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PHYSICOCHEMICAL, MECHANICAL & FILM FORMING STUDIES OF NOVEL BIOMATERIAL

A.M. Pethe* and S.B. Joshi

School of Pharmacy &Technology Management, SVKM'S NMIMS Shirpur Campus, Dist-Dhule- 425 405, Maharashtra, India

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Correspondence to Author:

Dr. A.M. Pethe

Associate Professor, HOD Quality Assurance, School of Pharmacy &Technology Management, SVKM'S NMIMS Shirpur Campus, Dist-Dhule-425 405, Maharashtra, India

Email: anilpethe@gmail.com

ABSTRACT: Gum Dammar is a natural resinous material of plant *Shorea javanica*, family–Dipterocarpaceae. In the present investigation Dammar gum (DG) and alcohol soluble fraction of Dammar gum (DA) are evaluated for their physicochemical, mechanical and film forming characteristics. Films were produced by casting/solvent evaporation method from plasticizer free and plasticizer containing solutions. Films prepared from different formulations were studied for their mechanical (tensile strength, percent elongation and Young's modulus), water vapor transmission and moisture absorption characteristics. Unplasticized DG, DA films were brittle and posed the problem of breaking during handling. Hydrophobic plasticizers, dibutyl phthalate (DBP), improved the mechanical properties of free films with plasticizer showing significant effects on film elongation. Thus present study proposes DG and DA as excellent film formers.

INTRODUCTION: Polymers are the youngest members of material family, and the recent progress in the fascinating area of applied polymer science is quite noteworthy.

The importance and pharmaceutical application of polymer films are established in providing controlling drug release from desired dosage form. These controlled release systems represent a relatively new development that evolves out of a continuing need to prolong and better control drug administration [1].

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One of the strongest and most rapidly growing areas of polymer is that of natural polymer and their derivatives. Natural materials have attracted considerable attention for drug delivery applications due to their abundant availability, compatibility with environment and degradation under natural or physiological conditions [3]. Natural resins have been used since ancient times for a wide range of applications: varnishes, sealant, binding media and waterproofing.

The most commonly used resins for paint and varnishes are Rosin, Sandarac, Dammar, Mastic and Copal. The composition of plant resins is quite complex and it is not entirely known yet for the majority of the plant species; it may also depend on the climate and on the characteristics of soil. Terpenoids are the main components of plant resins, and some characteristics diterpenoids and triterpenoids compounds allow the identification of the botanical origin of the resin [4].

Gum Dammar is a natural resinous material of plant *Shorea javanica*, family–*Dipterocarpaceae*. It contains about 40% alpha–resin (resin that dissolves in alcohol), 22% beta–resin, 23% dammarol acid and 2.5% water [5].

The present manuscript deals with the characterization of Dammar gum for various physicochemical, mechanical and film forming properties.

MATERIALS: Dammar gum (*Shorea javanica*, family – Dipterocarpaceae) purchased from Yucca, Enterprises, Mumbai. All other chemicals were of analytical grade and purchased locally.

METHODS:

Preparation of powdered Dammar gum: The dammar gum obtained was grinded in Ball mill and the powder gum was passed through sieve and used as Dammar gum (DG). The 20 g of gum was then dissolved in 100 ml 95% ethanol in 500 ml beaker. The supernatant liquid was collected and kept in an evaporating dish at room temperature. The resultant yellow color product after evaporation was collected and used as alcohol soluble fraction of dammar gum (DA) and alcohol insoluble fraction of gum dammar (DI).

Physiochemical characterization of Dammar: The Dammar gum (DG), its alcohol soluble fraction (DA) and alcohol insoluble fraction (DI) was characterized for the following physiochemical properties:

a. **Color:** Comparison of color of DG, DA and DI was made visually, against white background.

b. **Acid Value**: Acid value of each material was determined by using the method described in Indian Pharmacopoeia, 2010. An average of three readings was noted.

c. **Saponification Value**: Saponification value of the each material was estimated by the method described in Indian Pharmacopoeia, 2010. Average of three determinations was noted.

d. **Ester Value**: Ester value was calculated from the expression described in Indian pharmacopoeia, 2010.

