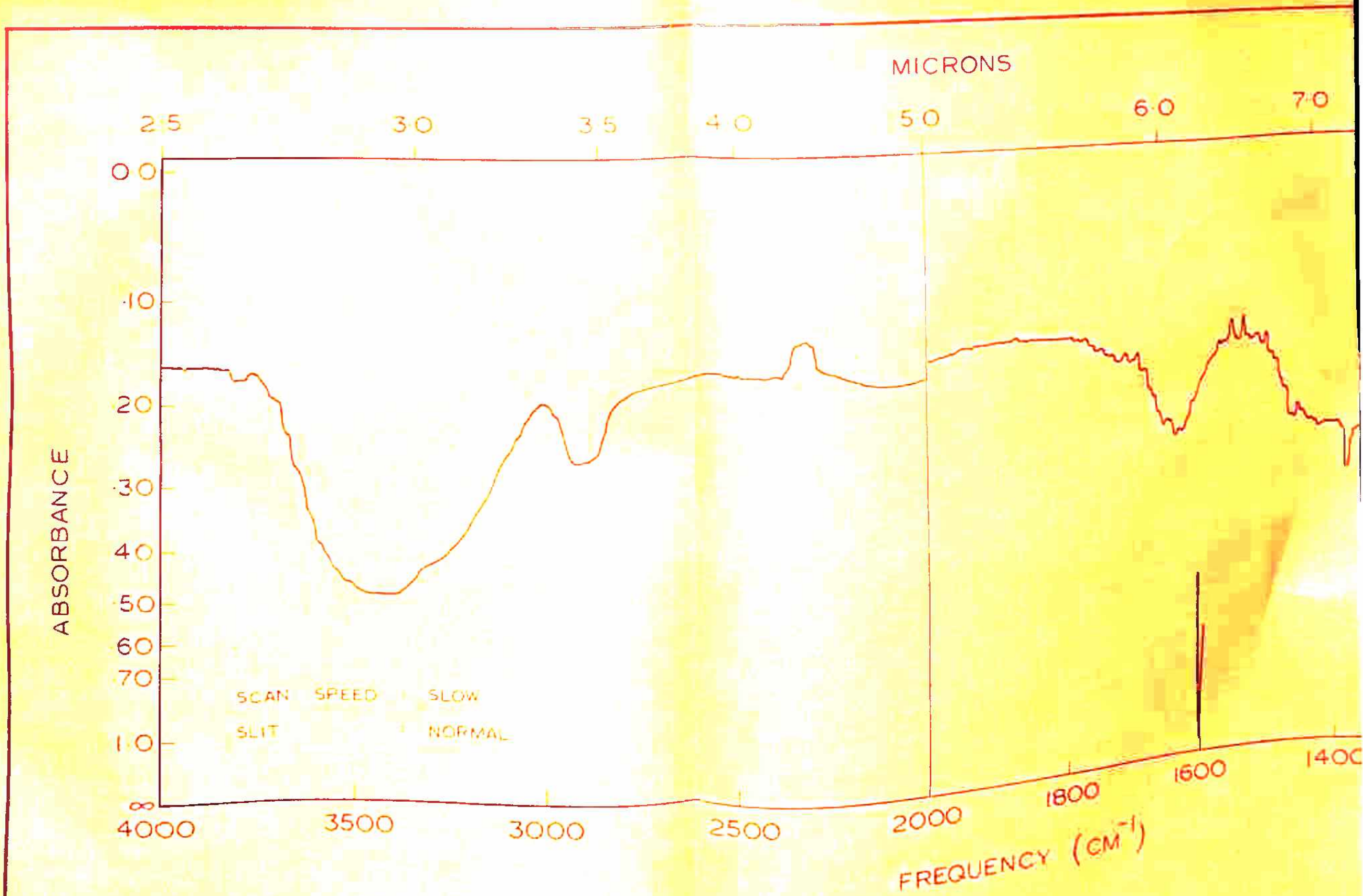


FIG. - 5

I. R. SPECTRA OF FRACTION A TO D. (IN KBr)

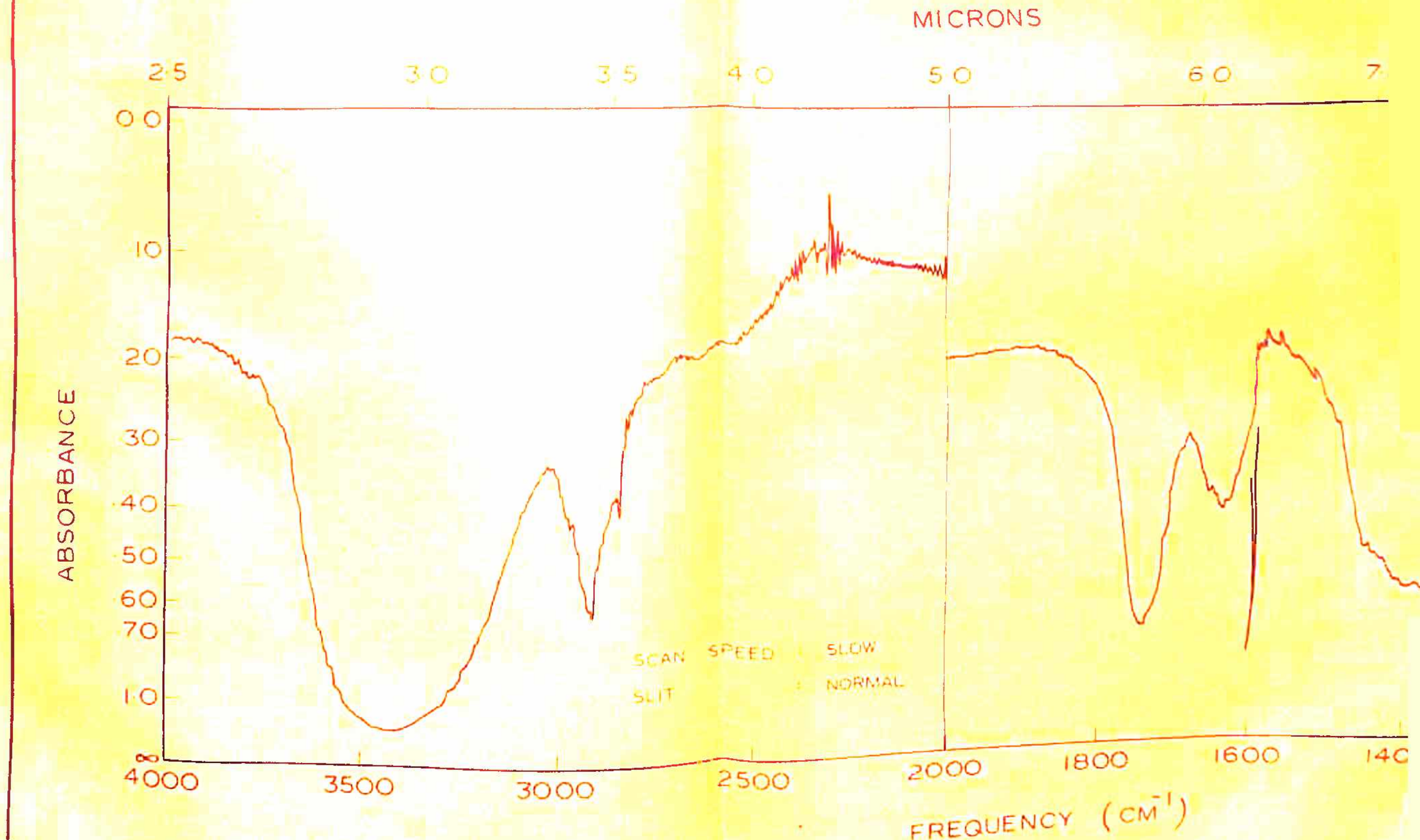


FIG - 6

I. R. SPECTRA OF FRACTION E. (IN KBr)

acid (2N, 100 ml) on a boiling water bath for 30 hrs. The resulting solutions, after complete hydrolysis, were neutralised with Barium carbonate (using Litmus paper) and filtered. The ppt. was washed with water. The filtrate and washings were combined, and passed through cationic exchange resin column (Dowex 50W, H^+ form) for the removal of barium ions. The eluates were concentrated under reduced pressure at 40° to a syrupy mass, and used for chromatographic work.

(B) Paper chromatography of the hydrolysates:

Descending paper chromatography was employed for the detection of component sugars[131]. Whatman (No. 1) chromatographic papers were cut according to the chamber size (20"×6"). Following solvent systems were tried for the separations:

(a) Ethyl acetate : Acetic acid : Water (9:2:2)

(b) Butanol : Pyridine : Water (9:2:2)

(c) Ethyl acetate : Methanol : Acetic acid : Water (60:15:15:10)

(d) Butanol : Ethanol : Water (4:1:5, organic phase)

(e) Ethyl acetate : Pyridine : Acetic acid : Water (5:5:1:3 by volume)

Among these solvent systems No. (a) and No. (e) were found to be the best, giving distinct separations. p-Anisidine hydrochloride was used as the detecting reagent[132]. The chamber was first saturated with the solvent systems by filling the solvent at the bottom and allowing it to stand undisturbed overnight. Samples (neutralized hydrolysate)

and authentic sugars were spotted by micropipette on the paper strips, by alternate spotting and drying, using hair dryer. After the spots were dried, the paper strips were positioned in the chamber and the solvents were allowed to traverse in the machine directions of the paper. The chromatogram were developed by spraying with p-anisidine hydrochloride reagent and heating at 110°C for 15 mts. The R_f values of the component sugars and that of authentic samples are recorded in Table-10 and Table-11 for solvent (a) and (e) respectively.

TABLE-10

R_f values of sugar components obtained after hydrolysis of fractions A to E using Ethyl acetate : Acetic acid : Water (9:2:2) as solvent system

Sample	No. of spots detected	R_f values				
		1	2	3	4	5
Fraction-A	5	0.28	0.35	0.48	0.21	0.40
Fraction-B	5	0.28	0.35	0.48	0.21	0.40
Fraction-C	5	0.28	0.35	0.48	0.21	0.40
Fraction-D	5	0.28	0.35	0.48	0.21	0.40
Fraction-E	3	-	0.35	0.48	-	0.40
D-Galactose	1	0.28	-	-	-	-
L-Arabinose	1	-	0.35	-	-	-
L-Rhamnose	1	-	-	0.48	-	-
D-Galacturonic acid	1	-	-	-	0.21	-
D-xylose	1	-	-	-	-	0.40

TABLE-11

R_f values of sugar components obtained after hydrolysis of fractions A to E using Ethyl acetate : Pyridine : Acetic acid : Water (5:5:1:3) as solvent system

Samples	No. of spots detected	R_f values				
		1	2	3	4	5
Fraction-A	5	0.74	0.60	0.54	0.46	0.20
Fraction-B	5	0.74	0.60	0.54	0.46	0.20
Fraction-C	5	0.74	0.60	0.54	0.46	0.20
Fraction-D	5	0.74	0.60	0.54	0.46	0.20
Fraction-E	3	0.74	0.60	0.54	-	-
L-Rhamnose	1	0.74	-	-	-	-
D-Xylose	1	-	0.60	-	-	-
L-Arabinose	1	-	-	0.54	-	-
D-Galactose	1	-	-	-	0.46	-
D-Galacturonic acid	1	-	-	-	-	0.20

Results and Discussion

Fractions A to D gave five spots corresponding to L-rhamnose, D-xylose, L-arabinose, D-galactose and D-galacturonic acid, whereas fraction E gave only three spots corresponding to L-rhamnose, D-xylose and L-arabinose.

From the above studies it is evident that fractions A to D are of similar characteristics. I.R. spectra of A to D are superimposable, hence these fractions (A to D) corresponding to water insoluble polysaccharide-copper complex are

of single entity, whereas Fraction-E corresponding to water soluble complex is entirely different. Hence it was concluded that *Plantago ovata* seed husk polysaccharide consists of at least two polysaccharides, one forming water insoluble complex (Fractions A to D) and the other water soluble complex (Fraction-E).

For further characterisation larger quantities of fractions were prepared. Fractions A to D were combined together to constitute one polysaccharide component (Fraction-I), whereas Fraction-E was reckoned to be another component (Fraction-II).

Various physico-chemical characteristics of both fractions I and II were studied and are discussed in ensuring chapter.

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CHAPTER IV

CHARACTERISATION OF FRACTIONS

The two fractions of purified husk (Fraction I and II) were characterised in the following respects.

(I) SUGAR CONTENTS

Accurate methods for quantitative determination of sugars by chromatographic techniques involve dissection of paper chromatogram into segments, elution of the sugar with the help of a suitable solvent, followed by microanalysis of the eluate[133,134]. Many colorimetric[133,134,139] and volumetric[135,136] methods have been suggested. Colorimetric method using Phenol-Sulphuric acid reagent[134] is sensitive, simple, rapid and reproducible. Sugar components of fractions I and II and purified husk were determined as follows.

Experimental

Weighed quantities of Fraction I, Fraction II and the purified husk (0.5 g. each) were hydrolysed using 2N sulphuric acid, neutralised with barium carbonate, filtered and passed through cationic exchange resin column (Dowex 50W, H^+ form) for removal of barium ions[138]. The eluates were concentrated to a syrupy mass and volume of each was made upto 25 ml. Descending paper chromatography of hydrolysates were carried out using Ethyl acetate : Pyridine : Acetic acid : Water (5:5:1:3) as solvent system and

p-anisidine hydrochloride as detecting agent as discussed earlier. Components detected are given in Table-12.

TABLE-12

Sugar components detected in the sample using Ethyl acetate: Pyridine : Acetic acid : Water (5:5:1:3) solvent system

Hydrolysate	L-Arabinose	D-Xylose	D-Galactose	L-Rhamnose	D-Galacturonic acid
1. Purified Husk	+	+	+	+	+
2. Fraction-I	+	+	+	+	+
3. Fraction-II	+	+	-	+	-
R_f values	0.54	0.60	0.46	0.74	0.20

(+) indicates presence, (-) indicates absence.

Quantitative Estimation

Two spots (40 µl) each of hydrolysate of Fraction I, II and purified husk were spotted on seperate chromatographic papers (6"×20", Whatman No. 1) using Lambda pipette. Chromatograms were irrigated with Ethyl acetate : Pyridine : Acetic acid : Water (5:5:1:3) solvent system for 30 hours, so that it could traverse whole length of the paper and then drip down. The bottom edge of the chromatographic paper was serrated previously for uniform solvent flow. The solvent system was allowed to drip off for 30 hrs. The wet chromatograms were then removed from the chamber, dried at room

temperature, and cut into two parts along long axis. On one portion p-anisidine hydrochloride was sprayed followed by heating at 110°C for 10 mts. This treatment revealed the positions of the sugar components, by reference to which, areas corresponding to each sugar component (on the second half of the same paper) were excised as segments.

The segments were placed in test tubes containing 20 ml of distilled water. After a 30 mts. wait and occasional swirling of the solution, the liquid was filtered through glass wool and volume made upto 25 ml.

2.0 ml each of above solutions were transferred to a tube and 1.0 ml of 5.0% w/v solution of Phenol (Analar grade, BDH) in water was added. 5.0 ml of conc. sulphuric acid (Analar grade, 95.5%, sp. gr. 1.84) was then added from a fast delivery pipet[137]. The stream of acid was directed against the liquid surface for proper mixing[137]. The absorbance of yellow-orange color was then determined using spectronic-20 at 500 mμ for L-Rhamnose and D-Galactose; 530 mμ for L-Arabinose, D-Xylose and D-Galacturonic acid. Values are shown in Table-13. A blank was also run simultaneously using 2.0 ml of water instead of sample. The amount of sugar in hydrolysate sample (Fraction I, II and purified husk) were then determined with reference to a standard absorbance curves (Fig.-7, Table-13). The percentages of sugar components of Fraction I, II and purified husk are shown in Table-14.

TABLE-13

Absorbance for standard sugar solutions

S.No.	Standard/Sample	Conc. (micro-gram)	D-Galac-turonic acid	L-Arabi-nose	D-Xylose	L-Rhamnose	D-Galactose
1	Standard-1	5	0.01	0.01	0.02	0.01	0.025
2	Standard-2	10	0.012	0.015	0.05	0.025	0.07
3	Standard-3	20	0.020	0.03	0.09	0.045	0.14
4	Standard-4	40	0.04	0.06	0.185	0.080	0.28
5	Standard-5	60	0.06	0.09	0.28	0.120	0.43
6	Fraction-I	-	0.018	0.02	0.18	0.02	0.02
7	Fraction-II	-	-	0.03	0.25	0.01	-
8	Purified Husk	-	0.015	0.028	0.18	0.015	0.022

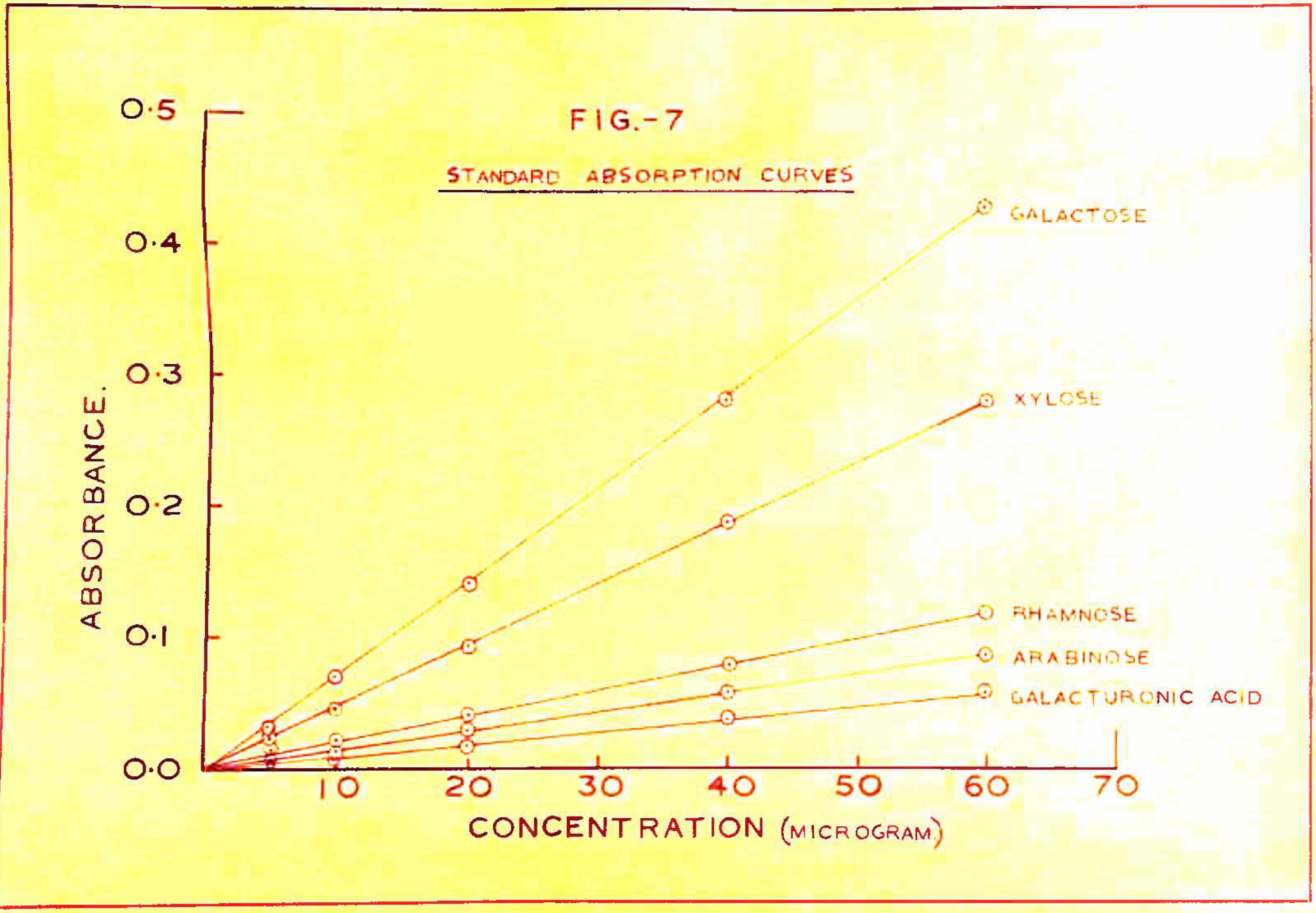


FIG.-7
STANDARD ABSORPTION CURVES
ABSORBANCE.
0·5
0·4
0·3
0·2
0·1
0·0
10
20
30
40
50
60
70
CONCENTRATION (MICROGRAM)
GALACTOSE
XYLOSE
RHAMNOSE
ARABINOSE
GALACTURONIC ACID

TABLE-14

Calculated percentages of sugar components

Sugars	Fraction-I		Fraction-II		Purified Husk	
	Amount calculated in hydrolysate (g.)	(%)	Amount calculated in hydrolysate (g.)	(%)	Amount calculated in hydrolysate (g.)	(%)
1. L-Arabinose	0.075	15.0	0.125	25.0	0.10	20.0
2. D-Xylose	0.250	50.0	0.325	65.0	0.25	50.0
3. D-Galactose	0.020	4.0	-	-	0.025	5.0
4. L-Rhamnose	0.042	8.5	0.025	5.0	0.040	8.0
5. D-Galacturonic acid	0.100	20.0	-	-	0.075	15.0
Total %		97.5		95.0		98.0

Results and Discussion

Fraction I was found to contain L-Arabinose (15%), D-Xylose (50%), D-Galactose (4%), L-Rhamnose (8.5%) and D-Galacturonic acid (20%), whereas Fraction-II consisted of L-Arabinose (25%), D-Xylose (65%) and L-Rhamnose (5.0%).

