



THE USE OF CHITOSAN TO FORMULATE IBUPROFEN GRANULES I: PHYSICO-CHEMICAL CHARACTERIZATION

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ABSTRACT

Granules of a combination of Chitosan and hydroxypropyl cellulose (HPC) were prepared by wet granulation method using 1% (w/v) lactic acid solution. The granules were physico-chemically characterized in terms of density, porosity, angle of repose, carr's index and hausner ratio and found to be free flowing with good compressibility. The observed results of content uniformity indicated that the drug was uniformly distributed throughout the four formulations. The angles repose for the formulations A1, A2 and A4 were found to be 24.1°, 23.7° and 23.31°, respectively, which are considered as very free flowing granules according to Carr's classification of flow-ability. To investigate the possible interactions between the API and the polymers contained in formulations A1-A4, FTIR spectroscopy was used. In order to detect the possible spectral changes due to the polymer/ Ibuprofen interaction (if any) and due to the formulation process the resulting spectra were compared to that of standard Ibuprofen, where no important shift of the vibrational band of the carbonilic moiety (1708 cm⁻¹) were occurred, and no important changes were recorded.

KEYWORDS: Granules, Chitosan, hydroxyl propyl cellulose, Physico-chemical characterization.

1. INTRODUCTION

Many attempts have been made to formulate controlled release drug dosage forms using Chitosan as a release controlling polymer. Kim et. al. attempted to design a novel type of porous chitosan scaffold containing transforming growth factor-beta1, TGF-beta1,^[1] Portero et. al. have described the preparation of re-acetylated chitosan microspheres for the controlled release of active anti-microbial agents such as amoxycillin and metronidazole.^[2] Pan et. al. prepared insulin-loaded Chitosan nanoparticles by ionotropic gelation of Chitosan with TPP anions.^[3] Mitra et. al. have encapsulated doxorubicin-dextran conjugate in chitosan nanoparticles prepared by reverse micellar method.^[4] Vila and coworkers have found out that a very useful feature of chitosan nanoparticles is their high protein loading capacity and used insulin as an example. Insulin was incorporated into chitosan nanoparticles very efficiently, which reached a final loading value of up to 50%. These particles were also found to keep the protein stable upon their incubation in the presence of lysozymes.^[5] Pavan Sriram et. al. have demonstrated the feasibility of effectively encapsulating Eplerenone into Chitosan microspheres to form potential sustained release drug delivery system.^[6] S. Senthil Kumar et. al. have developed a simple technique to prepare Clobazam loaded Chitosan microspheres using ionic gelation method.^[7]

1.2. Scope of the Study

The objective of this study is develop new formulations of Chitosan granules with the combination of a hydrophilic polymer namely hydroxypropyl cellulose (HPC). The granules are going to be prepared by wet granulation method using 1% (w/v) lactic acid solution. The granules will be physico-chemically characterized in terms of density, porosity, angle of repose, carr's index and hausner ratio, in addition to FTIR spectroscopy for ruling out any drug polymer interaction (if any).

2. Experimental

2.1-Materials: Chitosan from shrimp shells 75% deacetylation (SIGMA ALDRICH, ICLAND). Hydroxypropylcellulose, average Mw 100,000, HASS, CTRADINGHOUSE (BELLGIUM).

Ibuprofen, MW: 206.28 (SIGMA-ALDRICH, CHINA). lactic acid, (BDH CHEMICALS Ltd POOLE ENGLAND). Ethanol (99.6%), (RIEDEL-DEHAEN), Lot 83370. Sodiumhydroxide pellets (GRB, WINIAB LABORATORY CHEMICAL). Potassium dihydrogen orthophosphate, (BDH CHEMICALS Ltd POOLE ENGLAND). lactose, (E.MERCK, DARMSTADT, GERMANY).

2.2-Equipment: Balance- Sartorius BP121S-Sartorius BL600, CANADA. USP Dissolution apparatus-

Pharmatest DT70,, GERMANY. UV/VIS spectrophotometer- JENWAY 6305, GERMANY. PH meter handy-lab, SCHOTT GLASWERKE MAINZ, GERMANY. Granulator-Erweka AR 400, GERMANY. Drying oven-MEMERT UM 300, GERMANY. Magnetic stirrer -IKA LABORTECHNIK PH BASIC, USA. Scanning electron microscope (SEM)-LEO, GERMANY. Fourier Transform Infrared Spectroscopy (FTIR)-IR prestige, SHIMADZU, GERMANY.