Ester value = Saponification value – Acid Value

e. **Softening Point**: Herculus drop softening point method was employed for determining softening point. For this purpose thistle tube, partially fitted with liquid paraffin was used. A small amount of powered sample was filled in the capillary sealed at one end. It was then tied to precalibrated thermometer and the tube was heated gradually. The temperature at which the solid sample started softening was noted.

f. **Solubility**: Solubility study was conducted in water, organic solvents and buffer solutions of different pH values. About 1.0 g of the material was placed in different screw capped test tubes. 10 ml of solvent (water/organic solvent/buffer solution) was added to each of the test tube. All the tubes were then placed in mechanical shaker which was operated for 24 h at a speed of 50 r.p.m. and maintained at 25^{o}C. After 24 h 2.0 ml of solution was withdrawn, poured in tared dry petridish and the solvent was evaporated in an oven maintained at 50^{o}C. The increase in weight relative to the solvent blank was used to determine the solubility of the material in the given solvent. Buffers of different pH were prepared by method described in Indian Pharmacopoeia, 2010 [6].

g. **Glass transition temperature (Tg)**: Tg was estimated using a differential scanning calorimeter (DSC, Mettler–Toledo). Accurately weighed sample was placed in aluminum pans and heated at scanning rate of 5^{o}C/min over a temperature range of 20–200^{o}C. The Tg was taken as the midpoint of transition and read from the thermographs.

h. **FT–IR Spectroscopy**: Infrared (IR) spectra of the materials were recorded with FT–IR spectrophotometer (FT IR–8101 A, Shimadzu, Japan). The samples were prepared by processing compressed potassium bromide (KBr) pellets.

i. **Viscosity**: The viscosity of 10% and 20% w/v solution of DG in DCM/DA in acetone was measured at 25^{o}C. Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts) was used to determine the viscosity employing spindle number 1 at 100 rpm.

j. **Refractive Index**: The Abbe's refractometer was used for measurement of refractive index. Apparatus was calibrated against distilled water, which has a refractive index of 1.3325 at 25^{o}C. 5% w/v solution of DG was prepared in Dichloromethane (DCM) and DA in acetone and read on the refractometer at 25^{o}C.

k. **Molecular weight and polydispersity**: Molecular weight (Mw) and polydispersity (Mw/Mn) of the samples were estimated by Gel Permeation Chromatography with UV-Visible detector. Polystyrene was used as reference standard; samples were eluted through PL gel 5. Mixed D column at a flow rate of 1 ml/min using tetrahydrofuran as a solvent

Film forming studies of Dammar:

a. **Preparation of free films of dammar** [7]: Free polymeric films were prepared by solvent evaporation technique on mercury substrate. A 30% w/v solution of DG in DCM and DA in acetone was poured into mercury containing petridish of 20 cm diameter (area of casting 19.4 cm^2) and dried at room temperature for 24 h. Films were further stored in desiccators at ambient temperature for 24 h before analysis.

b. **Water vapor transmission rate studies** [8]: To measure the water vapor transmission, polymer films were cut into appropriate dimensions and mounted on permeation cell. The cell consisted of a glass body (2.25 cm internal diameter X 8.0 cm height) and a cap with an opening of 23.4 mm diameter (test area : 4.3cm^2), the two held in place by means of three screw clamps. A disk of the film under investigation was clamped tightly between the two to provide an effective surface area of 4.3 cm^2 for water vapor transmission. The RH was maintained with saturated salt solutions in contact with the undissolved salt: potassium carbonate (43% RH) and potassium nitrate (93% RH) [9]. The charged cell were weighed and placed in pre-equilibrated desiccators maintained at 0% RH. The cells were reweighed at the end of 24 h. The amount of water transmitted through the film was given by the weight loss of assembled cell. The water vapor transmission rate (WVTR) was computed using Utsumi's equation [10].

