CHAPTER II
MATERIALS AND METHODS
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CHAPTER-II
MATERIALS AND METHODS

II.1 MATERIALS

II.1.1 Solvents

The following laboratory reagent grade solvents were used without further purification: methanol, diethyl ether, petroleum ether (40-60°C), pyridine, n-butanol, acetone and glacial acetic acid.

Tetrahydrofuran was distilled over metallic sodium and used immediately. Dichloromethane, phenol and chlorobenzene, and commercial solvents like isopropanol, acetone and ethanol were distilled before use.

II.1.2 Reagents

The following chemicals were of laboratory reagent grade and were used without further purification.

Silver nitrate, sodium thiosulfate, sodium pentachlorophenate, ammonium thiocyanate, hydrogen peroxide (30%), citric acid, sodium salt of m-nitrobenzene sulfonic acid (resist salt), hexamethylene tetramine, hydrazine sulfate and potassium iodide.

The following reagents were used for the derivatization of guar gum. Ethylene oxide (Nocil), propylene oxide (Fluka) and monochloroacetic acid. Ethylene chlorohydrin, which was
also used for hydroxyethylation, was distilled (126-129°C) before use. Hydriodic acid (57% w/w; d 1.7), purchased from local market and also prepared in the laboratory, was further purified by the standard procedure to remove elemental sulfur and stabilizers.

II.1.3 Gum

Two batches of guar gum and two commercial printing gums (A and B) were purchased from the local market. Their analysis is given in Table-II.1:

**TABLE-II.1**
ANALYSIS OF GUAR GUMS

<table>
<thead>
<tr>
<th>Gum</th>
<th>Moisture</th>
<th>Nitrogen</th>
<th>Ash</th>
<th>Viscosity (1% Solution)</th>
<th>Molar Substitution</th>
<th>Turbidity (NTU) of 0.25% Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Viscosity Gum</td>
<td>12.8</td>
<td>2.1</td>
<td>0.6</td>
<td>8500</td>
<td>0.006</td>
<td>36.0</td>
</tr>
<tr>
<td>Low Viscosity Gum</td>
<td>12.8</td>
<td>1.5</td>
<td>2.0</td>
<td>400</td>
<td>0.003</td>
<td>20.0</td>
</tr>
<tr>
<td>Commercial Printing Gum A</td>
<td>9.6</td>
<td>—</td>
<td>17-19</td>
<td>140</td>
<td>0.06</td>
<td>21.0</td>
</tr>
<tr>
<td>Commercial Printing Gum B</td>
<td>10.8</td>
<td>—</td>
<td>17-25</td>
<td>180*</td>
<td>0.1</td>
<td>22.0</td>
</tr>
</tbody>
</table>

II.1.4 Fabric

For evaluating guar derivatives, prepared in the laboratory, as printing thickeners, polyester-cotton fabric (80:20) was used. The warp of this fabric was composed of spun polyester

* viscosity of 2% solution
and cotton in the ratio of 67:33 whilst the weft was pure polyester filament.

II.1.5 Dye

Printing was carried out with a blue disperse dye (C.I. dispersol blue B.R. 56, C.I. No. 63235) which is soluble in acetone, benzene and has the following structure:

![Chemical Structure](image)

II.2 INSTRUMENTS

The following instruments were used for the analysis of samples of gums, fabrics and prints:

- Synchro-Letric model-LVT Brook-Field viscometer
- Rheotest-2 viscometer
- Perkin Elmer model Infracord-137 double-beam infrared spectrophotometer
- Perkin Elmer model IR-180 double-beam infrared spectrophotometer
- Systronics Nephalo - turbidity meter type-131
- Cambridge Stereoscan model S4-10 scanning electron microscope
Bausch-Lomb Spectronic-100 visible spectrophotometer
Pretema-Spectromat model FS-3A
Warner Matnis high temperature steamer
High pressure steamer
Instron strength tester

Waters Associates high pressure liquid chromatograph having model 6000A solvent delivery system, model U6K injector and model R401 differential refractometer.