(II) PENTOSAN AND PENTOSE CONTENTS

Pentosans and pentoses can be estimated by determining the amount of furfural which they yield on distillation with 12% hydrochloric acid. The furfural can then be precipitated as furfural phloroglucide and from its weight the Pentosan and Pentose contents may be calculated using the following

formulae[140,141].

Pentosans = (a + 0.0052) × f

a = weight of furfural phloroglucide

f = 0.895 if 'a' is less than 0.03 g., or

= 0.887 if 'a' is between 0.03 and 0.3 g., or

= 0.882 if 'a' is above 0.3 g.

Pentose = (a + 0.0052) × f

a = same as above

f = 1.017 if 'a' is less than 0.03 g., or

= 1.0075 if 'a' is between 0.03 and 0.3 g., or

= 1.0026 if 'a' is above 0.3 g.

The factor 0.0052 represents the amount of phloroglucide remaining dissolved in the hydrochloric acid[142]. The pentosan and pentose contents of Fraction I, Fraction II and Purified Ispaghula husk were determined as discussed below.

Experimental

Weighed amount of samples (Fraction I, II and purified husk) were placed in a three neck distillation flask. 100 ml of 12% Hydrochloric acid was then added and the mixture distilled. The distillate was collected at the rate of 30.0 ml in 10 mts. As soon as 30.0 ml of the distillate were collected, another 30.0 ml of 12% Hydrochloric acid was added to the flask. Distillation was continued until the distillate was free from furfural when

tested with aniline reagent[140]. To the distillate 40.0 ml of Phloroglucin solution[140] (Riedel, free from Diresorcinol) was added and the liquid made upto 400 ml with 12% hydrochloric acid. The precipitate of furfural phloroglucide was allowed to stand undisturbed for 16 hrs, then filtered through tared gooch crucible, washed with water and finally dried at 105° to constant weight. The crucible was then placed in a narrow beaker and 20.0 ml of alcohol (95% v/v) added. The beaker and contents were heated for 10 mts on water bath (60°). Alcohol was removed by suction and the crucible dried at 105° to constant weight. From the weight of furfural phloroglucide (Table-15) Pentosan and Pentose contents of samples were calculated. Pentose contents found to be 69.65%, 81.70%, and 80.90% respectively for Fraction I, II and purified husk.

TABLE-15

Pentosans and Pentose contents

Sample	Weight taken (g.)	Weight of furfural Phloro-glucide (g.)	Pentosan content (%)	Pentose content (%)
1. Purified Husk	0.2	0.1460	68.60	80.90
2. Fraction-I	0.2	0.1250	57.70	69.65
3. Fraction-II	0.2	0.1475	67.70	81.70

(III) URONIC ACID CONTENT

Uronic acids in hydrochloric acid undergo decarboxylation and the amount of carbon-dioxide evolved can be stoichiometrically correlated with the amount of uronic acid present in the material[140-143]. Pentose or hexoses do not interfere in the estimation. Colorimetric methods have also been suggested for determination of uronic acid contents[144].

Experimental

Weighed amounts of material (Table-16) were taken in flask with a inlet tube for air stream and a vertical condenser. 100 ml of hydrochloric acid (12%) was added and stream of air (free from CO_2) was passed for some time. The other end of condenser was connected to an absorption train, which included in succession 4.0% Aniline-hydrochloride (to remove furfural, if carried over), Dil. sulphuric acid (to remove HCl) with copper sulphate; sulphuric acid (72%, to remove water) and calcium chloride tower. Ultimately it was connected to a absorption tube containing standard Barium hydroxide solution (0.0810N). The contents of the flask were heated to boiling for 3 hrs and carbon dioxide evolved carried to the absorption tube with the help of air current. At the end of the experiment excess of Barium hydroxide (0.0810N) solution was titrated with hydrochloric acid standard solution (0.0928N), using phenolphthalein as the indicator. From the amount of Barium hydroxide consumed, liberated carbon dioxide was calculated[147]. Carbon dioxide percentage multiplied by 4 gave the uronic acid

content of the samples calculated as uronic acid anhydride[146] (Table-16).

TABLE-16

Uronic acid anhydride content of Fractions

Sample	Weight taken (g.)	Volume of Barium hydroxide consumed (ml)	Yield of CO_2 (%)	Uronic acid anhydride content (%)
1. Fraction-I	0.2	5.2	4.63	18.52
	0.3	7.2	4.23	16.92
				Ave = 17.72%
2. Fraction-II	0.2	0.7	0.623	2.49
	0.3	1.1	0.646	2.58
				Ave = 2.53%
3. Purified Husk	0.2	6.1	5.46	21.84
	0.3	8.5	4.99	19.96
				Ave = 20.90%

Results and Discussion

Uronic acid anhydride contents of Fraction I, II and purified husk were found to be 17.22%, 2.53% and 20.90% respectively. Fraction-II contained very small amount of uronic acid anhydride in comparison to Fraction-I. Hence Fraction-I could possibly be acidic polysaccharide.

(IV) METHOXYL CONTENT

On the micro and semimicro scales modified Zeisel method is used for Methoxyl group determination[145,148]. In this method methoxyl group are reduced to alkyl iodide, which is converted into silver iodide and determined gravimetrically[140]. The distilled alkyl iodide can also be absorbed in bromine solution and the resulting iodic acid is determined iodometrically by titration with sodium thiosulphate. Methoxyl content of Fractions were determined using modified Zeisel method[146,149-151] and calculated by the following formula:

$$\% \text{ Methoxyl content} = \frac{(A - B) \times N \times 0.00517}{W} \times 100$$

where A = ml of Sod. thiosulphate required for the sample

B = ml of Sod. thiosulphate required for the blank

N = Normality of Sod. thiosulphate solution

0.00517 = Factor for methoxyl group determination (31.0/6000)

W = Weight of the sample taken.

Experimental

Weighed amount of sample was transferred to the reaction flask. Slurry of red phosphorous (0.06 g./100 ml of water) was filled up in side trap and in the receiver bromine solution was kept. 2.0 ml of Phenol and 6.0 ml of hydriodic acid (Analar grade, BDH, 57%, sp. gr. 1.70) were added to the reaction flask and it was connected to

the receiver. Side tube of the flask was connected to carbon dioxide source which was allowed to pass at the rate of two bubbles per second. The flask was heated in oil bath at 140-145° for 1 hr. Thereafter contents of the receiver were transferred to a flask containing 10.0 ml of sodium acetate solution and diluted with water (25 ml). Formic acid was added drop by drop, till brown color of bromine was discharged, and 4-5 drops were in excess. Potassium iodide (3.0 g.) and 15 ml of sulphuric acid (1:10) were then added and titration carried out with standard Sod. thiosulphate (0.1012N) solution. The blank was run in similar manner. Observations are recorded in Table-17.

TABLE-17

Methoxy contents of the fractions

Sample	Weight taken (W) (g.)	Vol. of Thiosul-phate (A-B) (ml)	Methoxyl content (%)	Average value (%)
Fraction-I	0.6125	4.1	0.349	
	0.6018	3.9	0.338	0.343
Fraction-II	0.8170	2.3	0.147	
	0.8110	1.9	0.122	0.144
Purified Husk	0.6010	4.0	0.348	
	0.6000	3.9	0.339	0.344

(V) SPECIFIC OPTICAL ROTATION

Specific rotations of Fraction I, II and purified husk (C = 0.5, in 0.1N Sod. Hydroxide) were found to be -60°, $+39^{\circ}$ and $+51^{\circ}$ respectively.

(VI) EQUIVALENT WEIGHTS

Equivalent weights of Fraction I, II were determined by direct titration with 0.1N Sodium hydroxide (0.0122N) using phenolphthalein as the indicator. Results are shown in Table-18.

TABLE-18

Equivalent weight of Fraction I and II

Sample	Weight taken (g.)	Volume of Sodium Hydroxide (ml)	Equivalent weight	Average value
Fraction-I	0.1038	5.8	1467	1479
	0.1032	5.7	1484	
	0.1015	5.6	1486	
Fraction-II	0.1024	1.8	4662	4706
	0.1020	1.7	4776	
	0.1028	1.8	4680	

(VIII) pH VALUES

0.1% w/v and 0.5% w/v solutions of Fraction I and Fraction II were prepared and their pH values recorded using Beckmann pH meter (Model H-2) and are given in Table-19.

TABLE-19

pH values of samples

Sample	Concentration	pH
Fraction-I	0.1%	6.2
	0.5%	6.3
Fraction-II	0.1%	6.7
	0.5%	6.7
Purified Husk	0.1%	6.7
	0.5%	6.6

(VIII) TOTAL REDUCING SUGAR CONTENTS

Weighed amount of Fraction I and Fraction II were hydrolysed with sulphuric acid (2N) by heating on boiling water bath for 30 hrs. The hydrolysate was neutralised with barium carbonate, filtered and washed with water. Filtrate and washings were concentrated under reduced pressure to a syrupy mass and volume made upto 100 ml.

0.2 ml of this was transferred to a tube and 2.0 ml of alkaline copper solution reagent[152,153] added. Contents were heated for 8 minutes on boiling water bath and cooled. To each tube 2.0 ml of phosphomolybdic acid reagent[152,154] was added. After standing for 2 mts, the absorbance (Table-20) was determined at 420 mμ using spectronic-20. Blank was run using water instead of sample and with this zero reading was adjusted. A standard curve (Fig.-8) was prepared using glucose as reference material. From the standard curve (Fig.-8) the total reducing sugars were calculated with reference to glucose equivalents.

TABLE-20

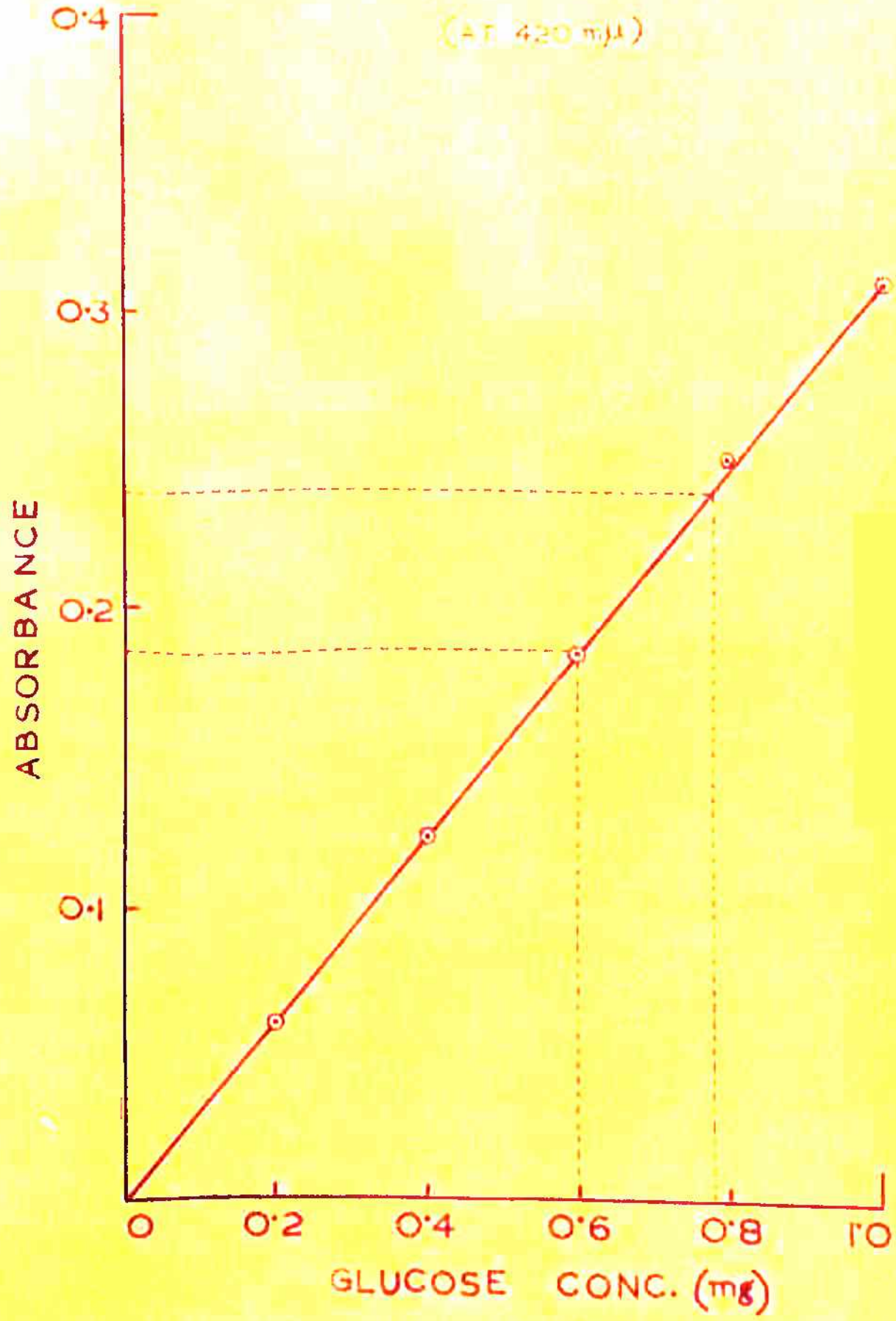
S.No.	Sample	Absorbance at 420 mμ		Mean
		I	II	
1	Glucose - 0.2 mg	0.06	0.06	0.06
2	Glucose - 0.4 mg	0.12	0.12	0.12
3	Glucose - 0.6 mg	0.18	0.18	0.18
4	Glucose - 0.8 mg	0.25	0.25	0.25
5	Glucose - 1.0 mg	0.31	0.31	0.31
6	Fraction-I	0.18	0.18	0.18
7	Fraction-II	0.24	0.24	0.24

Results

Reducing sugar contents of Fraction I and Fraction II were found to be 60% and 78% respectively calculated as glucose.

FIG. 8

STANDARD ABSORBANCE CURVE FOR GLUCOSE



(IX) ELECTROPHORESIS

Polysaccharides as such cannot be detected directly on the cellulose chromatographic paper and hence use of Glass fibre paper[122,155,156] and bleached pure silk[122] has been recommended as supports. Electrophoretic behaviour of Fraction I and II were studied as below:

Experimental

2.0 lit. each of Borate buffer (pH-12) and Acetate buffer (pH 4.0) were prepared. The Glass fibre paper[158] (Whatman Chromatographic Glass Fibre paper, Cat. No. GF-81, Thickness 0.25 mm, basic weight 53 g/m^2, supplied by H. Reeve Angle and Co., London) were cut in strips (6 × 28 cm). Samples (Fraction I and II, 0.1% w/v in water) were applied as a line not greater than 2 cm in length. Strip support was then suspended horizontally in the Electrophoresis chamber (Thomas Electrophoresis Unit). 250 V (50 mA) was applied for both the buffer systems for 3 hrs[159]. Strips were taken out carefully and dried in oven at 60°. The spots of polysaccharides were developed with modified p-anisidine reagent[122] and dried at 105° for 10 mts. The mobilities were calculated as a ratio of distance travelled by detected zone and actual distance travelled by Glucose (R_G values) and are shown in Table-21. Duplicate tests were run for each sample.

TABLE-21

Electrophoretic behaviour of Fractions on Glass Fibre paper

Sample	Buffer system	Movement	Distance from centre line (cm)	R_G value*
Fraction-I	Borate	Anode	5.0	0.68
	Acetate	Anode	3.0	0.60
Fraction-II	Borate	Cathode	-3.0	-0.39
	Acetate	Stationary	-	-

*Distance travelled by glucose (Borate - 7 cm, and Acetate - 5 cm)

Results and Discussion

Fraction I migrates towards Anode in both the buffer systems whereas Fraction II was stationary in acetate buffer. Since acetate buffer is non-complexing buffer this fraction may be neutral one.

(X) VISCOSITY AND RHEOLOGICAL BEHAVIOUR

Fisher improved MacMicheal viscometer (Fisher Scientific Co., USA, Cat. No. 15-346-500) a rotational covette type viscometer with variable shear rate settings was employed for the rheological studies of both the fractions and the purified husk. The r.p.m. of rotating hot-plate, corresponding to the different regular divisions were determined (Table-22) to begin with.

TABLE-22

R.P.M. corresponding to regular divisions of viscometer

Regular divisions of speed control	R.P.M.*
1	7.86
2	8.53
3	9.13
4	9.94
5	10.59
6	10.97
7	12.00
8	13.54
9	16.83
10	20.46
11	24.00
12	29.52

*Average of three readings

The uncertified wire (B and S Gauge - 30) was calibrated against certified wire (B and S Gauge - 30, Resistance - 0.01305 G.cm/^{o}M. Div.) using liquid paraffin as the standard. The values of Instrument constant (K) were determined (Table-23) using the following formula[162].

$$K = \frac{\mu \times H \times N}{^{o}M}$$

where K = Instrument constant

μ = viscosity in centipoises

H = depth of immersion of the plunger

N = Number of revolution of the cup per minute

^{o}M = dial reading in degree MacMicheal.

TABLE-23

Instrument constant (K) at different r.p.m. of cup

S.No.	R.P.M.	^{o}M	Instrument constant (K)
1	7.86	6	141.3
2	8.53	6	153.3
3	9.13	7	140.7
4	9.94	7	149.5
5	10.59	8	142.0
6	10.97	8	148.0
7	12.00	9	143.9
8	13.54	10	146.1
9	16.83	14	142.2
10	20.46	16	147.2
11	24.00	18	143.8
12	29.52	22	144.9

0.5% w/v dispersions of Fraction I, II and purified husk were prepared and transferred to the big size sample cup (6.98 cm I.D.). A cylindrical plunger of standard

dimensions (4.0 cm Dia.), suspended by a torsion wire (Gauge - 30) was immersed to 3.0 cm in the sample cup, which was then revolved at a constant speed of 20.46 r.p.m. on a rotating hot plate. The plunger and the supporting spindle turned because of the drag of the liquid, until the restoring force in the wire just balanced it. The spindle turn indicated by a pointer on a disc graduated in Degrees MacMicheal (Table-24), was noted and the viscosities computed using the following equation[102].

$$\mu = \frac{K \times {}^{o}M}{H \times N}$$

TABLE-24

Viscosities (centipoise) of 0.5% w/v dispersions

Sample	Disc Reading (^{o}M)					Average viscosity (cps.)
	I	II	III	IV	Mean	
1. Purified Husk	85	84	84	84	84.25	202.0
2. Fraction-I	71	73	71	71	72.00	172.7
3. Fraction-II	48	48	48	48	48.00	115.1

Rheological Characteristics

In order to study the rheological characteristics of fractions the sample cup (6.98 cm I.D.) was filled with 0.5% dispersions and left undisturbed for half an hour. The cup was rotated to begin with at the lowest rate of shear (7.86 r.p.m.) and the reading on the rotating disc

recorded. The rate of shear was then gradually increased to 29.52 r.p.m. and reading noted at each increase. Thereafter the rate was decreased at the same rate to the lowest shear rate (Tables 25, 26 and 27). The upward and downward curves of samples are shown graphically in Figs. 9, 10 and 11.