c. Taking the film thickness into consideration as shown below;
    1. WL
    2. Q = ----------
    3. S
    4. Where W = g of water transmitted per 24 h
    5. L = film thickness (cm)
    6. S = surface area (cm2)
    7. Q = WVT (g/cm/cm2)/24 h

d. **Moisture absorption by free films:** Films were cut into 25 x 10 mm2 strips. The strips were transferred to a tarred petridish and then to glass desiccators maintained at controlled relative humidities of 23, 43, 75 and 93% respectively. The RH in the chamber was controlled by the use of different saturated solutions containing excess solute. The film specimens were accurately weighed, placed in RH chamber, removed and weighed again at the end of 14 days. Changes in weight and physical appearance were noted. Percent moisture absorption was calculated in terms of the increase in the weight of the film (if any) over the initial weight of film specimen. Increase in the weight is indicative of moisture absorption.

e. **Mechanical properties of free films:** As the unplasticized free films of DG/DA were brittle to handle and susceptible to breakage while tensile testing, the free films plasticized with 2.5% w/w dibutyl phthalate (DBP) [with respect to DG/DA concentration] were tested for tensile properties. Film thickness was measured by a micrometer screw gauge (20 mm, UPISCO, India) and recorded as mean of three measurements.

   The prepared cast films were carefully cut into film strips (~ dry film thickness: 0.4 mm, 12 mm width x 120 mm length) and were evaluated for the stress strain parameters using Instron Instrument (Model 4467, Instron. Corp., Canton, MA) by ASTM standard test principle. The measurements were made at a guage length of 50 mm, cross head speed of 5 mm/min at 50% relative humidity (RH) and 23^{o}C. Stress-strain parameters including the ultimate tensile strength, percent elongation and Young's Modulus were determined for each film specimen with at least three repetitions.

RESULTS AND DISCUSSIONS:

Physiochemical characteristics of DG and DA: The physiochemical characteristics are the fundamental aspects in anticipating applications of the material in pharmaceutical technology. DG is dark brown in color, whereas DA possesses yellowish brown color.

The higher acid value (**table 1**) of DG indicates presence of large amount of free acid groups in the polymer, which could suggest the possible esterification of the DG. The lesser acid value of DA is an indication of partial esterification of acids present in dammar moiety. Higher acid value indicates the suitability of these polymers for enteric coating of drugs.

TABLE 1: PHYSICOCHEMICAL PROEPRTIES OF DG & DA

Product	Acid Value	Sap Value	Ester Value	Tg	Softening Point
	(n=3, ± S. D.)	(n=3, ± S. D.)	(n=3, ± S. D.)	(°C)	(°C)
D.G.	62.82 ± 0.80	89.22± 0.28	26.4±0.43	< 35	78 – 82
D.A.	34.24 ± 0.54	52.16± 0.18	17.92± 024	42.00	72 – 74

FT-IR spectroscopy was used to give information about the molecular state. **Figure 1A** show the FT-IR spectra of DG. The FT-IR spectra of DG depict characteristics absorption bands at 1707.2 cm-1, 2953.4 cm-1 and 3454.7 cm-1 for C=0, -CH and –OH stretching. This indicates the presence of carboxyl group. In DA the absorption bands at 2953.4 cm-1 indicates –CH stretching (**Figure 1B**).