II.3 METHODS

II.3.1 Derivatization

Guar gum was derivatized by wet (isopropanol slurry) and dry methods. In the slurry method, reaction ingredients were mixed in a 2-litre round bottom flask. After adding the reagents, the flask was sealed and heated in a water bath for required periods of time. The derivatized gum was obtained by neutralization, filtration, washing and drying.

In the dry method, guar gum was sprayed with a solution of sodium hydroxide, dissolved in minimum amount of water, thoroughly mixed in an electric mixer and aerated for 10 minutes to remove excess of water. To the alkali gum the reagent was added and the mixture was stirred in a mixer. After the reaction was over, the product was neutralized, washed, dried and sieved.
Alkaline degradation of guar gum was carried out in the dry state as well as in the slurry form. In these experiments, the preparation conditions were duplicated except that no derivatizing reagent was added. The amount of various ingredients (given as ratio) used in the two methods are given below:

**Slurry Method**

Gum + Water + NaOH + isopropanol

A: 1 : 1 : 1 : 0.04 or 0.08%

**Dry Method**

Gum + NaOH (dissolved in minimum amount of water)

B: 1 : 0.04 or 0.08%

Derivatization: A or B + reagent.

Following the above procedures, O-(2-hydroxyethyl, O-(2-hydroxypropyl and O-carboxymethyl derivatives of guar gum were prepared.

**II.4 ANALYSIS**

**II.4.1 Purification of Gum**

Different gum samples used in the experiment were purified in the following manner:

Gum, dispersed in water, was allowed to hydrate for 24 hrs and then filtered through muslin cloth. The gums was then precipitated by pouring the aqueous sol into 95% aqueous

* on the weight of the gum
ethanol. The precipitated product was filtered and washed successively with ethanol, solvent ether and petroleum ether (40-60°C) and air dried. The air dried samples were then dried over phosphorus pentoxide. These samples were taken for analysis.

11.4.2 Viscosity Measurement
Gum was dispersed in water, allowed to hydrate for 24 hrs and wherever necessary sodium pentachlorophenate (0.5-1% on the weight of the gum) was added to the paste. Viscosity of the gum was measured using a Brookfield rotational viscometer. Viscosity values reported in this thesis are of 1% concentration unless otherwise stated. Spindles 1-4 were used depending upon the viscosity of the solution.

11.4.3 Acid Hydrolysis
For qualitative sugar analysis by paper chromatography, the gum was hydrolysed with 1 N sulfuric acid for 6-8 hrs, and the hydrolyzate, after neutralization and concentration, was used.

11.4.4 Paper Chromatography
Qualitative paper chromatography was performed by the descending technique on Whatman No. 1 paper using the system n-butanol-pyridine-water (6:4:3). The sugars were visualized by alkaline silver nitrate.
II.4.5 Determination of Ash, Nitrogen, Moisture and Fat

o An accurately weighed amount of gum (1 gm) was heated for 1 hr in a muffle furnace maintained at a temperature of 600°C. From the difference in weights of ignited and unignited material percent ash content was calculated.

o Nitrogen content of gum was determined by the Kjeldahl method.

o Moisture content of gum was determined in a Brabender moisture tester.

o The lipid content of gum was determined by extracting it with petroleum ether (40-60°C) in a soxhlet apparatus.

II.4.6 Rheology

Rheology of the gum at different levels of molar substitution was measured at five concentrations using Rheotest - 2 viscometer under the following experimental conditions: Rate of shear ranged between 3 and 1312 reciprocal seconds for the ascending and the same rates in a reverse order for the descending shear rate. Readings were taken after shearing the paste after 30-40 sec at each shearing rate.