TABLE-25

Rheological behaviour of 0.5% w/v Purified husk

S.No.	R.P.M.	Disc Reading (^{o}M)	
		Upcurve	Down curve
1	7.86	38	25
2	8.53	40	26
3	9.13	45	29
4	9.94	49	33
5	10.59	50	34
6	10.97	52	35
7	12.00	54	39
8	13.54	58	44
9	16.83	72	58
10	20.46	85	77
11	24.00	90	88
12	29.52	95	95

FIG.– 9

RHEOLOGICAL BEHAVIOUR OF PURIFIED HUSK

(0·5% w/v)

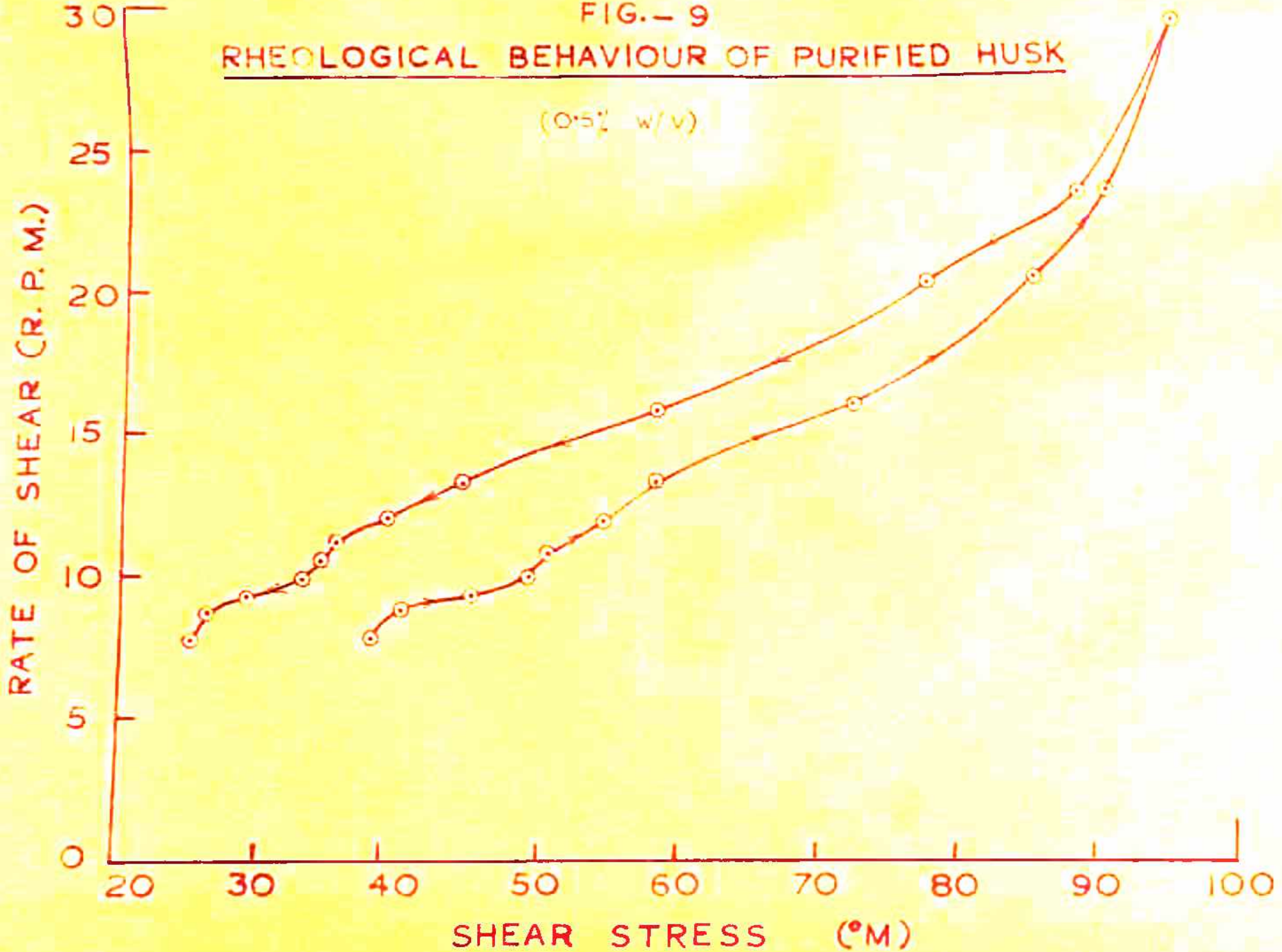


TABLE-26

Rheological behaviour of 0.5% w/v Fraction-I

S.No.	R.P.M.	Disc Readings (^{o}M)	
		Upcurve	Down curve
1	7.86	40	25
2	8.53	42	28
3	9.13	45	31
4	9.94	48	34
5	10.59	51	38
6	10.97	55	43
7	12.00	59	47
8	13.54	62	52
9	16.83	67	57
10	20.46	71	64
11	24.00	75	71
12	29.52	81	81

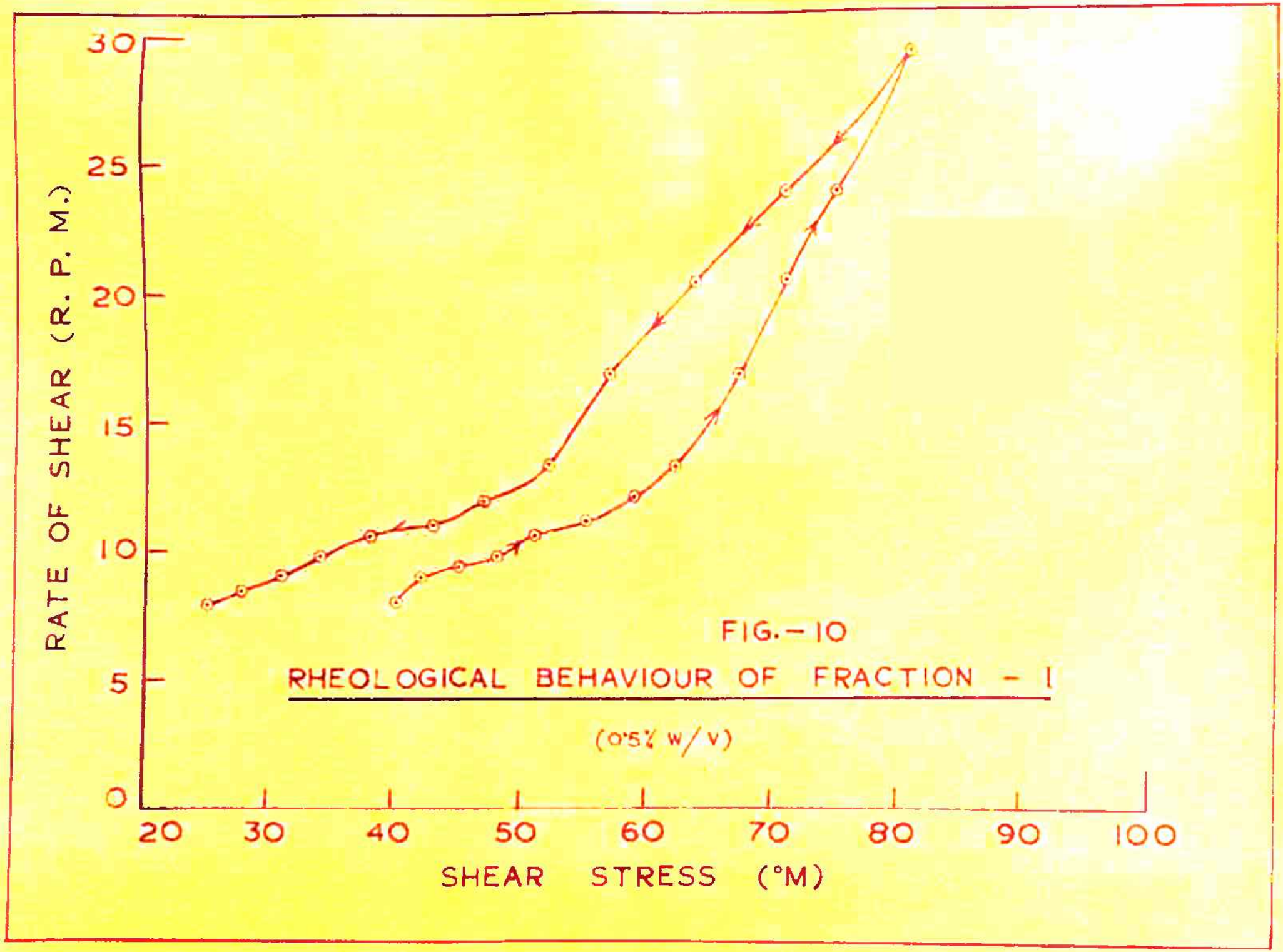


FIG.– 10

RHEOLOGICAL BEHAVIOUR OF FRACTION – I

(0·5% w/v)

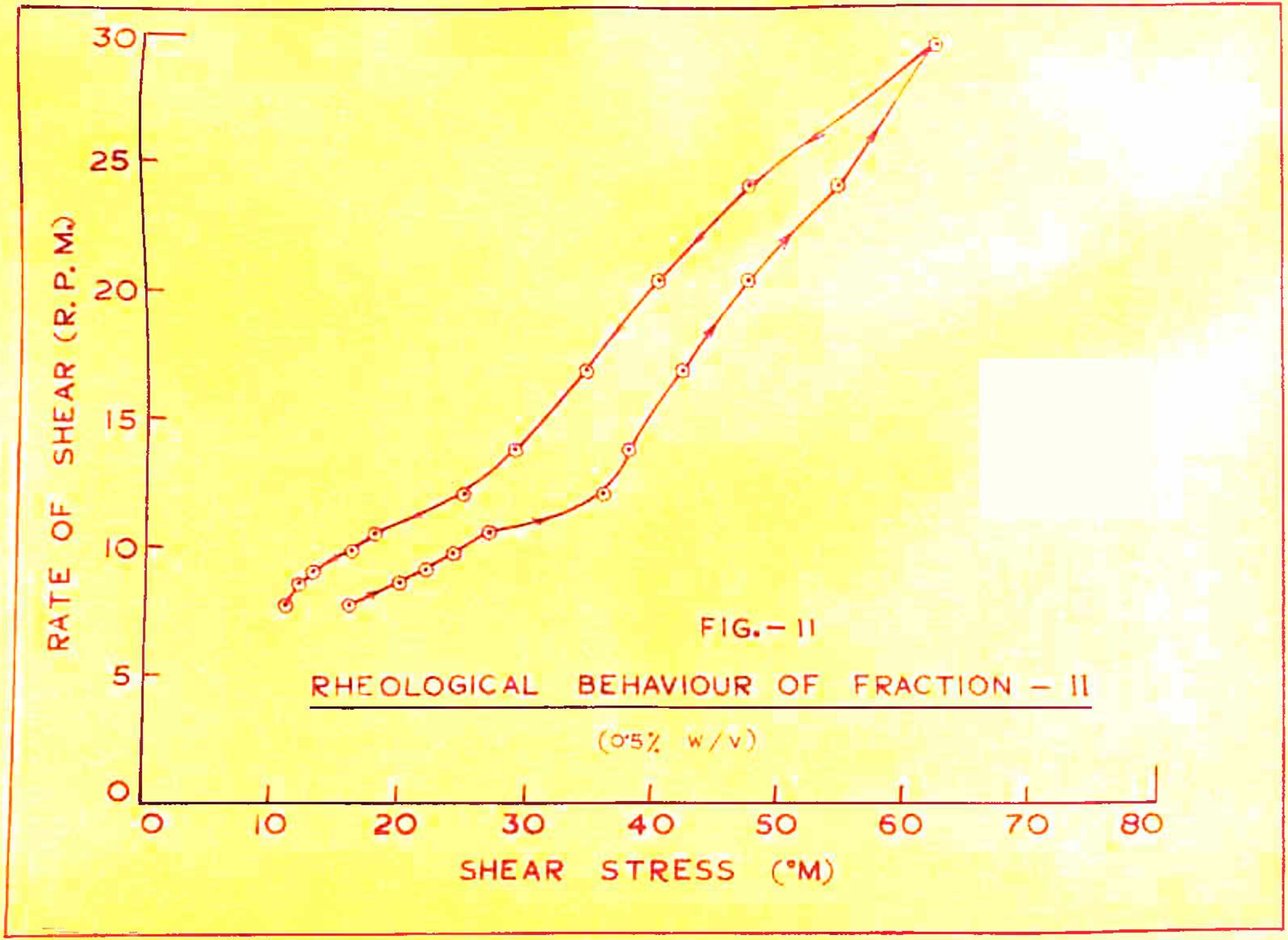


FIG. – 11

RHEOLOGICAL BEHAVIOUR OF FRACTION – II

(0·5% w/v)

TABLE-27

Rheological behaviour of 0.5% w/v Fraction-II

S.No.	R.P.M.	Disc Readings (°M)	
		Upcurve	Down curve
1	7.86	16	11
2	8.53	20	12
3	9.13	22	13
4	9.94	24	16
5	10.59	27	18
6	10.97	30	21
7	12.00	36	25
8	13.54	38	29
9	16.83	42	35
10	20.46	48	41
11	24.00	55	48
12	29.52	63	63

Results and Discussion

Viscosities of both fractions were found to be less than that of purified husk for 0.5% w/v concentration. Shear rate vs shear stress (°M) curve shows all the samples to be non-Newtonian (Pseudoplastic).

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CHAPTER V

CARBOXY METHYL DERIVATIVE (SODIUM SALT) OF P.OVATA HUSK

Chemical modification of polysaccharides alters their physical properties and some times their derivatives are more valuable than th original polysaccharides. Modification of polysaccharides may be achieved by the introduction of (a) neutral groups - e.g. methyl, ethyl, hydroxyethyl, etc., (b) acidic groups - e.g. carboxyl, sulphate, phosphate, etc., or (c) basic groups - e.g. amino acids. Introduction of certain ether groups into the polysaccharide molecule generally enhances its hydrophilic properties. Methyl, hydroxy ethyl and carboxymethyl group when present in the proper amounts make the polysaccharide more water soluble. Cellulose and starch have been structurally modified by substitution of methyl, ethyl, carboxy methyl, hydroxy ethyl groups and some of their derivatives are potential substitutes for the many natural gums in the pharmaceutical industry.

Very few carbohydrate derivatives have enjoyed such widespread popularity as the carboxy methyl derivatives. Carboxy methyl derivatives of acacia[10,33], tragacanth[10,33], guar gum[10,33], locust bean gum[10,33], cellulose[34,35,163] and starch[36,37] have been reported to be of value as additives for pharmaceutical formulations. Hence it was considered worthwhile to prepare carboxy methyl derivative of Ispaghula husk and study its properties with a view to ascertain possibilities of its use in formulations.

Carboxy methyl derivatives (sod. salt) can be prepared according to the method suggested by Moe[10]. The gum is treated with a cold aqueous solution of an alkali (NaOH) and then with an etherifying agent such as sodium chloroacetate or Monochloro acetic acid, at an elevated temperature. The alkali is neutralized with dilute acetic acid and the salts removed by washing with hot aqueous alcohol or ethanol-methanol mixture. This method has been used for the preparation of carboxy methyl derivatives of acacia, tragacanth, guar and locust bean gums[10,33]. Klug and Tinsley[164] suggested yet another method, in which a non-aqueous solvent such as 2-propanol is used for maintaining the alkaline-swollen gum and the viscous product in dispersed form during the reaction[105,160].

The procedure involves the following variables:

1. Concentration of alkali,
2. Temperature and duration of heating, and
3. Amount of etherifying agent.

These variables were standardized as follows:

SELECTION OF ALKALI CONCENTRATION

Treatment of the carbohydrate gum with a cold solution of alkali serves to disperse the gum in water without formation of lumps[107,168]. Incorporation of alkali is also warranted for the ease of penetration of the etherifying agent and uniformity of etherification. The effect of alkali concentration on the preparation of carboxy

methyl derivative (sodium salt) of Ispaghula were studied and the products characterised on the basis of their sodium contents. The carboxy methyl derivative (sodium salt) of Ispaghula is henceforth referred to as Sod. CMD Ispaghula.

(a) Preparation of Sod. CMD Ispaghula

Sodium hydroxide solutions (5% to 50% w/v) were prepared and kept in Refrigerator. 2.0 g. of Ispaghula husk were dispersed in cold sodium hydroxide solution (200 ml) by stirring at 4,000 r.p.m. for 30 mts. Dispersions were allowed to stand for another half an hour and then heated to 45° for 30 mts and cooled. To this mixture sodium chloro acetate (2% w/v sol., 100 ml) was added slowly in small quantities at a time and stirring continued for 5-10 mts. The reaction mixture was again heated to 50° for 30 mts, cooled, and diluted with water. Excess of alkali was then neutralised with dil. acetic acid. The Sod. CMD Ispaghula was then precipitated with 4 times the volume of Ethyl alcohol (90% v/v) and filtered. The precipitate was washed with alcohol and then with alcohol-methanol mixture to remove salt impurities, dried under suction and finally in vacuum oven, powdered and weighed. Results are recorded in Table-28.

(b) Determination of Sodium Content

For compounds of this type the degree of substitution may be assessed by determining the sodium content[169,171]. A

non-aqueous titration method for the determination of sodium content of carboxy methyl cellulose has be n reported[170] and is now official in U.S.P.[172]. Other methods suggested for the determination of carboxy methyl group are (a) conversion to free acid by decomposition of sodium salt, followed by the determination of the neutral equivalent[173,174], and (b) conductometric titration of the alkaline sodium carboxy methyl cellulose[175]. Sodium content of Sod. CMD Ispaghula were determined by non-aqueous titration with 0.1N Perchloric acid (Normality 0.0710N) in glacial acetic acid media[172]. Results are recorded in Table-28.

TABLE-28

Effect of Sodium hydroxide concentrations

S.No.	Concentration of NaOH (% w/v)	Weight of husk (g.)	Yield (g.)	Sodium content (%)
1	5.0	2.0	1.2	6.039
2	10.0	2.0	1.4	6.366
3	20.0	2.0	1.8	6.692
4	30.0	2.0	2.1	7.345
5	40.0	2.0	2.1	7.345
6	50.0	2.0	1.9	7.183

Results

Sodium hydroxide concentration when used above 30% w/v gives maximum Sod. content, but at 50% w/v Sodium content falls.

TEMPERATURE OF ETHERIFICATION

Suitable temperature and duration of etherification were worked out, keeping other variables such as conc. of alkali and amounts of Sod. chloroacetate : husk constant.

2.0 g. of Ispaghula husk were dispersed in sodium hydroxide solution (200 ml, 30% w/v). To this sodium chloroacetate solution (2% w/v, 100 ml) was added gradually with stirring. The reaction mixture was heated at different temperatures (40°, 50°, 60° and 70°) for different periods of time (15, 30 and 60 mts.). Excess of alkali was then neutralised with dil. acetic acid, and precipitated with ethyl alcohol (90% v/v). The final products were weighed out and the sodium content determined. Results are recorded in Table-29.

Results

It was observed that maximum etherification was obtained when the reaction mixtures were heated at 50° for 30 mts duration. At 40° and at 70° yields were less.

EFFECT OF SODIUM CHLOROACETATE

Carboxy methyl derivatives (Sod. salt) were prepared using different amounts of Sodium chloroacetate and their sodium contents determined.

Experimental

2.0 g. Ispaghula husk were dispersed in cold sodium hydroxide solution (200 ml, 30% w/v). To this different

TABLE-29

Effect of Temp. and duration of heating on Etherification

S.No.	Temperature (°C)	Time (mts.)	Yield of Sod. CMD Ispaghula (g.)	Sodium content (%)
1	40	15	1.50	2.285
2	40	30	1.50	2.939
3	40	60	1.42	5.714
4	50	15	1.85	6.855
5	50	30	1.98	7.509
6	50	60	1.98	7.428
7	60	15	1.71	7.020
8	60	30	1.78	7.020
9	60	60	1.60	6.855
10	70	15	1.62	6.530
11	70	30	1.62	5.714
12	70	60	1.60	5.714

amounts of Sod. chloroacetate (Table-30) were added with stirring. Reaction mixtures were heated to 50° for 30 mts, cooled and diluted. Sod. CMD Ispaghula were recovered as discussed earlier and then subjected to determination of sodium content. Results are shown in Table-30.