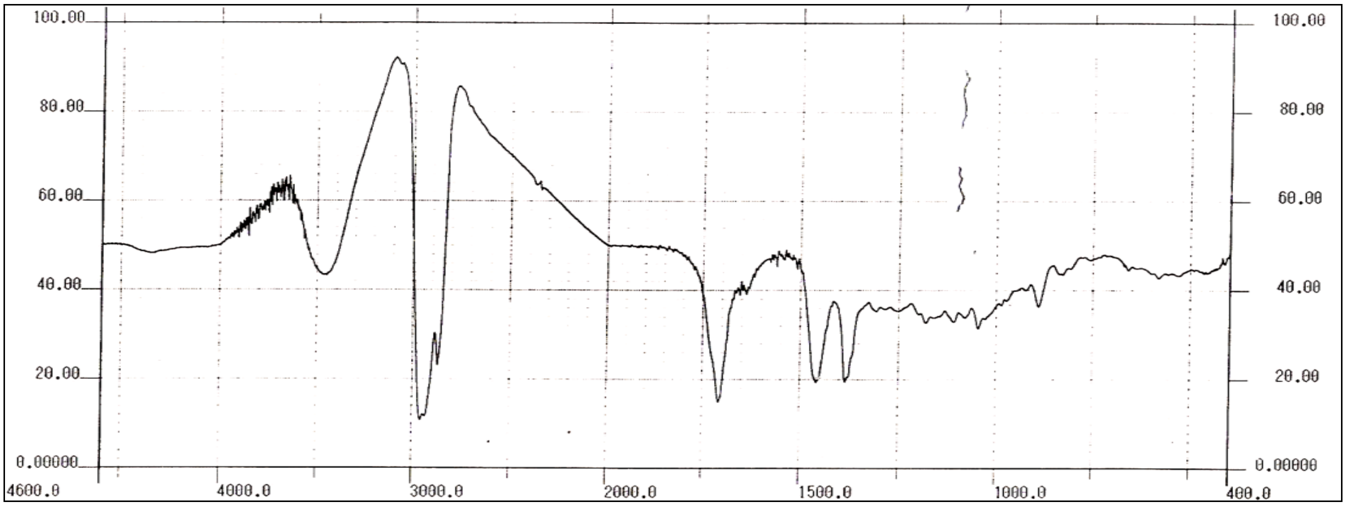


FIG. 1A: FT-IR SPECTRA OF DG

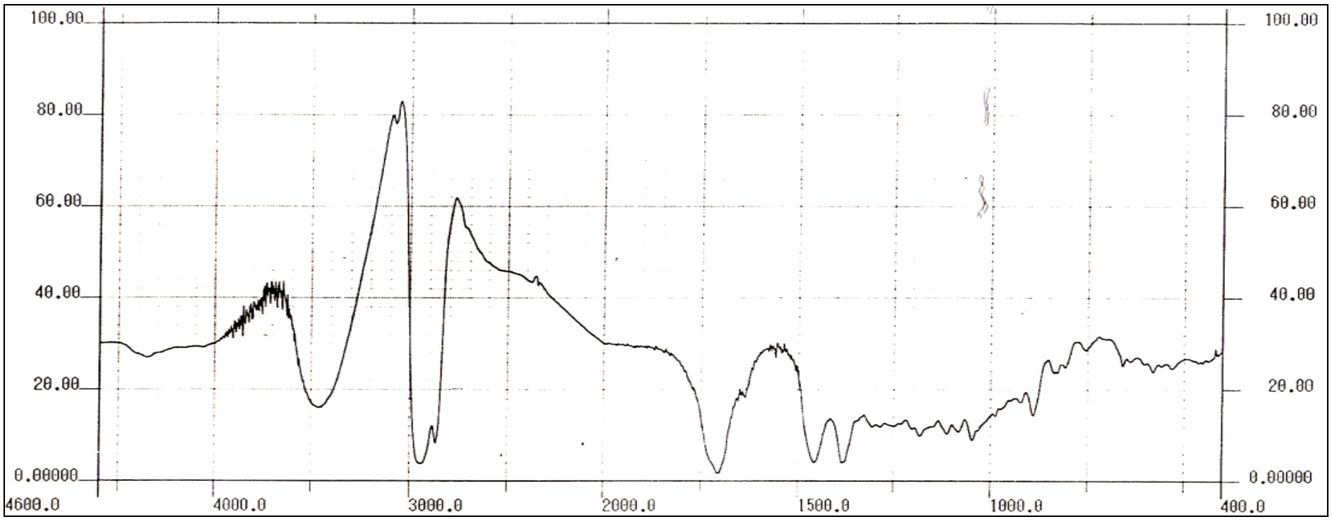


FIG. 1B: FT-IR SPECTRA OF DA

Interest in the concept of glass transition has increased during recent years. The applicability of glass transition principles has permeated from polymer and material science into such areas as food science, pharmaceutics and biotechnology. The physical and possibly chemical stabilization of foods, drugs and lyophilized biological materials is a primary reason for the increase in glass transition research. Tablet properties, crystallization of excipients or drugs, free flowability of powders are aspects of pharmaceutical formulations influenced by the glass transition [11]. Glass transition temperature (Tg) is one of the important characteristics associated with a material and can provide valuable information regarding the end use performance of a product. It is the temperature at which there is an absorption or release of energy as the temperature is raised or lowered. At Tg a solid, brittle, amorphous material undergoes its transformation to a soft, rubbery state. Materials for coating must be used near to their Tg so that they have some flexibility but are not rubbery [12]. DG has Tg less than 35°C whereas Tg of the DA is 42°C. (As seen from **figures 2A and 2B**) DG due to its tackiness at ambient temperature (approx. 30°C) is difficult to handle. But DA is free from this undesired property. The softening point of DG (Table 1) is comparatively high (78-82°C) to that of DA (72-74°C). The Tg value and the softening point range are indicative of soft nature of the polymer.