II.4.7 Solubility

Solubility of the gum was measured using a Nephaloturbidity meter, which measures the light scattered by colloidal particles in a solution, at 90° angle to the path of incident light. The instrument was calibrated using an aqueous
standard colloidal suspension of formazin. Formazin is a
substance which has particles of uniform size and shape. It
is an ideal colloidal suspension when prepared and thoroughly
mixed. It is prepared as follows:

5 grams of reagent grade hydrazine sulfate \((\text{NH}_2\text{NH}_2\text{H}_2\text{SO}_4)\) was
dissolved in 400 ml of distilled and deionized water. To it,
hexamethylene tetramine in 400 ml of water was added and the
mixture was made up to 1000 ml in a 1 litre volumetric flask.
After allowing it to stand for 48 hrs, during which time
complete and fine particle suspension of formazin was deve­
loped, it was thoroughly shaken. This standard aqueous
colloidal suspension called "Stock solution" was further
diluted with distilled water to standards of different
Nephaloturbidometric units (NTU) which is as follows:

<table>
<thead>
<tr>
<th>Nephathoturbidometric units (NTU)</th>
<th>Volume (ml) of stock solution diluted to 1000 ml with water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>250.00</td>
</tr>
<tr>
<td>100</td>
<td>25.00</td>
</tr>
<tr>
<td>10</td>
<td>2.50</td>
</tr>
<tr>
<td>1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Following the recommended procedure, the instrument was cali-
brated at the above NTU ranges. Turbidity of gums were
measured at 0-100 range only. For all the operation, deioni-
sed and doubly distilled water was used.
II.4.8 Measurement of Foam Height

Solutions containing guar gum and its derivatives were foamed by the following methods:

Gum was added carefully, avoiding lump formation, to 200 ml of water contained in a polythene jar (h = 15 cm; r = 3.5 cm), while the whole solution was stirred. After the addition, the rate of stirring was adjusted to 4000 ± 100 rpm and this rate was maintained for 4 minutes. The height of foam column in the jar was measured using a foot-rule. Revolutions of the stirrer were measured by "Spintronics" speedometer having a noncontact sensor. The readings were cross-checked by a Stroboflash speedometer.

Measurement of foam height was also attempted by the pour method⁶ (Rose and Solash) but because of the high viscosity of solutions, and low and inconsistent foam height, it was not followed.

II.4.9 Foam Stability

Foam, obtained after stirring the gum solution for 4 minutes at 4000 ± 100 rpm, was transferred to a 500 ml measuring cylinder. The rate of foam breakdown to original volume of the unfoamed liquid was monitored at different intervals of time.
II.4.10 Moisture Regain

Weighed amount of the gum (1.5-2 gm), contained in a weighing bottle was desorbed by heating in an oven maintained at 110°C till a constant weight was obtained. The dried gum was then placed in desiccators containing saturated solutions of electrolytes having desired relative humidity in equilibrium. The following electrolytes were used for obtaining the relative humidities mentioned against them:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Electrolyte</th>
<th>% Relative Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CaCl$_2$.6H$_2$O</td>
<td>32.3</td>
</tr>
<tr>
<td>2</td>
<td>NaN$_2$O$_2$</td>
<td>66.0</td>
</tr>
<tr>
<td>3</td>
<td>NaCL</td>
<td>75.7</td>
</tr>
<tr>
<td>4</td>
<td>Pb(NO$_3$)$_2$</td>
<td>98.0</td>
</tr>
</tbody>
</table>

Moisture regain of the gum was monitored to a constant equilibrium value and percent moisture regain was calculated using the formula:

$$ R = \frac{100W}{D} $$

Where, $W$ is the weight of moisture absorbed and $D$ is the dry weight of the gum.

II.4.11 Infrared Spectroscopy

Infrared spectroscopy was performed using the infrared spectrophotometers mentioned earlier. Samples were scanned
II.4.12 Gel Permeation Chromatography

For gel permeation chromatography the following materials and experimental conditions were used:

**Samples**: 5-7 mg of the gum was dispersed in water, heated in a water bath for 15 minutes, and centrifuged to remove the insolubles.

**Column**: M. Bondagel, E. 300 (30 cm length)

**Mobile Phase**: Deionized and distilled water

**Amount of solution injected**: 50 μl of clear gum solution

**Flow rate**: 1 ml/min

**Detector**: Refractive index

**Chart speed**: 12.5 cm/min

**Attenuation**: 8x
II.5 REFERENCES