TABLE-30

Etherification with varying amounts of Sod. chloroacetate

S.No.	Amount of husk (g.)	Amount of Sod. chloroacetate/ 100 ml. (g.)	Yield (g.)	Sodium content (%)
1	2.0	0.25	1.80	1.796
2	2.0	0.50	1.82	2.285
3	2.0	1.00	1.90	4.571
4	2.0	1.50	1.95	5.714
5	2.0	2.00	2.05	7.428
6	2.0	3.00	2.05	7.428
7	2.0	4.00	2.051	7.345
8	2.0	5.00	2.05	7.428

PREPARATION AND CHARACTERISATION OF SODIUM CMD ISPAGHULA

Having worked out optimum conditions, three different samples (A, B and C) of Sod. CMD Ispaghula were prepared, starting with 4.0 g. of husk every time. Yields of 4.2, 4.18 and 4.2 g. were obtained for samples A, B and C respectively. The three samples of Sodium CMD Ispaghula were characterised

with respect to their sodium content, IR spectra and viscosity.

(i) Sodium Content

Weighed amounts of samples A, B and C were taken in flasks, glacial acetic acid added to them and heating done for a few minutes. After cooling the mixture were titrated potentiometrically against 0.1N Perchloric acid (0.0710N) using Beckmann pH meter (Model H-2). Calomel electrode was dipped in KCl (saturated) solution and Glass electrode in the sample. Both the electrodes were connected by a salt bridge[176] consisting of an Agar-KCl gel. Results are recorded in Table-31 and titration curves shown in Figs. 12, 13 and 14. Sodium contents of all samples were also determined by direct titration with standard Perchloric acid in glacial acetic acid media using methyl rosaniline chloride (crystal violet in Glacial acetic acid) as the indicator[177,178]. Both the results are compared in Table-31. Similar direct titration was conducted on Ispaghula husk which served as the blank.

Results

The sodium contents of three samples (A,B,C) were found to be similar and hence it was concluded that method used for the preparation of Sod. CMD Ispaghula gives reproducible substitution in the husk polysaccharides.

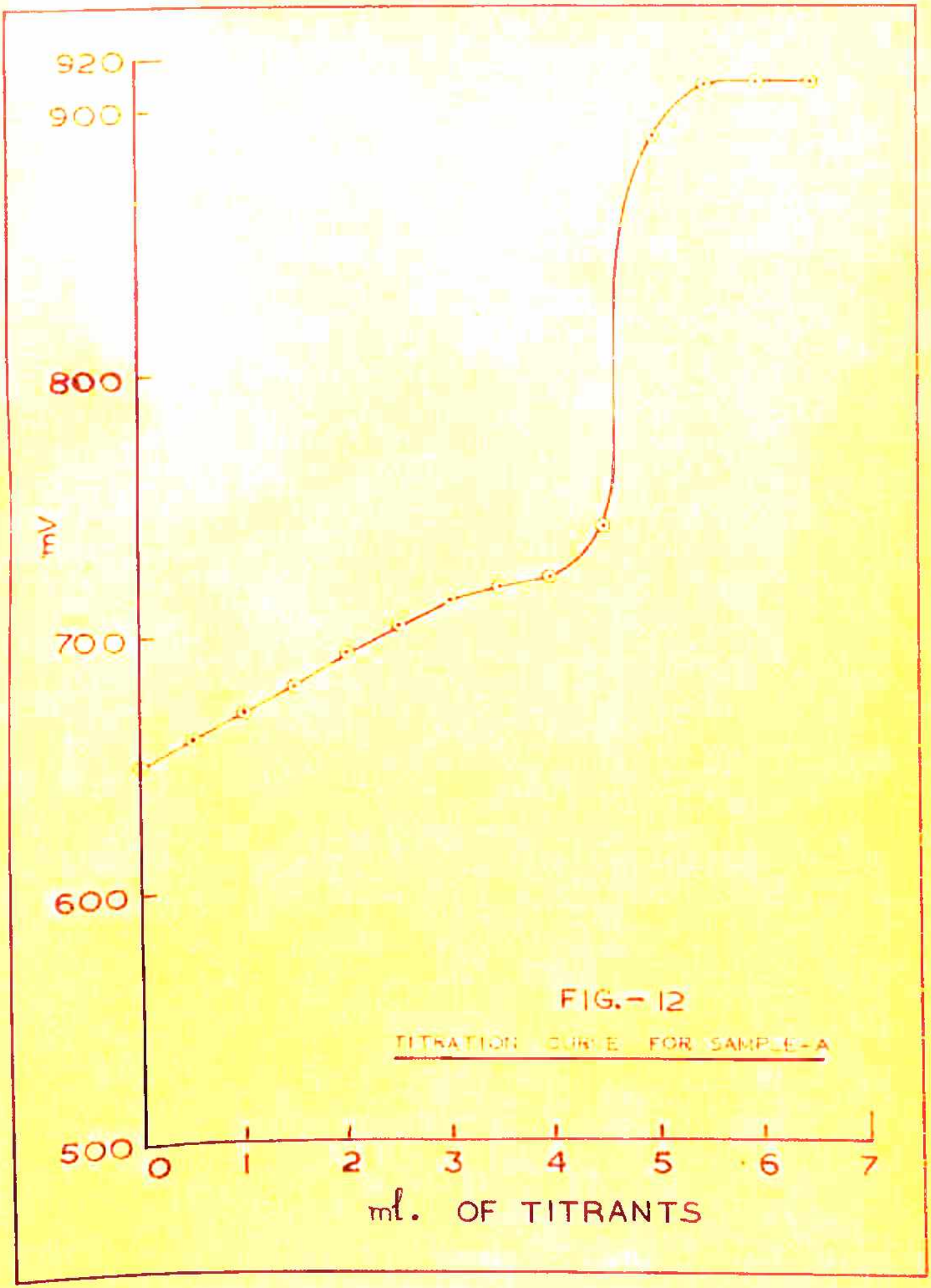


FIG.- 12

TITRATION CURVE FOR SAMPLE-A

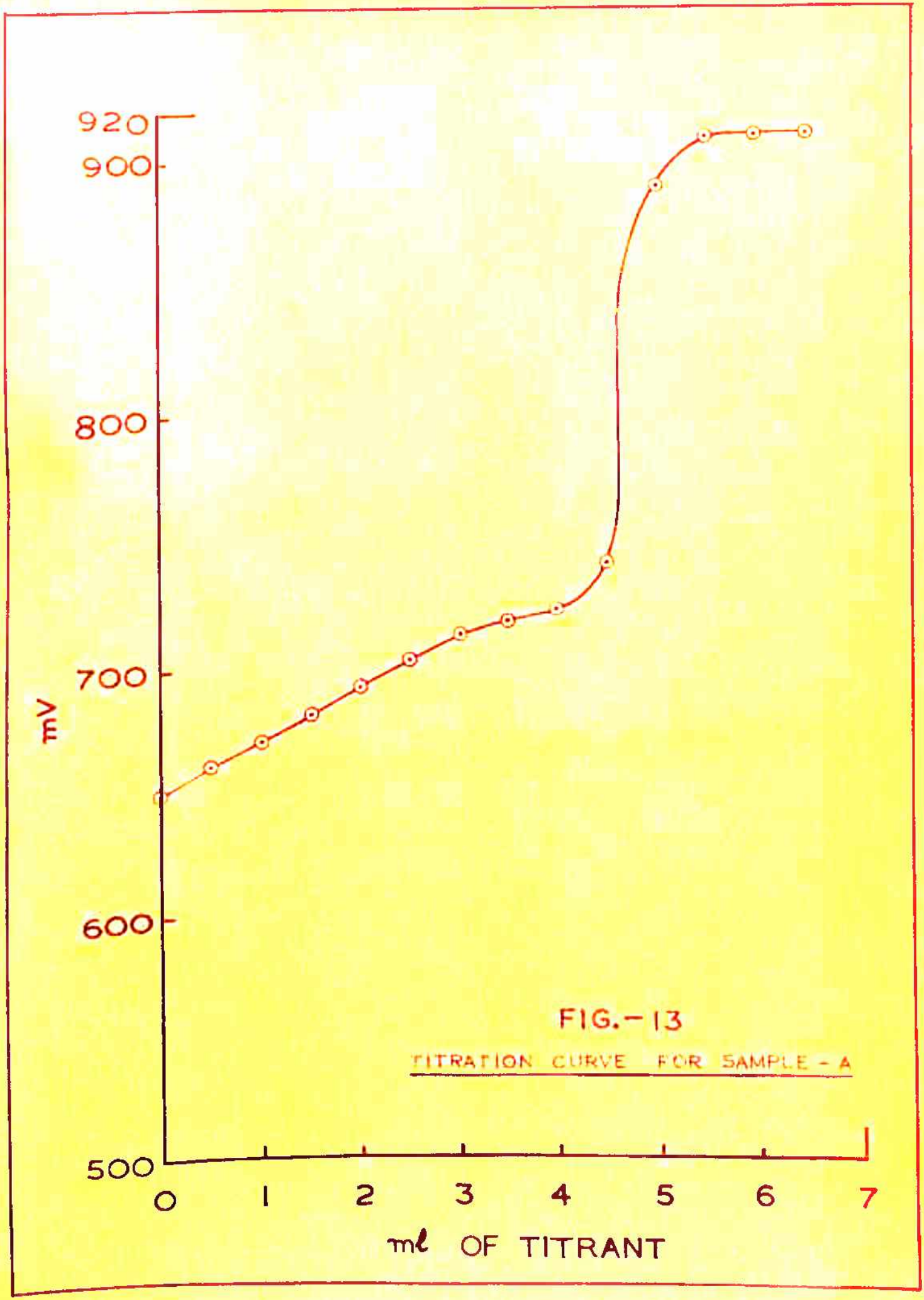


FIG.– 13

TITRATION CURVE FOR SAMPLE – A

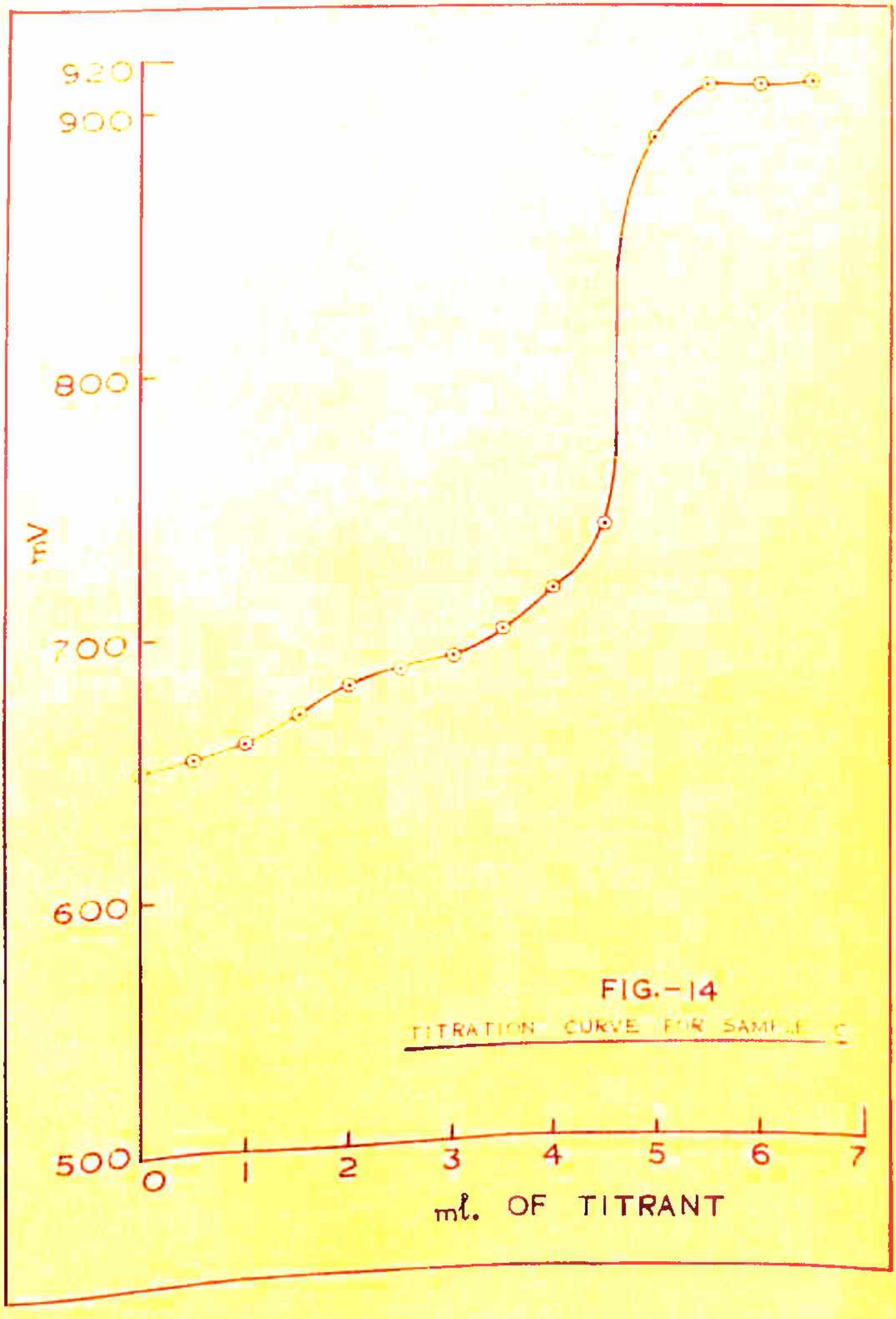


FIG.-14

TITRATION CURVE FOR SAMPLE C

TABLE-31

Sodium contents of Sod. CMD Ispaghula

Sample	Direct titration		Potentiometric titration	
	Volume consumed (ml)	Sodium content (%)	Volume consumed (ml)	Sodium content (%)
Ispaghula husk	0.2	0.32	-	-
Sample - A	4.65	7.59	4.6	7.50
Sample - B	4.60	7.50	4.6	7.50
Sample - C	4.60	7.50	4.6	7.50

(ii) I.R. Spectra

I.R. spectra of the samples were recorded in Perkin-Elmer Infrared Spectrophotometer using KBr disc technique and compared with the spectra of husk. Broad peaks at 1650-1550 cm^{-1} and 1450-1400 cm^{-1} for carboxylic salt[179,180] (COO^- stretching, Sod. salt) and in regions 1150-1025 cm^{-1}, 1250 cm^{-1} for alkyl ether linkage[179,180] (C–O–C) were observed, giving proof that carboxy methyl ether group have been introduced in the molecule. IR spectra of samples is shown in Fig.-15 for Sod. CMD Ispaghula and for husk in Fig.-2.

(iii) Viscosity

Viscosity of samples A. B and C (0.5% and 0.75% w/v) was determined using Fisher improved MacMicheal viscometer

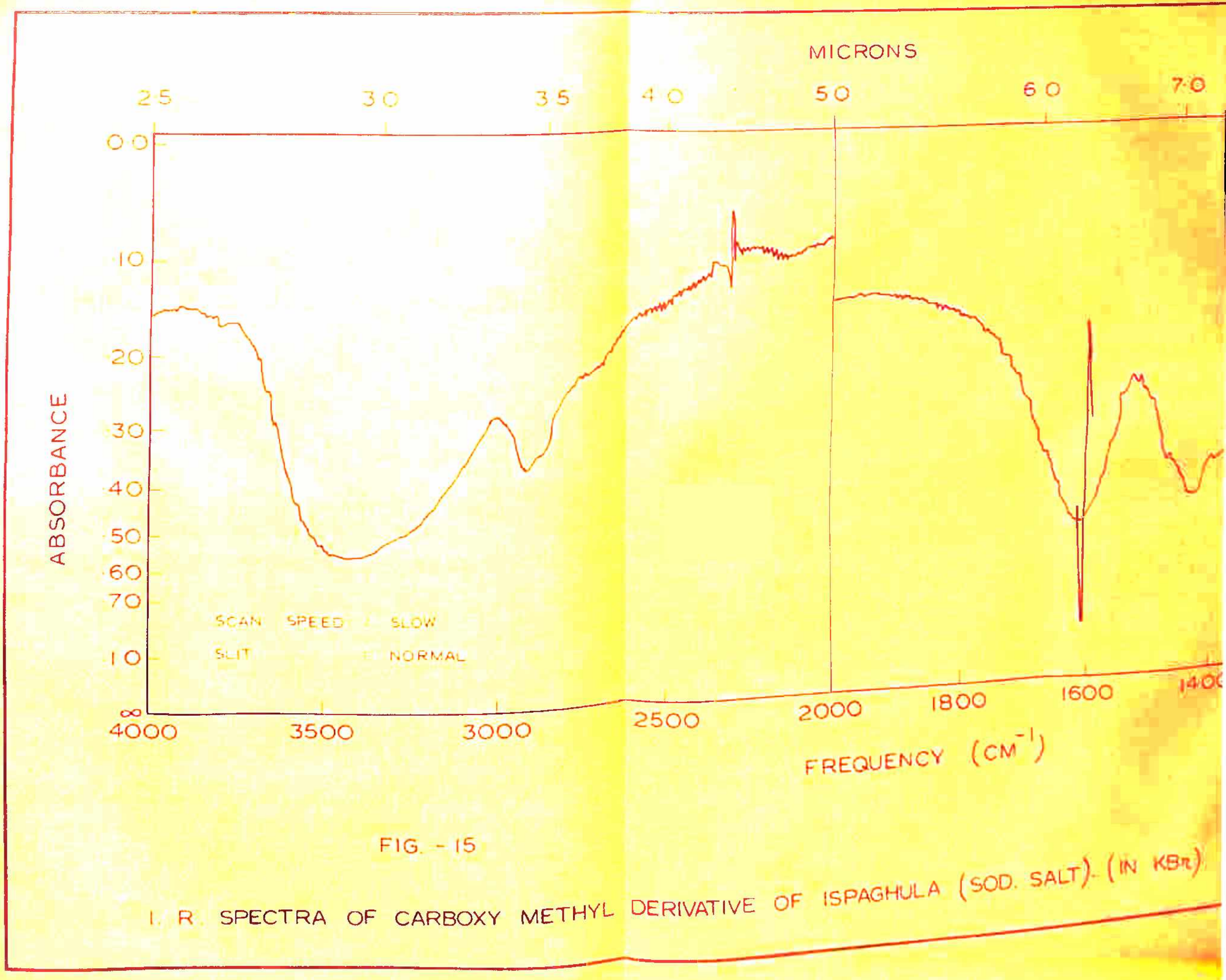


FIG. - 15

I. R. SPECTRA OF CARBOXY METHYL DERIVATIVE OF ISPAGHULA (SOD. SALT). (IN KBr)

TABLE-33

Rheological behaviour of Sod. CMD Isphghula

S.No.	R.P.M.	Conc. 0.5% w/v		Conc. 0.75% w/v	
		Upcurve	Down curve	Upcurve	Down curve
1	7.86	55	50	70	65
2	8.53	58	56	72	68
3	9.13	60	58	76	72
4	9.94	73	69	81	78
5	10.59	76	73	85	83
6	10.97	80	78	88	85
7	12.00	83	81	95	92
8	13.54	86	82	99	96
9	16.83	90	87	109	107
10	20.46	96	93	120	116
11	24.00	105	100	132	130
12	29.52	115	115	150	150