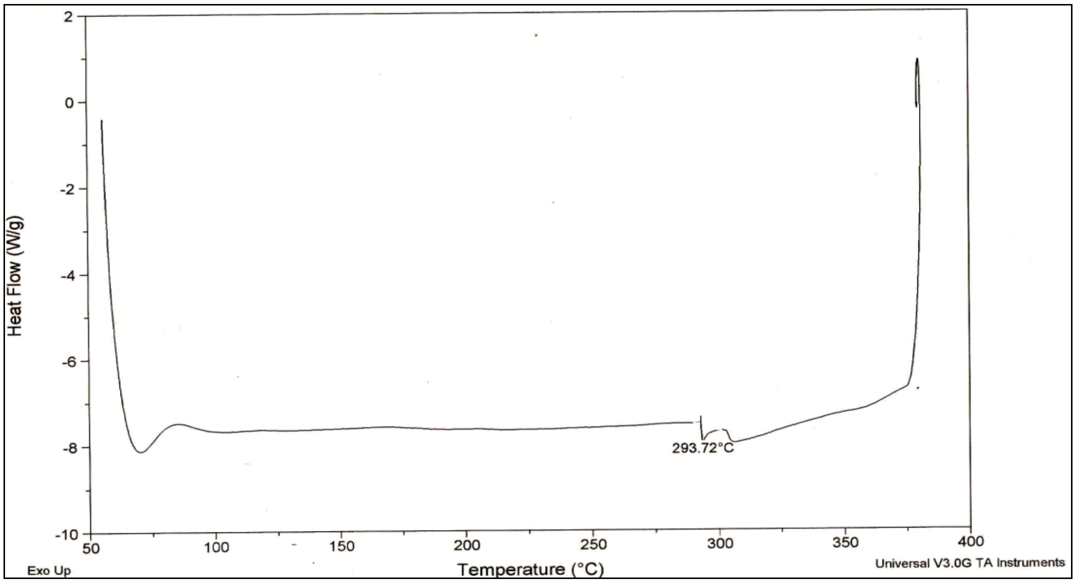


FIG. 2A: DSC THERMOGRAM OF DG

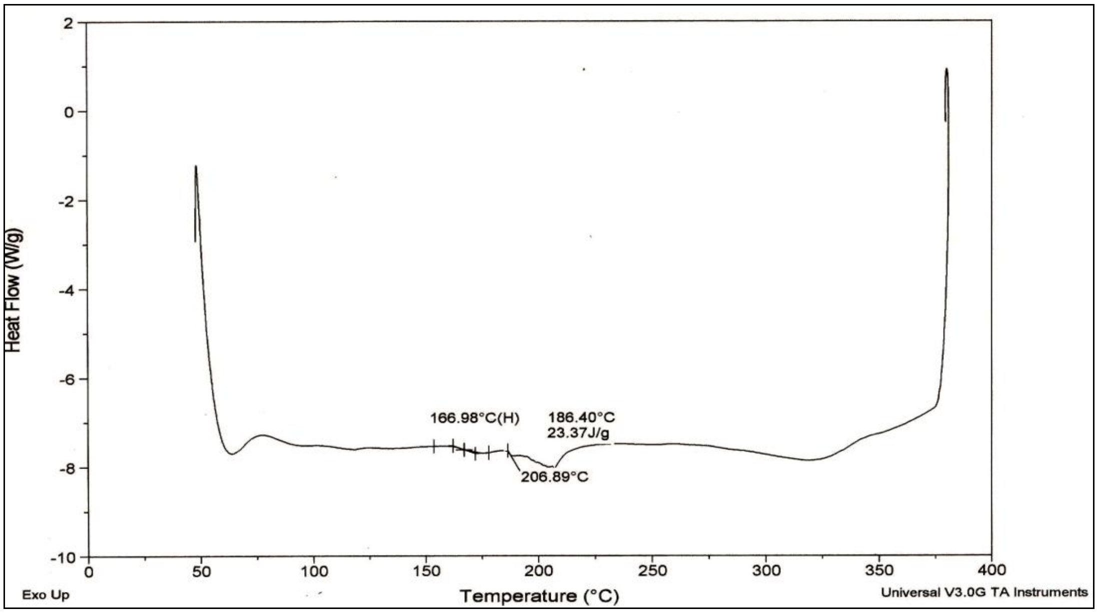


FIG. 2B: DSC THERMOGRAM OF DA

The solubility data of a material suggest its suitability in drug delivery applications. It has been recognized that the solubility of polymer decreases when strong intermolecular forces are present in the polymer solution. The process of dissolving a polymer in a solvent is regained by the free energy of mixing [1]. Solubility of DG and DA is shown in **table 2**. DG and DA showed free solubility in organic solvents.

TABLE 2: SOLUBILITY OF DG AND DA (n = 4, ± S. D.) IN DIFFERENT SOLVENTS & BUFFERS

Solvents/Buffers	Solubility, mg/ml	
	DG	**DA**
Chloroform	870.22 ± 42.40	845.26 ± 27.46
Carbon tetrachloride	866.18 ± 18.42	826.77 ± 34.20
Dichloromethane	840.00 ± 36.66	860.75 ± 43.32
Acetone	205.12 ± 12.18	542.00 ± 16.85
Isopropyl alcohol	407.06 ± 21.08	662.00 ± 26.96
Ethanol	450.00 ± 10.77	604.20 ± 24.84
Distilled Water	1.50 ± 0.28	1.92 ± 0.19
pH 1.5	3.99 ± 0.72	3.7 ± 0.46
pH 4.5	12.95 ± 0.86	7.63 ± 1.57
pH 6.8	32.20 ± 0.77	11.8 ± 0.16
pH 8.0	36.34 ± 1.35	15.36 ± 0.66
pH 10.0	38.66 ± 2.06	14.0 ± 0.74

However, the solubility was highest in chlorinated solvents (chloroform and dichloromethane). The solubility of DA is more in most of the solvents compared to DG. As shown in table 2, the solubility of DG and DA increased with pH of the aqueous media, which indicates their pH dependent solubility. Above pH 6.8, DG showed comparatively greater solubility than DA. This could be due to greater acid value.

Most of the synthetic polymers and many naturally occurring materials consist of molecules with different molecular weights and are said to be polydisperse. The molecular weight is expressed as the number average molecular weight, Mn or weight average molecular weight, Mw. The Mn is highly sensitive to small molecules and is independent of molecular size.

The Mw is more dependent on the total number of heavier molecules. The ratio Mw/Mn is a measure of polydispersity and called polydispersity index [12]. The polydispersity index of DG and DA indicates a narrow range of molecular weight distribution (**Table 3**).

TABLE 3: MOLECULAR WEIGHT AND POLY-DISPERSITY INDEX OF DG AND DA

Product	Molecular weight		Polydispersity Index (Mw/Mn)
	Mw	**Mn**	
DG	799.07	573.86	1.392
DA	703.02	514.44	1.366

Viscosity is the measure of resistance to flow of a material solution. This is a critical parameter while coating or layering of the material/polymer on a substrate (tablet or pellet). The most commonly used process in the pharmaceutical coating is the spraying of coating solution, which is greatly influenced by the solution viscosity [13]. The DG and DA shows considerably low viscosity value (< 10 cps) even at 20% w/v concentrations **(Table 4)** and thus, good atomization even at high concentration can be achieved. The refractive index n is the measure of the ability of a material to refract or bend light as it passes through the material. The refractive index is equal to the ratio of the sin of the angles of incidence (sin i) and refraction (sin r) of light passing through the material:

$$n = \sin i / \sin r$$

The magnitude of n is related to the density of the substance [12]. Although insignificant, the n values of the DA were found to be higher than DG.

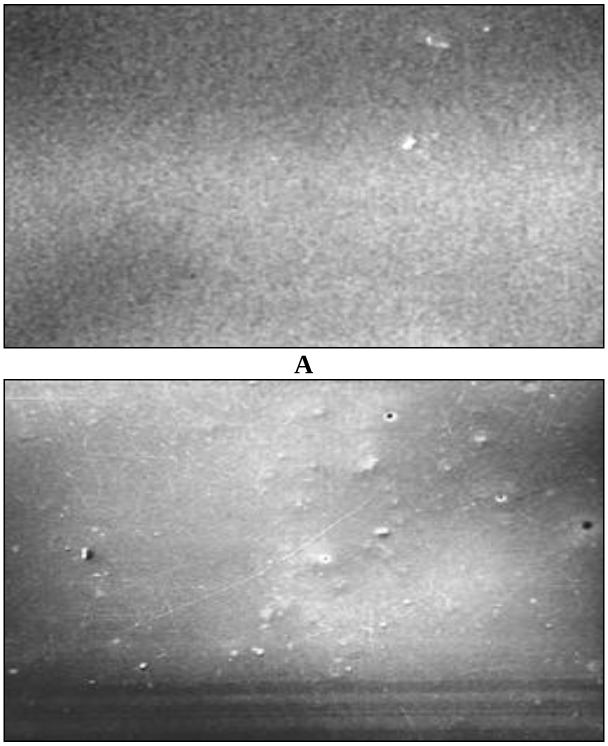
TABLE 4: VISCOSITY AND REFRACTIVE INDEX OF SOLUTION OF DG IN DCM AND DA IN ACETONE AT 25°C

Product	Viscosity (cps), n = 3		Refractive index n = 4
	10% w/v	**20% w/v**	**5%w/v**
DG	4.5	8.0	1.385
DA	5.0	9.0	1.388

Film Characteristics: Preliminary study showed that DG and DA possesses excellent film forming property. The films of DG and DA were suitably cast on mercury. The DG and DA were readily soluble in dichloromethane and acetone respectively. Therefore respective solvents were selected throughout the studies. Films produced from the plasticizer free solution were smooth and transparent but slightly brittle. Most polymers used alone for film formation are commonly brittle at room temperature and require plasticizer or another additive to improve their process ability and flexibility [14]. Therefore plasticizer was added with the aim of improving their mechanical properties.

Film formulations containing, 2 and 2.5% w/w Dibutyl phthalate (DBP) as plasticizer was prepared. Plasticizer is an important formulation factor affecting mechanical properties of films. It shifts the glass transition temperature to lower temperature [15, 16]. Also reduces the aggregate force caused by intermolecular attraction of the polymer and results in an increase in the adhesive strength of the film [14].

1. **Scanning electron microscopy of free films**: No micropores on surface of the films were observed under SEM (**figure 3 A &B**). This indicates that DG and DA under investigation formed non-porous films.