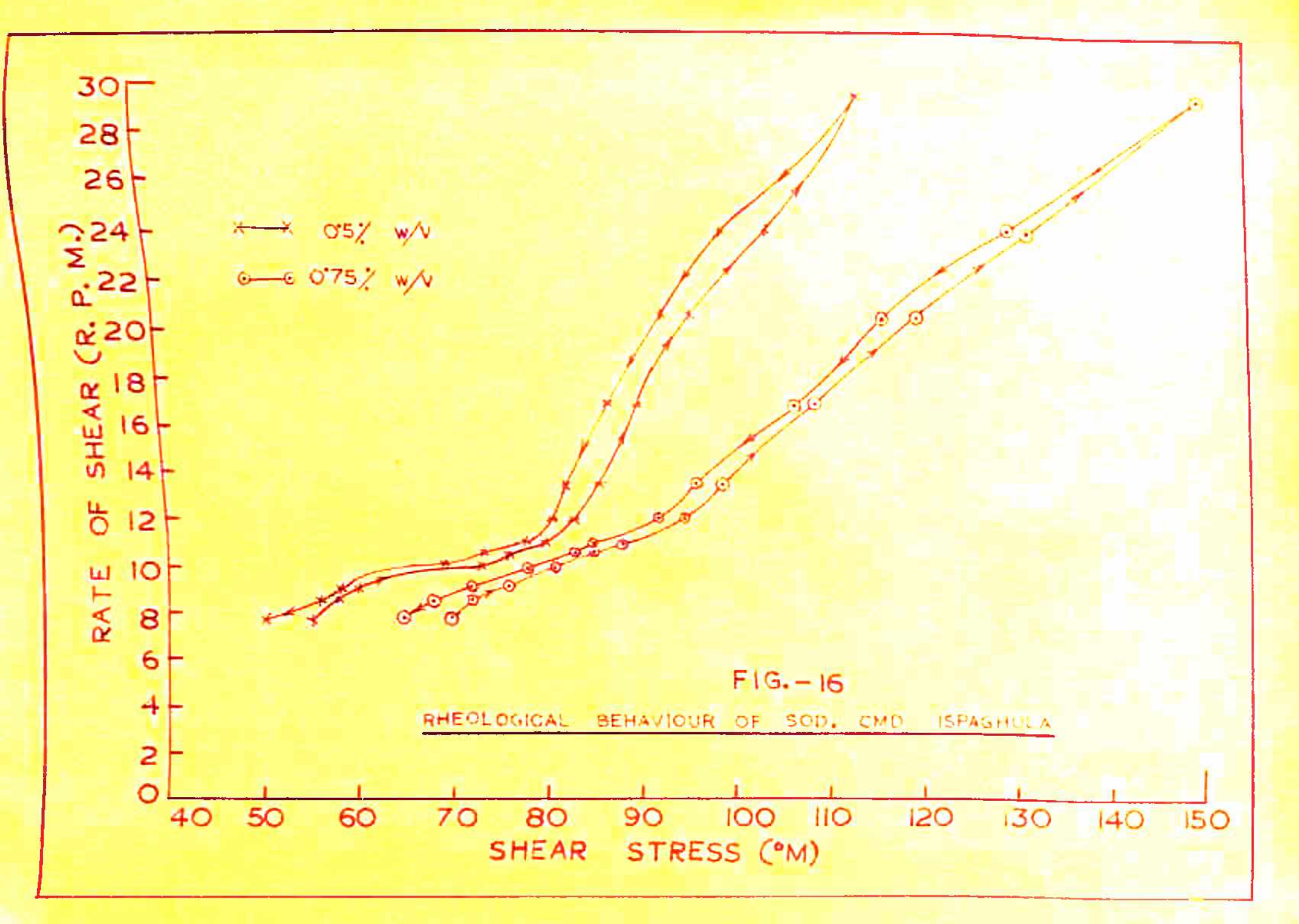


FIG. – 16

RHEOLOGICAL BEHAVIOUR OF SOD. CMD ISPAGHULA

TABLE-34

Rheological behaviour of Ispaghula husk

S.No.	R.P.M.	Conc. 0.5% w/v		Conc. 0.75% w/v	
		Upcurve	Down curve	Upcurve	Down curve
1	7.86	39	23	58	44
2	8.53	40	26	62	58
3	9.13	45	30	68	63
4	9.94	48	36	75	72
5	10.59	50	43	81	79
6	10.97	52	48	90	85
7	12.00	54	50	96	93
8	13.54	58	52	100	96
9	16.83	72	66	103	98
10	20.46	86	75	110	105
11	24.00	90	86	125	120
12	29.52	98	98	138	138

Results and Discussions

The above study indicates that Sod. CMD Ispaghula possesses greater viscosity as compared to Ispaghula husk of similar concentrations. Further the plot of rate of shear vs shear stress (Fig.-16) shows that Sod. CMD Ispaghula possesses non-Newtonian characteristics like Ispaghula husk (Fig.-17).

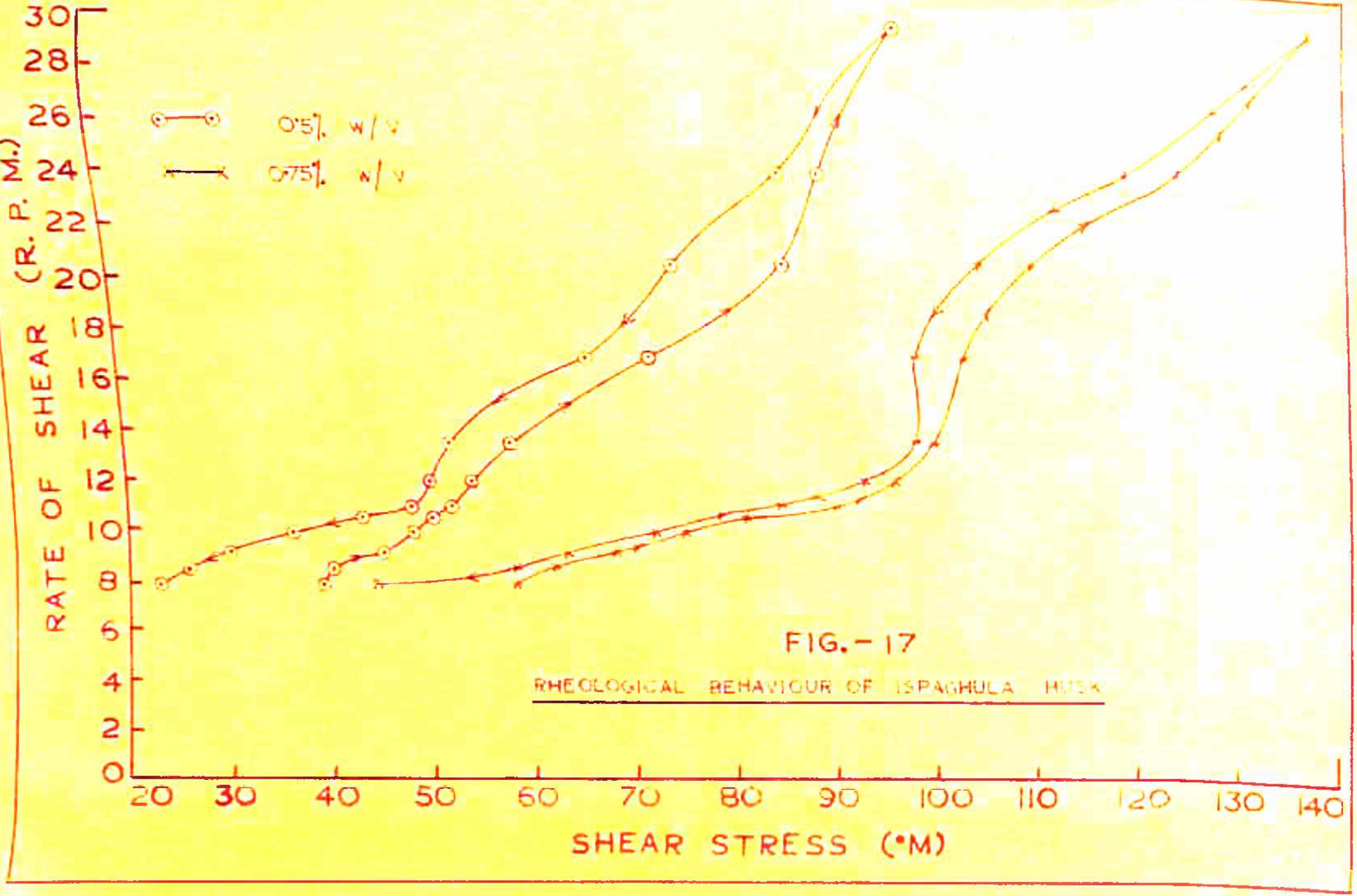


FIG.- 17

RHEOLOGICAL BEHAVIOUR OF ISPAGHULA HUSK

On the basis of Sodium contents, IR spectra and viscosities it is evident that all the three samples (A, B and C) of Sod. CMD Ispaghula possesses similar characteristics. Hence following the above mentioned procedure bulk quantities of Sod. CMD Ispaghula was prepared for further studies.

EFFECT OF PHYSICO-CHEMICAL VARIABLES ON THE VISCOSITY OF SOD. CMD ISPAGHULA

Viscosities and stabilities of the mucilage of gums are known to be influenced to appreciable extents by factors such as concentrations, temperatures, prolonged heating, pH, added substances, ultrasonic vibrations, etc. Baveja and Gupta[66-68,76] have reported the effect of various variables on the rheology of *Plantago ovata* seed husk mucilage. Mithal and Gupta[74,75] reported the effect of pH, heat, aging, added substances such as alcohol, surfactant, humectants and ionic substances. Study of the effects of above mentioned variables on the viscosity of Sod. CMD Ispaghula mucilage was undertaken and is discussed below.

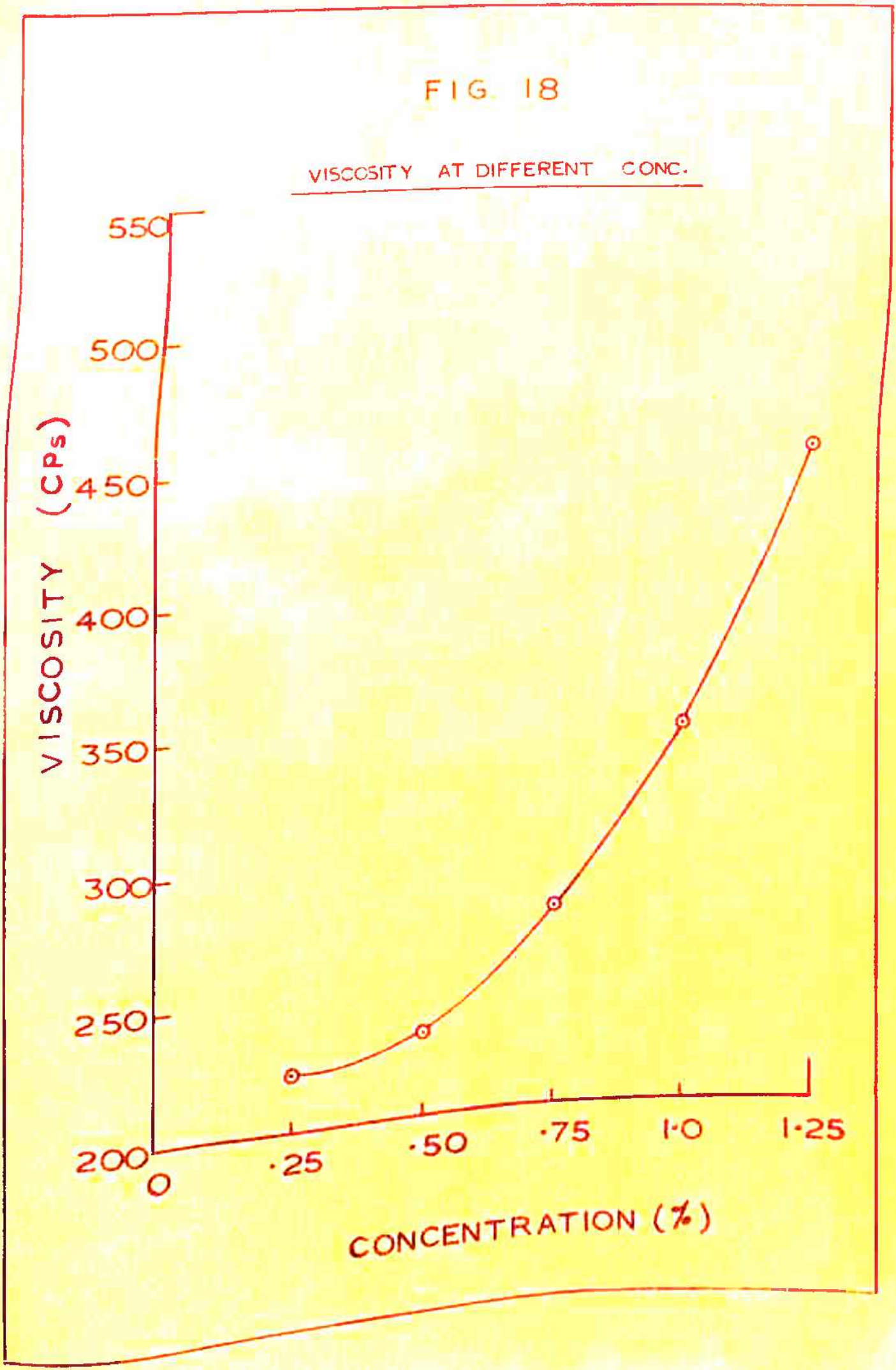
(i) Effect of Concentrations

Viscosity is a function of concentration and hence change in viscosities at various concentration of Sod. CMD Ispaghula were studied as below.

Experimental

0.25%, 0.5%, 0.75%, 1.0% and 1.25% w/v dispersions

FIG. 18


VISCOSITY AT DIFFERENT CONC.
VISCOSITY (CPs)
550
500
450
400
350
300
250
200
0
·25
·50
·75
1·0
1·25
CONCENTRATION (%)

of Sod. CMD Ispaghula were prepared and viscosities determined using 30 gauge torsion wire and cylindrical plunger (4.0 cm dia.) as discussed earlier. Results are recorded in Table-35 and a rheogram of concentration vs viscosities shown in Fig.-18.

TABLE-35

Viscosities at different concentrations

S.No.	Concentrations (% w/v)	Disc Reading (^{o}M)				Viscosity (cps)
		I	II	III	Mean	
1	0.25	93	91	92	92	220.7
2	0.50	96	96	96	96	230.2
3	0.75	116	116	116	116	278.2
4	1.00	145	146	144	145	347.7
5	1.25	190	190	190	190	455.7

(ii) Effect of pH

pH is known to affect the viscosity and stability of hydrophilic and semi-synthetic gums to a considerable extent. pH values at which the viscosity is maximum differs from gum to gum. The effect of pH on the viscosity of Sod. CMD Ispaghula was therefore studied.

Experimental

Buffer solutions ranging from 2.6 to 11 pH were prepared[181]. 0.5%w/v dispersions of Sod. CMD Ispaghula

were prepared using buffer solutions and viscosities determined as usual. Values are given in Table-36 and graphically represented in Fig.-19.

TABLE-36

Effect of pH values on the viscosity

S.No.	pH	Disc Reading (^{o}M)			Mean (^{o}M)
		I	II	III	
1	Control	96	96	96	96.0
2	2.6	74	73	72	72.0
3	3.0	77	77	77	77.0
4	4.0	82	84	83	83.0
5	5.0	95	95	95	95.0
6	6.0	95	95	95	95.0
7	7.0	95	95	95	95.0
8	8.0	95	97	96	96.0
9	9.0	95	95	95	95.0
10	10.0	93	94	92	93.0
11	11.0	90	89	90	89.6

Results

Viscosity of Sod. CMD Ispaghula remains almost constant from pH 5.0 to pH 9.0, but get reduced below pH 5 and above pH 9.0.

(iii) Effect of Temperature

Studies of the effect of heat on mucilage of natural gums have revealed that while limited amounts of heat may cause an increase in their viscosities, the viscosities decrease at higher temperatures due to degradation of the gum molecules or due to rupture of hydrogen bonds. Effect of temperature on the viscosity of Sod. CMD Ispaghula were studied as follows.

Experimental

0.5% dispersion of Sod. CMD Ispaghula was prepared and disc reading (^{o}M) was recorded at room temperature (26°). The temperature was then raised to 30° and the sample allowed to stand for 30 mts. Thereafter cup was rotated at 20.46 r.p.m. and disc reading recorded (Table-37). In this manner temperature was gradually increased to 60° and readings recorded after 30 mts wait at each temperature. Results are shown in Table-37 and graphically represented in Fig.-20.

Results and Discussion

There was a small increase in disc reading on raising the temperature from 26° to 30°, thereafter a steady fall in viscosity was observed.

TABLE-37

Effect of temp. on the viscosity of Sod. CMD Ispaghula

S.No.	Temperature (°C)	Disc Reading (°M)			Mean (°M)
		I	II	III	
1	26	94	94	94	94.0
2	30	96	95	96	95.6
3	35	82	81	80	81.0
4	40	68	67	67	67.3
5	45	56	56	56	56.0
6	50	47	46	46	46.3
7	55	38	38	38	38.0
8	60	36	36	36	36.0

IV. Effect of Prolonged Heating at High Temperature

100 ml of 0.5% w/v Sod. CMD Ispaghula dispersion was taken in a tared beaker and heated on a water bath (98°±1) for 15 minutes. It was then cooled to room temperature and water lost by evaporation added and mixed up. The disc readings were noted at 20.46 r.p.m. (Table-38). In the same way three samples were heated for 30, 45 and 60 minutes, and the disc readings determined. Results are recorded in Table-38 and represented in Fig.-21.

FIG. – 21

EFFECT OF HEATING AT HIGH TEMPERATURE

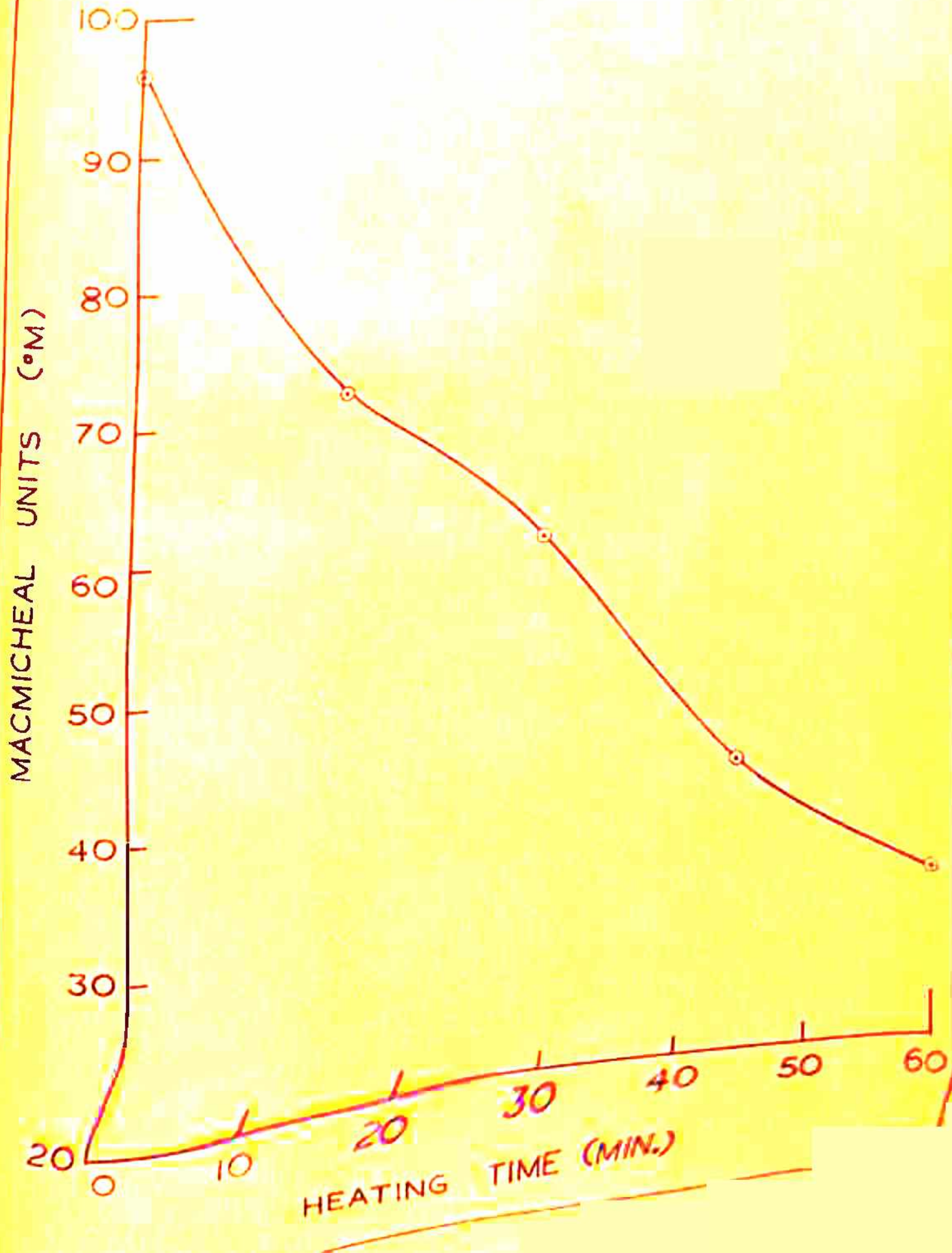


TABLE-38

Effect of heating at High Temperature ($98^{o}\pm1$)

S.No.	Time of Heating	Disc Reading (^{o}M)			Mean (^{o}M)
		I	II	III	
1	Control	96	96	96	96
2	15 mts.	73	72	71	72
3	30 mts.	60	60	60	60
4	45 mts.	42	43	41	42
5	60 mts.	33	33	33	33

(V) Effect of Surfactants

To study effect of surfactants, sodium lauryl sulphate (SLS) representing anionic, cetyl trimethyl ammonium bromide (CTAB) representing cationic and Tween 80 (Polyoxy ethylene sorbitan mono oleate) representing non-ionic type were selected in different concentrations.

Experimental

0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/v solutions of SLS, CTAB and Tween 80 were prepared separately and used as solvents for preparing Sod. CMD Ispeghula (0.5% w/v) dispersions. The disc readings of each sample are shown in Table-39. A blank was run simultaneously without any surfactant. Results are represented graphically in Fig.-22.

FIG.– 22

EFFECT OF SURFACTANTS

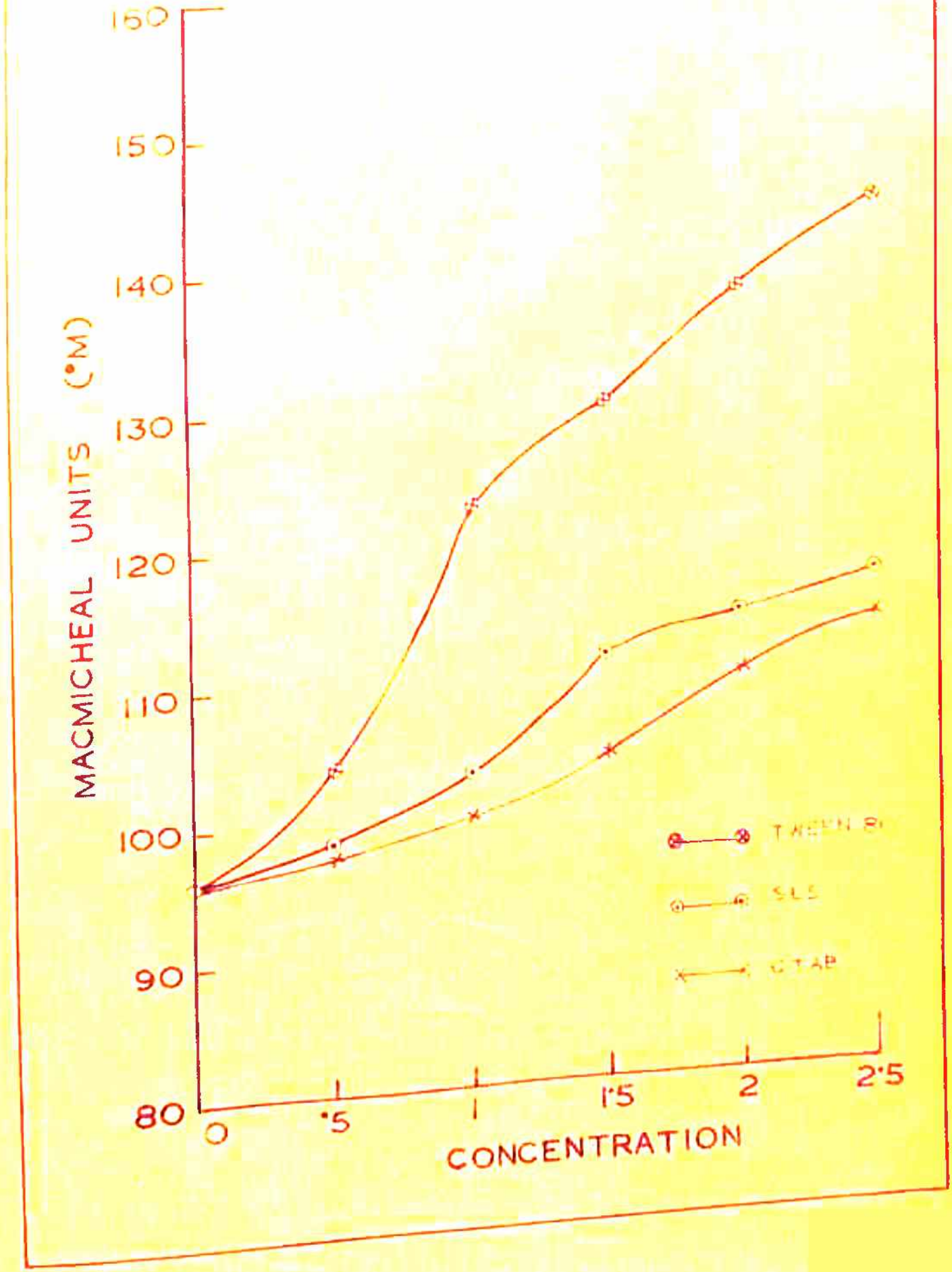


TABLE-39

Effect of Surfactants on the Viscosity

Surfactant	Conc. (%)	Disc Reading (°M)			Mean (°M)
		I	II	III	
Blank	-	96	96	96	96.0
SLS	0.5	99	99	99	99.0
	1.0	103	103	103	103.0
	1.5	111	112	111	111.3
	2.0	114	114	114	114.0
	2.5	117	117	117	117.0
CTAB	0.5	98	98	98	98.0
	1.0	100	100	100	100.0
	1.5	105	105	102	104.0
	2.0	109	110	110	109.6
	2.5	113	114	113	113.3
Tween 80	0.5	103	105	104	104.0
	1.0	122	123	123	122.6
	1.5	130	130	130	130.0
	2.0	138	138	138	138.0
	2.5	145	145	145	145.0

(VI) Effect of Ultraviolet Irradiation

Carla Zucca[182] studied the effect of UV irradiation on the viscosity of gum mucilages. Effect of UV rays on the viscosity of Sod. CMD Ispaghula was studied as follows.

Experimental

0.5% w/v dispersion of Sod. CMD Ispaghula was prepared and its initial disc reading at 20.46 r.p.m. recorded. The dispersion was then divided in three parts. First was exposed to UV rays for 15 mts., second for 30 mts. and the third for 45 mts. and then their disc readings determined at the same r.p.m. Results are recorded in Table-40 and shown graphically in Fig.-23.

TABLE-40

Effect of Ultraviolet Irradiation

S.No.	Time of exposure	Disc Reading (^{o}M)			Mean (^{o}M)
		I	II	III	
1	Control	96	95	96	95.6
2	15 mts.	88	88	89	88.3
3	30 mts.	75	75	75	75.0
4	45 mts.	67	67	67	67.0

Results and Discussions

Viscosities of Sod. CMD Ispaghula decrease when exposed to UV rays. The decrease in viscosity may be due to structural breakdown of the polysaccharide molecule.

(vii) Effect of Ultrasonic Vibrations

Effect of ultrasonic waves[183,184] on the viscosity of Sod. CMD Ispaghula dispersion was studied as follows.

FIG-23

EFFECT OF ULTRAVIOLET IRRADIATION

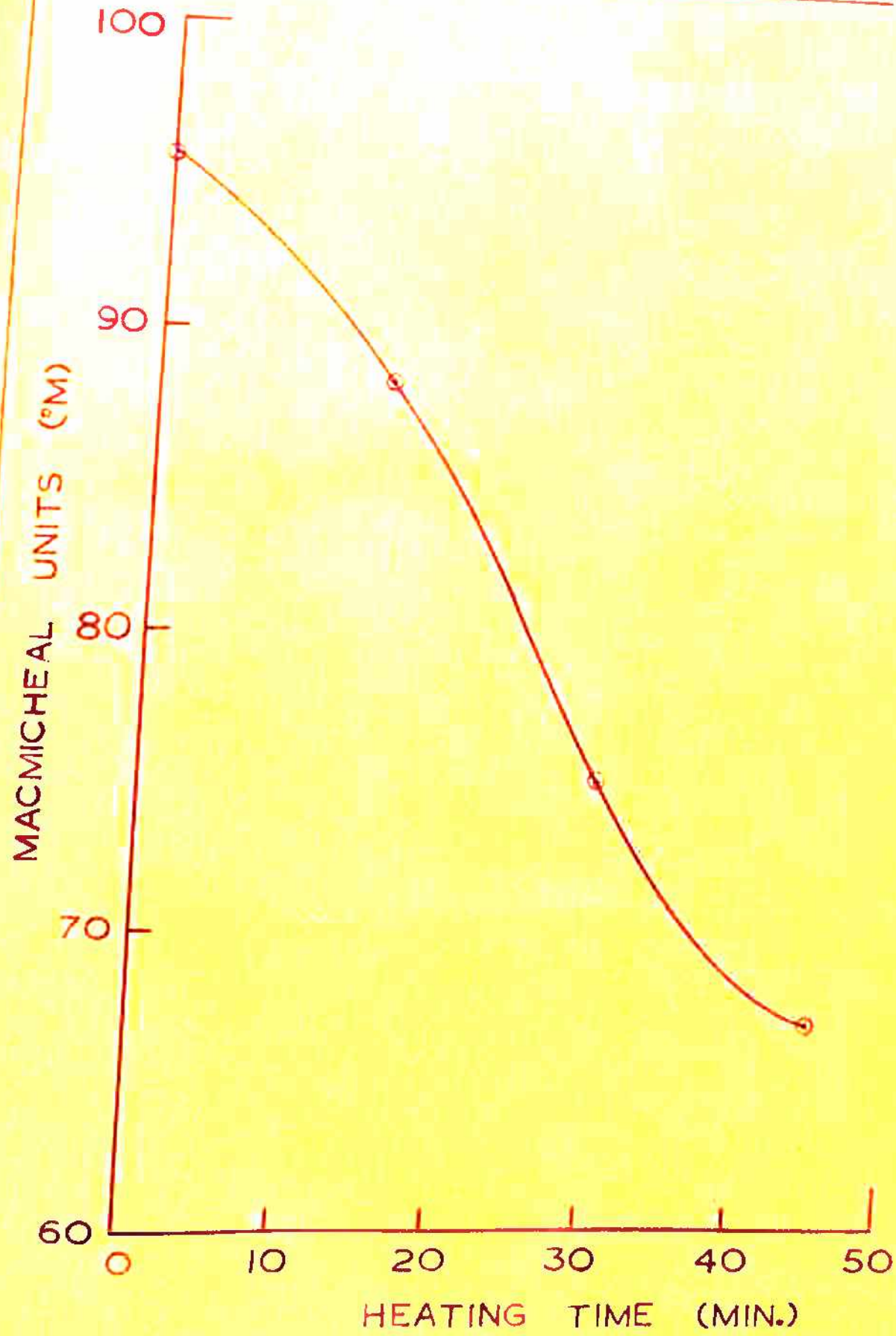
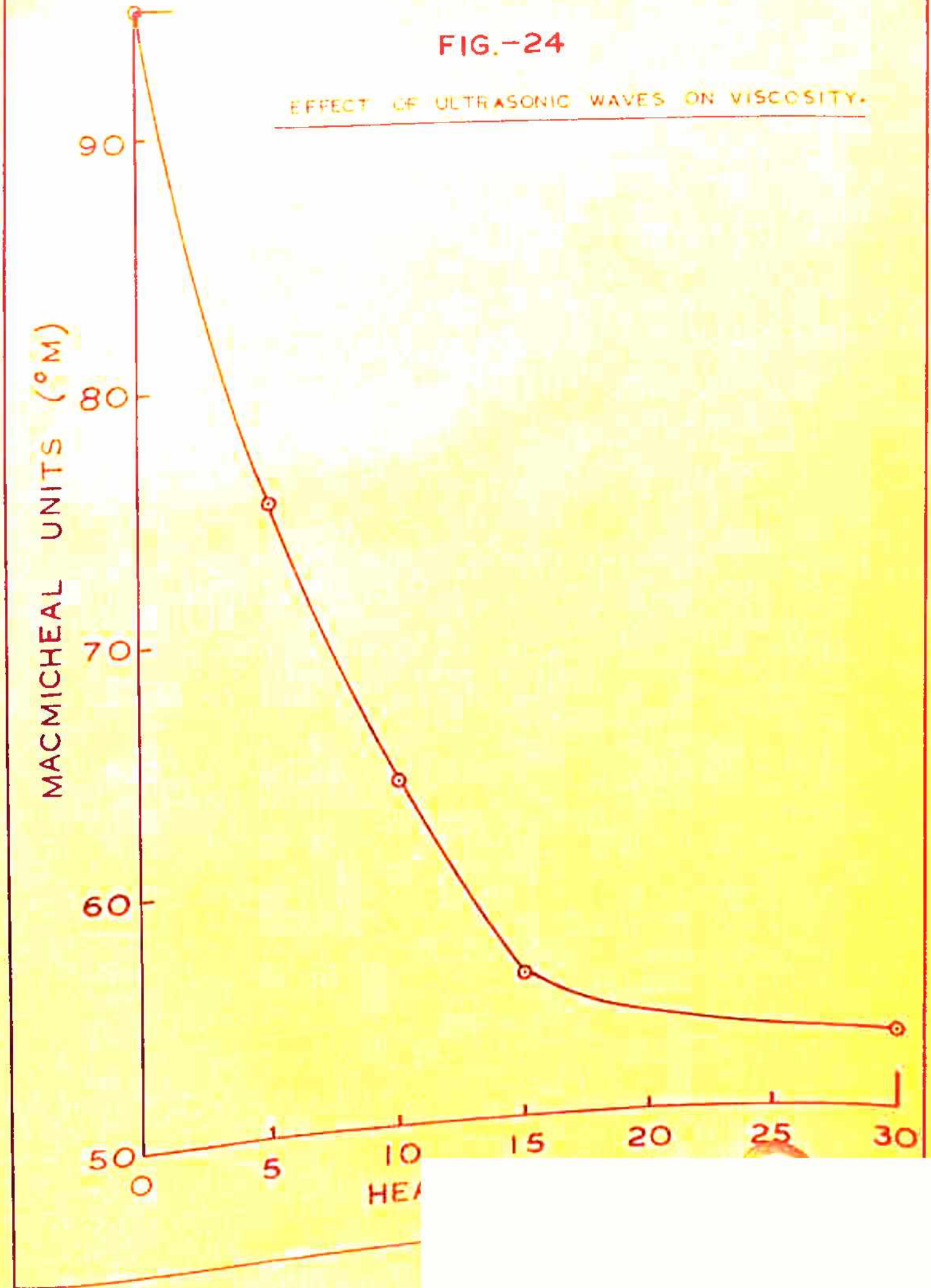


FIG.-24

EFFECT OF ULTRASONIC WAVES ON VISCOSITY.



Experimental

0.5% w/v dispersions of Sod. CMD Ispaghula was prepared and its initial disc reading recorded. This was then divided in 4 parts. One portion was subjected to ultrasonic vibrations (Frequency - 90 Kcs) for 5 minutes (using Systronic Ultrasonic Unit), the second for 10 minutes, third for 15 minutes and fourth was given 30 minutes treatment with ultrasonic vibrations. Thereafter disc readings for each sample were determined. Results are recorded in Table-41 and shown in Fig.-24.

TABLE-41

Effect of Ultrasonic waves on viscosity

S.No.	Time of exposure	Disc Reading (^{o}M)			Mean (^{o}M)
		I	II	III	
1	Control	95	95	95	95.0
2	5 mts.	76	75	76	75.6
3	10 mts.	65	64	64	64.3
4	15 mts.	56	56	56	56.0
5	30 mts.	54	53	53	53.3

Results

The viscosity of Sod. CMD Ispaghula falls considerably when subjected to ultrasonic vibrations.

++++++++++

CHAPTER VI

BINDING PROPERTIES OF CARBOXYMETHYL DERIVATIVE OF ISPAGHULA (SOD. SALT)

Many natural and semisynthetic hydrocolloids such as acacia, gelatin, starch, methyl cellulose, carboxymethyl cellulose, etc. are used as binding agents for the wet granulation of medicaments. Mithal and Bhutiani[80] reported Ispaghula husk to be a good binding agent. The feasibility of employing Sod. CMD Ispaghula as binding agent for wet granulation was therefore investigated.

PRELIMINARY EVALUATION OF BINDING CAPACITY

In order to assess the binding capacity of Sod. CMD Ispaghula, Lactose and calcium carbonate granules of following compositions were prepared:

Lactose	30.0 g.	Cal. carbonate	30.0 g.
Soluble starch	1.5 g.	Soluble starch	1.5 g.
Binding agent	q.s.	Binding agent	q.s.

Three lots each of Lactose and Cal. Carbonate were weighed and mixed homogeneously with soluble starch and granulated with 0.5% and 1.0% w/v Sod. CMD Ispaghula. Third lot of granules was prepared with water alone for control. The granules were passed through sieve No. 16 and dried. Percentage fines of powder were then determined by arranging sieve No. 22, 40, 60 mesh in order and putting granules on sieve No. 22. Fractions of (a) material passing through

sieve No. 22 but retained by sieve No. 40 mesh, (b) passing through sieve No. 40, but retained by sieve No. 60, and (c) passing through sieve No. 60 mesh, were recovered and weighed. Percentage fines above 60 mesh and above 40 mesh were then calculated using the following formula. Results are shown in Table-42.

$$\% \text{ Fines above 40 mesh} = \frac{B + C}{A + B + C} \times 100$$

$$\% \text{ Fines above 60 mesh} = \frac{C}{A + B + C} \times 100$$

where A = weight of granules which are retained by sieve No. 40.

B = weight of granules which passed through sieve No. 40, but retained by sieve No. 60 mesh.

C = weight of fines passed through sieve No. 60.

TABLE-42

Percentage fines of granules using Sod. CMD Ispaghula

Material	Conc. (% w/v)	Percentage fines	
		Above 40 mesh (%)	Above 60 mesh (%)
Lactose	Control	56.46	39.87
	0.5%	34.43	11.48
	1.0%	12.90	3.22
Cal. carbonate	Control	55.73	36.07
	0.5%	33.65	14.24
	1.0%	16.11	6.05

Since the preliminary work showed Sod. CMD Ispaghula dispersion to possess good binding capacity the following further studies were carried out on its binding properties.

SELECTION OF SUITABLE CONCENTRATION

For conversion into granules, powders have to be moistened with water or a weak adhesive or a strongly adhesive mixture[185]. Hence tablets with different amounts of Sod. CMD Ispaghula (0.2% to 1.5% w/v) were prepared in order to arrive at a concentration giving best results. Control granules were also prepared for each series using water as the granulating agent.

Three sets of tablets using calcium gluconate (500 gm/Tab), sodium bicarbonate (300 mg/Tab) and lactose (350 mg/Tab), all of pharmaceutical grade, were prepared using water and 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.25% and 1.50% w/v Sod. CMD Ispaghula dispersions as binding agents. In all eight lots of granules were prepared for each material. Soluble starch was used as disintegration agent and talc as the lubricant.

Preparation of granules

Wet granulating method was employed for the preparation of granules[186,187]. In each lot half the amount of the soluble starch was incorporated prior to granulation and the remaining half before the compression. Weighed quantities of materials were triturated thoroughly and

binding agents added gradually to convert the powder mass into damp state. This was then passed through sieve No. 16. Granules were dried and the % fines above 40 and % fines above 60 mesh sieve were determined as discussed earlier. Observations on percentage fines for all the three types of granules are shown in Table-43. The relationship of concentration and the percentage fines are shown graphically in Figs. 25, 26 and 27.