A

B

FIG. 3: SCANNING ELECTRON MICROGRAPH OF FREE FILM OF A: DG, B: DA

2. **Water vapor transmission rates (WVTR) of free films**: The rate of water vapour transmission through free films could be an important quantitative parameter to predict the performance of film applied on the substrate in terms of moisture protection. The results of WVTR study are depicted in **Table 5**. The WVTR is strongly dependent on the experimental humidity. Hence, humidity conditions of 43 and 93% were employed for all the studies.

   Films of DG and DA showed extremely low WVTR. This indicates the low permeability of the films to moisture and thus can act as an excellent protective coating agent. The humidity transmitted through DG and DA films decreased with increasing plasticizer concentration.

   As the film thickness is likely to affect the WVTR, Utsumi's equation was employed for determination of transmission rate taking the film thickness into consideration.

3. **Moisture absorption study of free films**: **Table 5** records the percent moisture absorbed by free polymeric films at different relative humidity conditions. The percent moisture absorbed increases with increase in percent relative humidity.

   The higher percentage moisture absorption may be attributed to the higher hydrophilic property of the material whereas lower moisture absorption indicates hydro-phobicity.

TABLE 5: WATER VAPOUR TRANSMISSION RATES (WVTR) & MOISTURE ABSORPTION STUDIES OF FREE FILMS

Films	Film thickness (cm)	Area (cm^2)	Q ($g.cm/cm^2/24h$) x 10^{-5} at		% moisture absorbed at			
			43% RH	93% RH	23% RH	43% RH	75% RH	93% RH
DG	0.042	4.32	1.12 ± 0.10	4.01 ± 0.35	0.108	0.412	0.948	1.099
DA	0.043	4.34	1.10 ± 0.07	3.92 ± 0.27	0.099	0.384	0.896	1.033

4. **Mechanical Properties of free films**: The mechanical properties of free films are a critical tool to anticipate the performance of new material as film former. The most basic and important of all mechanical properties is an elastic modulus, which is a measure of film stiffness [17]. Elastic modulus is a proportionality constant of stress to strain and is computed from the slope of linear portion of the stress-strain curve. Percentage elongation is the deformation capacity of the film and its lower value indicates brittleness of film.

5. Addition of suitable plasticizers reduces the brittle state and increases the film flexibility [18]. The plasticizer free films of the DG and DA were brittle and slight deformation results in breakage. Thus, the tensile studies were performed on films plasticized with 2.5% w/w dibutyl phthalate (with respect to derivative content). The results of estimated mechanical properties of free films of DG and DA are shown in **table 6**.

TABLE 6: MECHANICAL PROPERTIES OF THE FREE FILMS OF DG AND DA CONTAINING 2.5% W/W DIBUTYL PHTHALATE

Mechanical Property	DG	DA
Thickness (mm)	0.41	0.43
Tensile strength (MN m^{-2})	0.271	0.397
Elongation (%)	26.060	40.120
Young's Modulus (MN m^{-2})	1.012	0.840

It was observed that the film elongation was more for DA films compared to DG. The DA films were comparatively less brittle than DG films and addition of some concentration of plasticizer may affect the % elongation. Similar results were obtained by Fulzele *et al* [19].

The tensile strength results obtained with DG and DA films indicates the risk of film cracking. Addition of plasticizers to the films decreased the values of Young's modulus consequent to increased percent elongation. This may contribute to increased adhesion between film and coating surface. The results reveal that addition of hydrophobic plasticizer DBP is efficient for positively modifying the free films of DG and DA.

CONCLUSIONS: The low molecular weight film forming biopolymers, DG and DA were investigated for their physiochemical, mechanical and film properties. It was observed that the DA was comparatively non-tacky as compared to DG at room temperature and was convenient for handling. Both showed pH dependent solubility in aqueous media, however, not to a greater extent. Films prepared from the novel biopolymer, containing plasticizer, were studied to assess the mechanical, WVT and moisture absorption characteristics. Free DG and DA films have low mechanical properties and plasticizers are required to positively modify these properties. DBP was found to improve film characteristics. The DG and DA showed extremely low rate of water vapour transmission and was influenced by the relative humidity conditions. Thus, above studies could propose DG and DA as excellent film formers.

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