TABLE-43

Percentage fines of granules prepared with different concentration of Sod. CMD Ispaghula

Concentration (% w/v)	Soda. bi-Carb. Tablets.		Lactose Tablets		Cal. gluconate Tablets	
	A	B	A	B	A	B
Control	64.49%	45.08%	64.86%	40.55%	58.49%	30.18%
0.2%	49.18%	32.78%	55.42%	32.43%	48.18%	24.39%
0.4%	44.40%	25.40%	41.92%	21.92%	31.05%	11.43%
0.6%	33.65%	10.90%	34.25%	16.43%	27.11%	9.61%
0.8%	14.73%	4.80%	21.92%	8.21%	20.19%	4.80%
1.0%	14.19%	3.55%	16.58%	4.14%	17.35%	4.03%
1.25%	13.07%	3.27%	16.58%	4.14%	17.66%	3.83%
1.50%	12.94%	3.15%	15.83%	3.61%	17.47%	3.83%

A = Percentage fines above 40 mesh sieve

B = Percentage fines above 60 mesh sieve

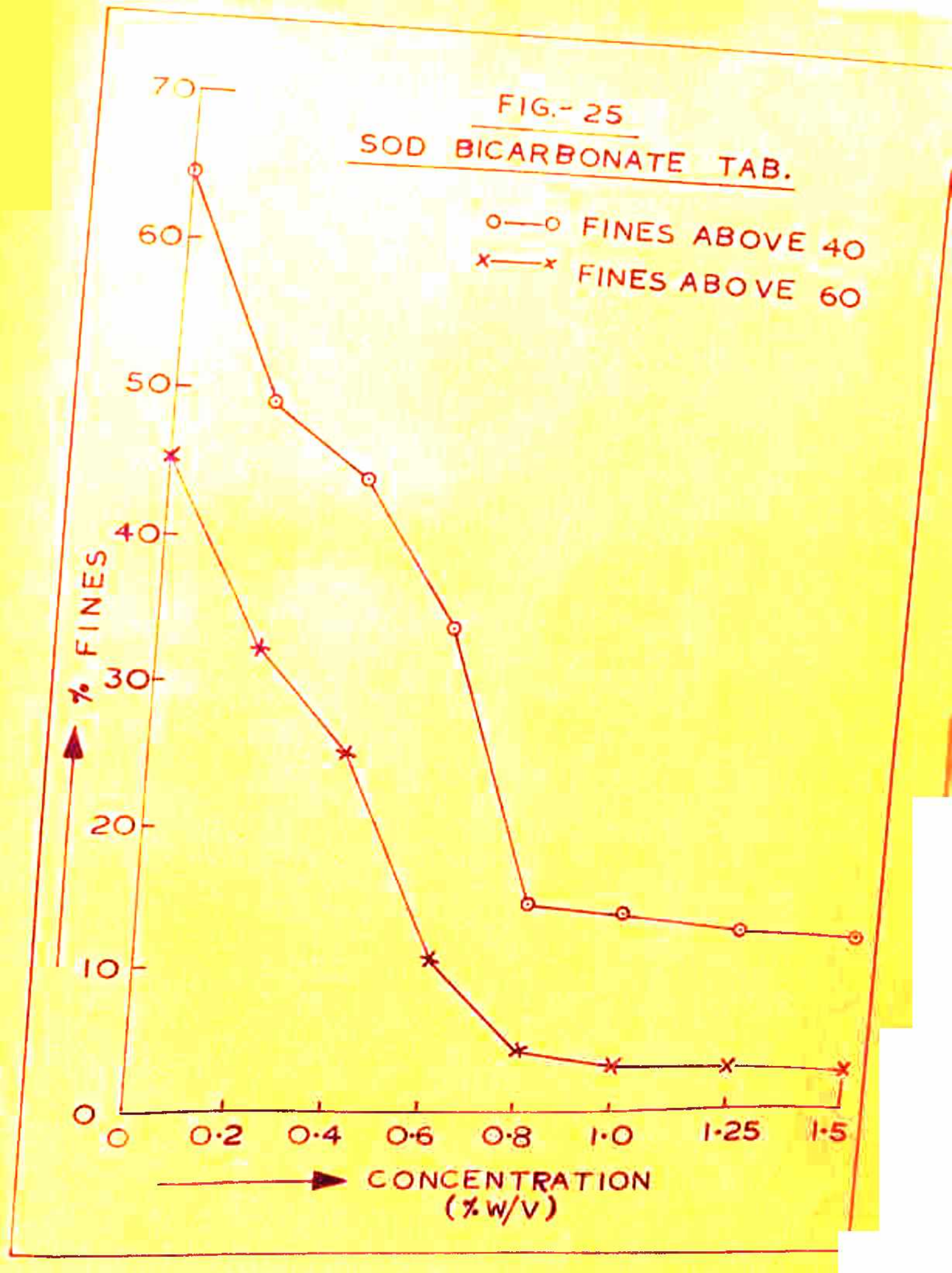


FIG.- 25
SOD BICARBONATE TAB.
o—o FINES ABOVE 40
x—x FINES ABOVE 60
% FINES
70
60
50
40
30
20
10
0
0
0·2
0·4
0·6
0·8
1·0
1·25
1·5
CONCENTRATION
(% W/V)

FIG.-26

LACTOSE TAB.

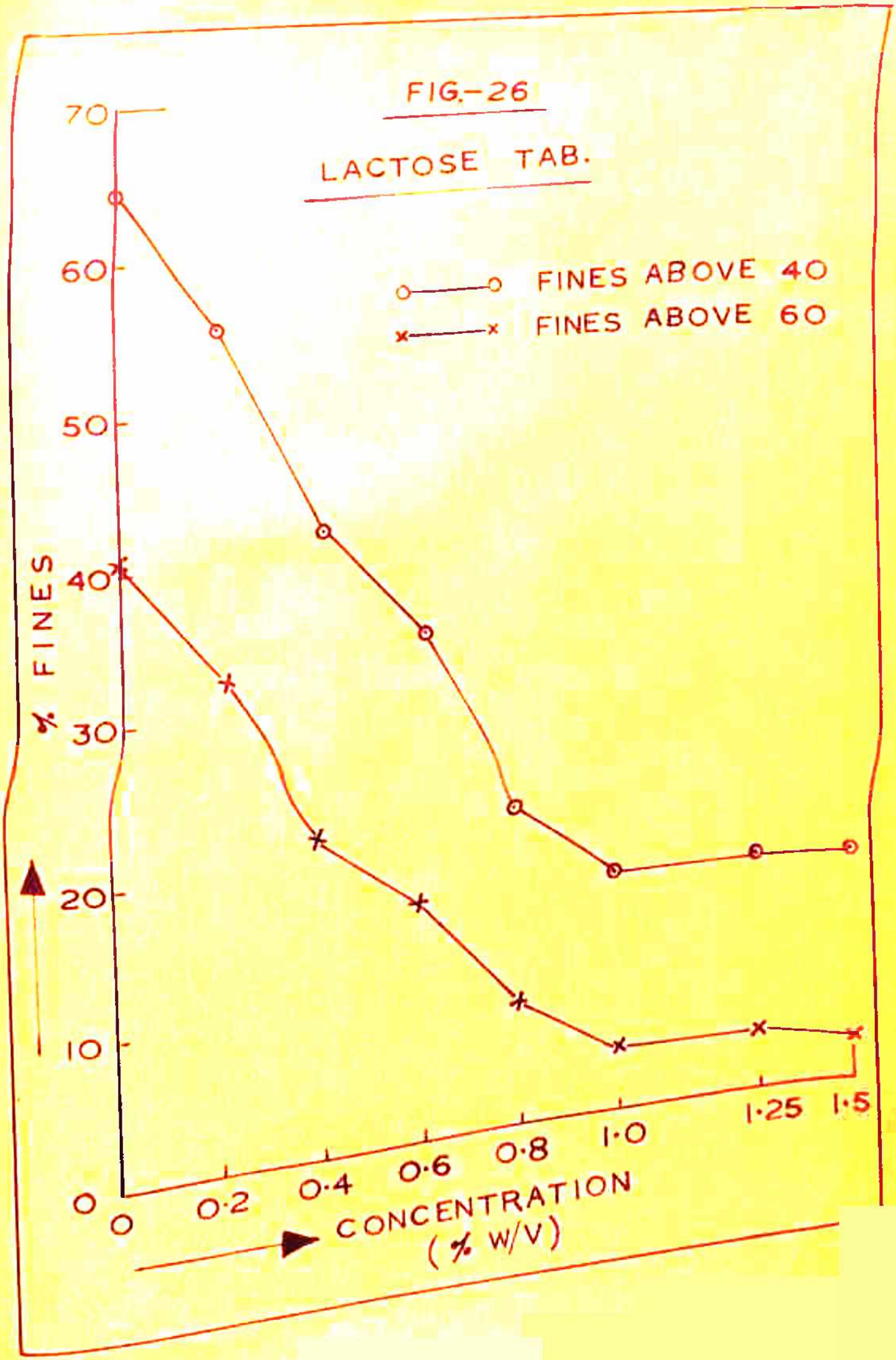
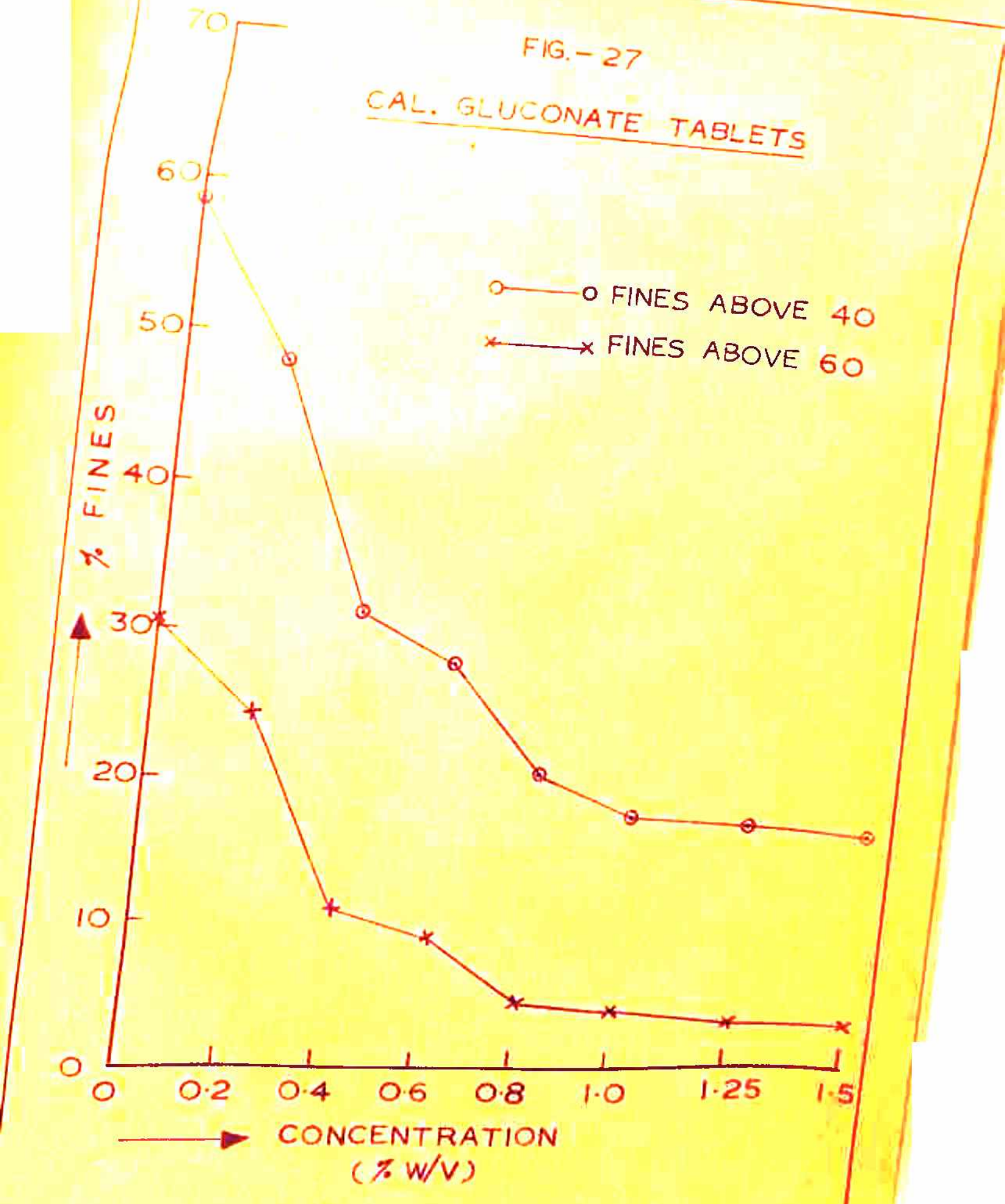


FIG. – 27

CAL. GLUCONATE TABLETS



Preparation of Tablets

Granules passing through sieve No. 16 but retained on sieve No. 60 mesh (16/60) were compressed using DIAF (H) Single Stroke Tablet Compression machine. Lactose and Sod. bicarbonate tablets were compressed with 9.5 mm diameter die and punches, whereas for Cal. gluconate tablets 12 mm diameter die and punches were used. The capacity of die was adjusted to 385 mg for Lactose, 330 mg for Sodium bicarbonate. The compression pressure and the speed of the machines was kept constant for each lot.

Evaluation of Compressed Tablets

Tablets were evaluated in the following respects:

(i) Average weight and weight deviation:

Ten tablets in each lot were weighed and average weight calculated. The tablets were then weighed individually and percentage deviation calculated[188,189]. The average weights and deviation are shown in Tables-44, 45 and 46.

(ii) Hardness:

Hardness of tablets was determined by Campbell (Thermonik) Tablet hardness tester (in kg/cm^2). Average values are recorded in Tables 44, 45 and 46, and shown graphically in Fig.-28.

(iii) Disintegration time:

Disintegration time of tablets were determined using Campbell Tablet Disintegration Test machine at 37°±1. The tablets were regarded as completely disintegrated when whole of the tablet broke down and granules passed through the gauge. Average values of disintegration time are recorded in Tables 44 to 46 and represented graphically in Fig.-29.

TABLE-44

Average weights, weight deviation, hardness and disintegration time of Sod. bicarbonate tablets

S. No.	Concentration (% w/v)	Average weight* (g.)	Percentage deviation		Hardness** (kg/cm^2)	Disintegration time** (sec.)
			Upper limit (%)	Lower limit (%)		
1	Control	0.3356	+3.337	-2.680	1.4	34.6
2	0.2	0.3325	+2.256	-3.458	1.8	38.0
3	0.4	0.3346	+1.908	-3.161	2.6	41.0
4	0.6	0.3328	+2.704	-3.846	2.9	51.0
5	0.8	0.3312	+2.657	-3.080	3.8	52.0
6	1.0	0.3312	+1.449	-0.9661	4.8	52.0
7	1.25	0.3322	+0.7827	-0.8128	5.0	54.6
8	1.50	0.3302	+0.8484	-1.515	5.4	55.3

*Average of ten tablets

**Average of five values

TABLE-45

Average weight, weight deviation, hardness and disintegration time of Lactose tablets

S. No.	Concentration (%)	Average weight* (g.)	Percentage deviation		Hardness** (kg/cm^2)	Disintegration time** (sec.)
			Upper limit (%)	Lower limit (%)		
1	Control	0.3892	+4.130	-3.525	1.6	47.6
2	0.2	0.3871	+3.641	-2.712	2.2	51.3
3	0.4	0.3862	+3.314	-2.329	2.6	57.6
4	0.6	0.3861	+2.642	-2.072	2.7	62.3
5	0.8	0.3845	+1.473	-1.047	3.4	67.3
6	1.0	0.3855	+1.445	-0.7263	4.6	70.0
7	1.25	0.3851	+1.462	-0.5712	4.8	73.0
8	1.50	0.3861	+1.036	-0.5180	5.0	73.3

*Average of ten tablets

**Average of five values

FIG.-28

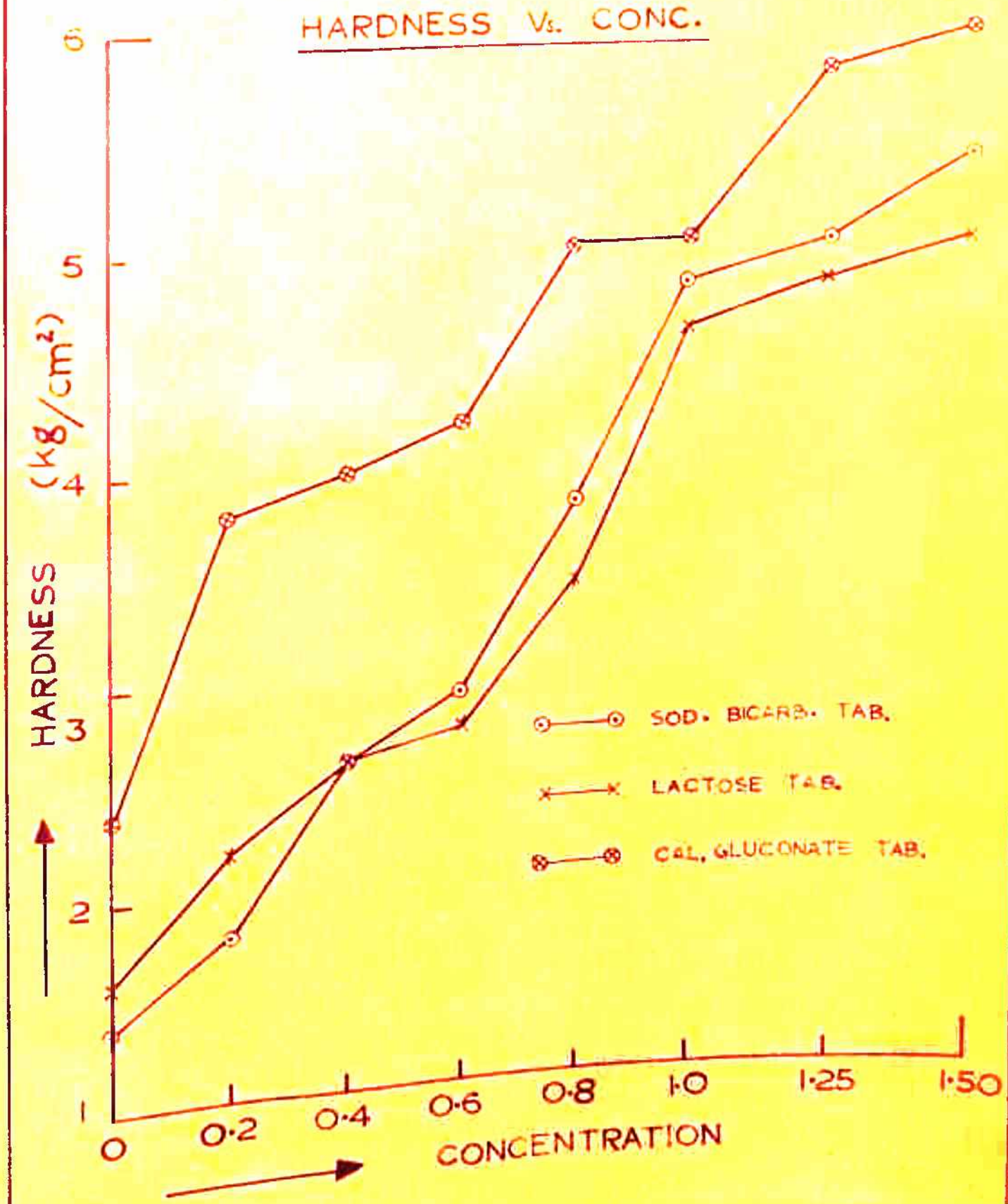

HARDNESS Vs. CONC.
HARDNESS (kg/cm²)
6
5
4
3
2
1
0
0·2
0·4
0·6
0·8
1·0
1·25
1·50
CONCENTRATION
SOD. BICARB. TAB.
LACTOSE TAB.
CAL. GLUCONATE TAB.

FIG.-29

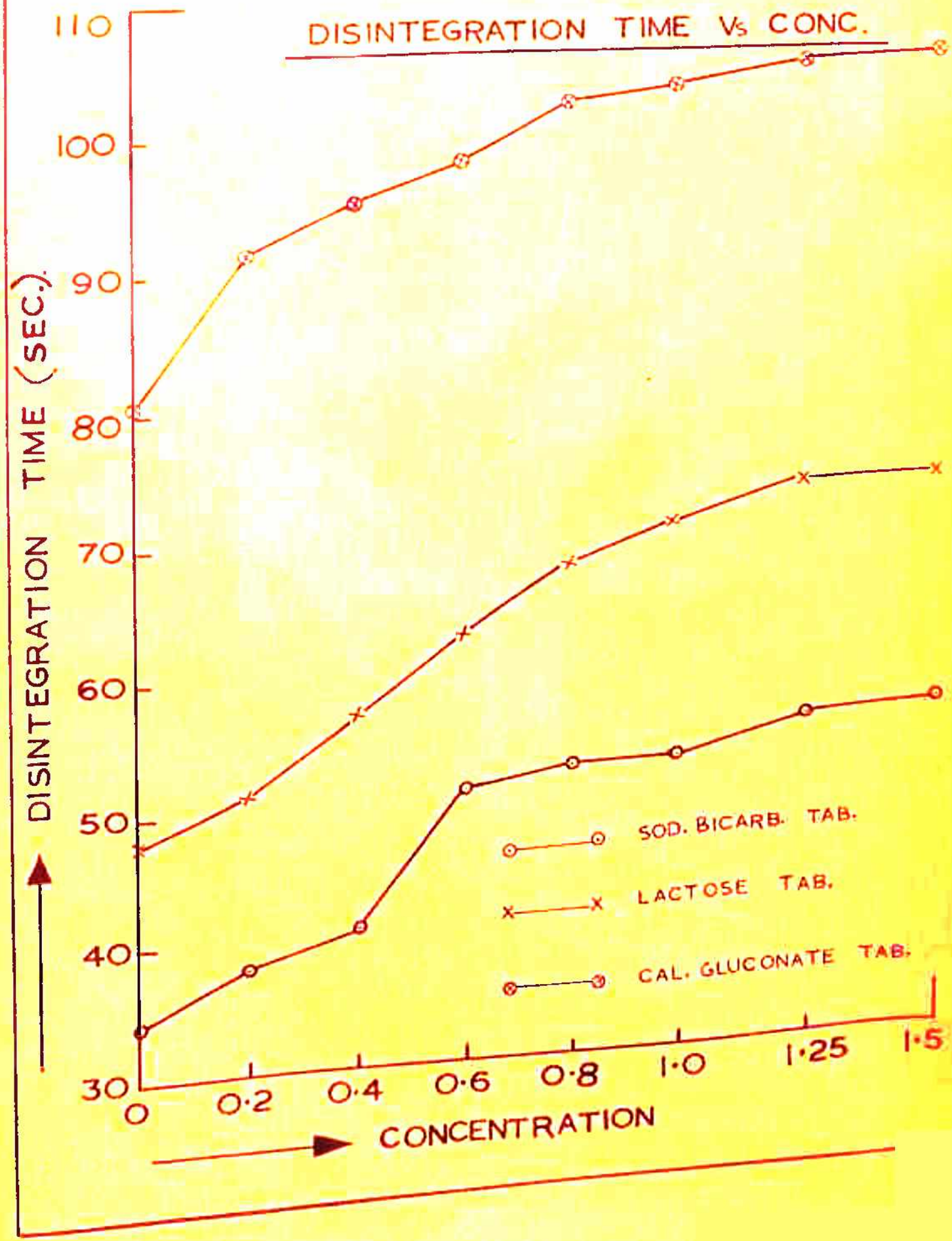

DISINTEGRATION TIME Vs CONC.
DISINTEGRATION TIME (SEC.)
110
100
90
80
70
60
50
40
30
0
0·2
0·4
0·6
0·8
1·0
1·25
1·5
CONCENTRATION
SOD. BICARB. TAB.
LACTOSE TAB.
CAL. GLUCONATE TAB.

TABLE-46

Average weight, weight deviation, hardness and disintegration time of Cal. gluconate tablets

S. No.	Concentration (% w/v)	Average weight* (g.)	Percentage deviation		Hardness** (kg/cm^2)	Disintegration time** (sec.)
			Upper limit (%)	Lower limit (%)		
1	Control	0.5462	+4.430	-3.843	2.4	80.6
2	0.2	0.5486	+3.918	-3.609	3.8	91.3
3	0.4	0.5488	+3.287	-2.278	4.0	95.3
4	0.6	0.5488	+1.859	-1.785	4.2	98.0
5	0.8	0.5514	+1.451	-1.541	5.0	102.3
6	1.0	0.5512	+1.223	-1.161	5.0	103.6
7	1.25	0.5508	+1.089	-1.089	5.8	105.0
8	1.50	0.5509	+1.125	-1.180	6.0	105.6

*Average of ten tablets

**Average of five values

Results and Discussion

As the concentration of Sod. CMD Ispaghula dispersion increases, the percentage fines values decrease up to a conc. of 0.8%, beyond which there is little change in values of percentage fines. Weight deviation of tablets, prepared with 0.8% and above conc. is less compared to lower concentrations. So it was concluded that 0.8% Sod. CMD Ispaghula is probably the minimum conc. above which it has good binding action. So, for further studies only two

concentrations (0.8% and 1.0% w/v) were selected and their binding action compared with some commonly used binding agents.

COMPARATIVE STUDY OF BINDING PROPERTIES

Binding capacity of Sod. CMD Ispaghula was compared with Acacia mucilage (15%), Methyl Cellulose mucilage (2.0% w/v), Starch paste (10% w/v) and Ispaghula husk (1.0% w/v). Thiamine hydrochloride (10 mg), Acetyl salicylic acid (300 mg) and compound Sodium bicarbonate (320 mg) tablets having following compositions were compressed.

(a) Thiamine hydrochloride Tablets[190,191]:

Thiamine HCl (B.P./U.S.P. Grade)	...	5.0 g.
Lactose	...	133.0 g.
Soluble starch	...	7.5 g.
Talc	...	4.5 g.

(b) Acetyl salicylic acid (Aspirin) Tablets[190]:

Acetyl salicylic acid (I.P.)	...	150.0 g.
Soluble starch	...	7.5 g.
Talc	...	5.0 g.

(c) Comp. sodium bicarbonate Tablets[190]:

Sod. bicarbonate	...	160.0 g.
Mentha oil	...	2.0 ml
Talc	...	5.0 g.

All these tablets were prepared according to I.P.[190] procedure. Granules were prepared by wet granulation method and tablets compressed in DIAF (H) Tablet compression machine using die and punches of 9.5 mm diameter. Capacity of die was regulated to give Thiamine HCl Tablets of 300 mg, Acetyl salicylic acid (Aspirin) Tablets of 325 mg and Comp. sodium bicarbonate tablets of 330 mg.

Evaluation of Tablets

Tablets were then evaluated[190,192,193] for average weight, weight deviation, hardness, disintegration time, drug content and compression ratio. For determination of compression ratio[194,195] each tablet was weighed (in g.) and its thickness determined (in cm) along the axis of rotation by vernier calliper. The ratio of weight by the thickness of tablets gave the compression ratio (g./cm). The observations are given in Tables 47, 48, 49 and 50.

Results and Discussion

Table-47 indicates that tablets prepared with Sod. CMD Ispaghula and other binding agents did not deviate beyond the limits prescribed in the Pharmacopoeia[190]. However, it was noticed that deviations in every lot were higher when Sod. CMD Ispaghula (0.8% w/v) was the binding agent, except for one lot of acacia where it was less.

TABLE-47

Average weight and weight deviation of tablets

S. No.	Binding agent	Conc. (% w/v)		Thiamine HCl tablets	Aspirin tablets	Comp. Sod. bicarb. tablets
1	Sod. CMD Ispaghula	0.8%	A	0.3050	0.3304	0.3251
			B	+1.690	+1.150	+3.353
			C	-1.640	-3.087	-5.261
2	Sod. CMD Ispaghula	1.0%	A	0.3031	0.3270	0.3324
			B	+1.617	+0.458	+1.684
			C	-1.020	-0.306	-1.323
3	Acacia Mucilage	15.0%	A	0.3080	0.3300	0.3351
			B	+1.623	+0.364	+1.761
			C	-1.689	-0.605	-1.523
4	Methyl Cellulose Mucilage	2.0%	A	0.3038	0.3210	0.3382
			B	+0.230	+0.199	+0.828
			C	-0.990	-0.436	-1.832
5	Starch Paste	10.0%	A	0.3061	0.3320	0.3410
			B	+0.620	+1.265	+2.052
			C	-0.620	-1.205	-1.173
6	Ispaghula husk	1.0%	A	0.3064	0.3321	0.3391
			B	+0.261	+1.77	+0.864
			C	-0.456	-3.61	-0.855

A = Average weight (g.)
B = Weight deviation upper limit (%)
C = Weight deviation lower limit (%)

TABLE-48

Hardness of tablets prepared with different binding agents

S. No.	Binding agents	Conc. (% w/v)	Hardness* (kg/cm^2)		
			Thiamine HCl Tablets	Aspirin Tablets	Comp. Sod. bicarb. Tablets
1.	Sod. CMD Ispaghula	0.8	2.7	2.8	3.2
2.	Sod. CMD Ispaghula	1.0	3.6	3.2	3.6
3.	Acacia Mucilage	15.0	3.8	2.9	3.8
4.	Methyl cellulose Mucilage	2.0	4.0	4.0	3.8
5.	Starch paste	10.0	3.6	4.4	3.2
6.	Ispaghula husk	1.0	3.8	2.8	3.9

*Average of five values

TABLE-49

Disintegration of tablets prepared with different binding agents

S. No.	Binding agent	Conc. (% w/v)	Disintegration time* (sec.)		
			Thiamine HCl Tablets	Aspirin Tablets	Comp. Sod. bicarb. Tablets
1.	Sod. CMD Ispaghula	0.8	47	191	65.0
2.	Sod. CMD Ispaghula	1.0	64	219	75.6
3.	Acacia mucilage	15.0	121	212	97.6
4.	Methyl cellulose	2.0	72	160	81.6
5.	Starch paste	10.0	55	221	75.0
6.	Ispaghula Husk	1.0	81	210	79.2

*Average of five values

The results in Table-50 show that the tablets with Sod. CMD Ispaghula as a binding agent have comparative compression ratios, except for tablets prepared with methyl cellulose, where it is slightly less.

From weight variation and hardness data it may be concluded that Sod. Ispaghula (1.0% w/v) compares favourably with acacia (15%), starch paste (10%), Ispaghula husk (1.0%) and methyl cellulose (2.0%) as binding agents.

ANALYSIS FOR ACTIVE INGREDIENTS

In order to ascertain that Sod. CMD Ispaghula did not have any deleterious effects on the active ingredients, tablets of Thiamine HCl, Aspirin and Comp. Sod. bicarbonate were assayed for the active ingredients.

(a) Thiamine HCl Tablets

Fluorometric[191,196,197] and gravimetric methods[190] are official in U.S.P. XVIII and I.P. 1966 respectively. The I.P. Gravimetric method was used for the determination of Thiamine HCl. The results are recorded in Table-51.

(b) Acetyl salicylic acid (Aspirin) Tablets

Aspirin content of Acetyl salicylic acid (Aspirin) Tablets were assayed according to I.P. method[190] and results are recorded in Table-51.

(c) Comp. Sodium bicarbonate Tablets

The weight of total carbonate in Comp. Sodium bicarbonate Tablets, calculated as $NaHCO_3$ in each tablet of average weight were estimated according to I.P. procedure[190]. The results are recorded in Table-51.

TABLE-51

Binding agents	Calculated amount of active ingredients (Average value) in each tablet of average weight*		
	Thiamine HCl tablets+ (mg)	Aspirin tablets+ (mg)	Comp. Sod. bicarbonate tablets+ (mg)
1. Sod. CMD Ispaghula (0.8%)	9.70	295.6	310.0
2. Sod. CMD Ispaghula (1.0%)	9.87	298.65	315.5
3. Acacia mucilage (15%)	9.86	297.6	316.0
4. Methyl cellulose mucilage (2.0%)	9.89	300.5	314.4
5. Starch paste (10%)	9.78	297.0	315.2
6. Ispaghula Husk (1.0%)	9.79	299.5	315.8

+ = Amount added : Thiamine hydrochloride (10 mg/tab.), Aspirin (300 mg/tab.) and Comp. Sod. bicarbonate (320 mg/tab.).

* = I.P. requirements : Thiamine Hydrochloride Tablets - (Not less than 93% and not more than 107.0% of the stated amount); Aspirin Tablets (Not less than 95% and not more than 105% of the stated amount); Comp. Sod. bicarbonate Tablets (total carbonate calculated as Sodium bicarbonate in each tablet of average weight is equivalent to not less than 0.30 g. and not more than 0.35 g. of $NaHCO_3$.

Results

From Table-51 it is evident that on the basis of drug content analysis Sod. CMD Ispaghula (1.0%) dispersion compare with other binding agents, and are also within the upper and lower limits specified in the Pharmacopoeia[190].

EFFECT OF AGING

One of the requirements for the stability of compressed tablets is that their qualities should not change when stored under varying environmental conditions. With this end in view tablets prepared with Sod. CMD Ispaghula were tested for any changes in appearance, hardness and disintegration time as compared to tablets prepared with acacia, methyl cellulose, starch and Ispaghula husk as binding agents.

Tablets were transferred to small sample tubes and kept on shelf at room temperature (varying from 26° to 34°) for 12 weeks. Another lot of tablets was transferred to small sample tube, plugged and kept at 50°±2 in a hot air oven for 12 weeks. At regular intervals of 2, 4, 6, 8 and 12 weeks, tablets were evaluated for change in color, hardness and disintegration time. The hardness of tablets and their disintegration time at different storage periods are recorded in Tables 52, 53 and 54. No appreciable change in color, hardness and disintegration time of tablets were noticed showing that aging of tablets under these condition did not affect their qualities.

TABLE-52

Effect of storage on hardness and disintegration time of Thiamine hydrochloride tablets

Binding agents	Conc. (% w/v)		Hardness (Kg/cm^2)					Disintegration time (Sec)				
			Storage periods in weeks									
			2	4	6	8	12	2	4	6	8	12
1. Sod. CMD Ispaghula	0.8	A	2.7	3.0	3.0	3.0	3.0	48.0	49.0	52.0	52.0	52.0
		B	3.3	3.0	3.0	3.0	3.3	48.5	50.0	50.0	50.0	55.0
2. Sod. CMD Ispaghula	1.0	A	3.6	3.6	3.6	3.6	3.6	64.5	66.0	66.0	66.0	66.0
		B	3.6	4.0	4.0	4.0	4.0	64.0	65.0	65.0	66.0	66.0
3. Acacia Mucilage	15.0	A	4.0	4.0	4.0	4.0	4.0	120.5	123.0	124.0	124.0	124.0
		B	4.0	4.0	4.0	4.0	4.0	123.0	124.0	124.0	124.0	124.0
4. Methyl cellulose	2.0	A	4.3	4.3	4.3	4.3	4.3	74.0	76.0	76.0	76.0	76.0
		B	4.6	4.6	4.6	5.0	5.0	74.0	76.0	76.0	76.0	76.0
5. Starch paste	10.0	A	3.6	3.6	3.6	3.6	3.6	55.0	58.0	58.0	58.0	58.0
		B	4.6	4.6	4.6	5.0	5.0	56.5	57.0	57.0	58.0	58.0
6. Ispaghula husk	1.0	A	4.0	4.0	4.0	4.0	4.0	82.5	86.0	87.0	88.0	88.0
		B	4.0	4.3	4.3	4.3	4.3	83.0	85.0	86.0	88.0	88.0

TABLE-53

Effect of storage on Hardness and Disintegration time of Aspirin tablets

Binding agents	Conc. (% w/v)		Hardness (Kg/cm^2)					Disintegration time (Sec)				
			Storage periods in weeks									
			2	4	6	8	12	2	4	6	8	12
1. Sod. CMD Ispaghula	0.8	A	3.0	3.0	3.0	3.0	3.0	191.0	192.0	193.0	194.0	194.0
		B	3.0	3.0	3.0	3.0	3.3	195.0	196.0	196.0	196.0	199.0
2. Sod. CMD Ispaghula	1.0	A	3.3	3.3	3.6	3.6	3.6	221.0	222.0	230.0	230.0	230.0
		B	3.3	3.3	3.3	3.3	3.6	219.0	220.0	220.0	220.0	220.0
3. Acacia mucilage	15.0	A	3.0	3.3	3.3	3.3	3.3	212.0	214.0	220.0	220.0	220.0
		B	3.0	3.0	3.0	3.0	3.0	216.0	220.0	220.0	221.0	222.0
4. Methyl cellulose	2.0	A	4.2	4.3	4.3	4.3	4.6	163.0	164.0	168.0	168.0	168.0
		B	4.3	4.3	4.3	4.0	4.3	164.0	168.0	168.0	168.0	168.0
5. Starch paste	10.0	A	4.4	4.4	4.4	4.4	4.4	222.0	224.0	229.0	230.0	230.0
		B	4.3	4.3	4.3	5.0	5.0	224.0	226.0	226.0	226.0	226.0
6. Ispaghula husk	1.0	A	3.0	3.3	3.3	3.3	3.3	223.0	224.0	230.0	230.0	230.0
		B	3.0	3.0	3.0	3.0	3.3	214.0	220.0	220.0	223.0	224.0

A = Storage at room temperature (26 to 34°) B = Storage at high temperature (50°±2)

TABLE-54

Effect of storage on Hardness and Disintegration time of Comp. Sodium bicarbonate tablets

Binding agents	Conc. (% w/v)		Hardness (Kg/cm^2)					Disintegration time (Sec)				
			Storage periods in weeks									
			2	4	6	8	12	2	4	6	8	12
1. Sod. CMD Ispaghula	0.8	A	3.3	3.3	3.3	3.3	3.3	67.0	67.0	68.0	70.0	71.0
		B	3.3	3.3	3.3	3.3	3.6	68.0	68.0	68.0	68.0	68.0
2. Sod. CMC Ispaghula	1.0	A	4.0	4.0	4.0	4.0	4.0	78.0	78.0	78.0	78.0	81.0
		B	4.0	4.0	4.0	4.0	4.0	79.0	80.0	80.0	80.0	80.0
3. Acacia mucilage	15.0	A	4.0	4.0	4.0	4.0	4.0	98.0	98.0	98.0	98.0	98.0
		B	4.0	4.0	4.0	4.0	4.0	102.0	102.0	102.0	102.0	102.0
4. Methyl cellulose	2.0	A	4.0	4.0	4.0	4.0	4.0	84.0	85.0	86.0	86.0	86.0
		B	4.0	4.0	4.0	4.0	4.0	85.0	85.0	89.0	89.0	89.0
5. Starch paste	10.0	A	3.6	3.6	3.6	3.6	3.6	78.0	78.0	78.0	78.0	78.0
		B	3.6	3.6	4.0	4.0	4.0	78.0	78.0	80.0	83.0	83.0
6. Ispaghula husk	1.0	A	4.0	4.0	4.0	4.0	4.0	80.0	82.0	82.0	82.0	82.0
		B	4.0	4.0	4.0	4.0	4.0	82.0	82.0	82.0	82.0	82.0

A = Storage at room temperature (26 to 34°) B = Storage at high temperature (50°±2)

CHAPTER VII

SUMMARY AND CONCLUSION

Earlier studies of the pharmaceutical potentialities of Plantago ovata (Ispaghula) seed husk have shown it to be a valuable emulsifying, suspending, binding, disintegrating and gel forming agent. The present study was undertaken to determine its homogeneity and prepare and test its carboxymethyl derivative (Sodium Salt).

Electrophoretic studies of purified husk indicated it to be heterogeneous and to consist of at least two polysaccharide components. Fractionation with copper acetate gave two types of complexes, one water insoluble and the other water soluble. The two fractions were characterised on the basis of their optical rotation, equivalent weights, I.R. and constituents sugars. Water insoluble complex was found to consists of L-rhamnose (8.5%), D-xylose (50%), L-arabinose (15%), D-galactose (4.0%) and D-galacturonic acid (20%), whereas fraction corresponding to water soluble copper complex consisted of D-xylose (65%), L-arabinose (25%) and L-rhamnose (5%). The fractions were further characterised on the basis of Pentose and Pentosan contents, uronic acid and methoxyl contents, electrophoresis on glass fibre paper, reducing sugar contents, viscosity and rheological characteristics. Both these fractions were found to differ from each other in every respect.

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