2.3-Methods: 1-Preparation of the granules

1.1-Formulations prepared by wet granulation (A1-A4)

Table 1, shows the composition of four formulations which are prepared following the method reported earlier (8) as follows: Accurately weighed quantity (pre-sieved through 250 μ sieve) of Chitosan, hydroxypropyl

cellulose (HPC), lactose, starch and Ibuprofen were mixed together using a mortar and pestle for 15 minutes. Sufficient quantity of 1% w/v lactic acid was added gradually to form a wet mass and mixed for 5 minutes. The mixture was then granulated using oscillating granulator. The granules were collected and dried for 1 hour using a drying oven at 55°C and then allowed to dry at room temperature for 24 hrs. The granules were sieved through 1000 μ -500 μ sieve and were stored in desiccators using anhydrous calcium chloride for further use. The required dose was filled into hard gelatin capsules for the release studies.

Table 1: Composition of formulations (A1-A4).

Ingredients	Formulations			
	A1	A2	A3	A4
	%(w/w)	%(w/w)	%(w/w)	%(w/w)
Ibuprofen	50%	50%	50%	50%
Chitosan	40%	30%	20%	10%
H.P.C	-	10%	20%	30%
Lactose	10%	10%	10%	10%
1% lactic acid	Qs	Qs	Qs	Qs

2- Physiochemical characterization of the granules

2.1- Determination of density

Several parameters are utilized to describe the density of the powders namely; true density, bulk density, tapped density and porosity.

2.1.1-Bulk density

The bulk density of the powder is defined as a given mass of the powder that occupies a volume. This volume includes both the particulate volume and the pore volume. Hence, the bulk density essentially depends on both, the density of powder particles and the spatial arrangement of particles in the powder bed. Thus, bulk density can be defined according to the following relationship (9):

$$\rho = \frac{w}{v_s} \dots \dots \dots \text{Eq 6}$$

Where: w: weight of granules (g), v_s : is the volume(cm^3), ρ : density(g/cm^3).

The bulk density of the granules were determined by pouring a given mass (0.7g) of each patch into a graduated cylinder. The volume that the granules occupied was noted and the bulk density was calculated according to equation 6. This procedure was repeated in triplicate and the mean value was calculated.

2.1.2-Tapped density

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. The tapped density can be calculated

according to equation 7. The tapped volume does not include pore volume, since the air trapped between the particles has been displaced during the tapping process. The tapped density was determined by placing a given mass (0.7g) of the granule into 5-ml dry graduated cylinder and the volume, V_o of the granules without tapping was determined, the cylinder was then given 100 taps using a tap density apparatus and the resulting volume, V_{100} , was determined (9)

$$\rho_{tap} = \frac{w}{V_{100}} \dots \dots \dots \text{Eq. 7}$$

2.1.3- Porosity

Porosity could be considered as the packing efficiency of a powder. Therefore, the porosity of a powder is defined as the proportion of a powder bed that is occupied by pores. And it was calculated as described by the following equation:

$$\text{porosity} = 1 - (\rho_B / \rho_T) \dots \dots \dots \text{Eq. 8}$$

Where: ρ_B =bulk density g/cm^{-3} and ρ_T = tapped density g/cm^{-3}

The measurements of bulk and tapped density were carried out in triplicate.

2.2- Determination of flow properties of the granules

The determination of the flow properties of the powder is of a critical value, since it aids in the prediction of the die filling efficiency. During these experiments the

Carr's index, angle of repose and Housner ratio were used to quantify the flow ability of the granules.

2.2.1-Angle of repose

Measuring the angle of repose is a well-known technique to determine the flow characteristics of the granules. The Angle of repose is the tan inverse of angle between height (h) of a pile of granules and the radius (r) of the base of the conical pile that formed when the bulk granular material are poured onto a horizontal surface at this angle, the material on the slope face is on the verge of sliding. For the determination of the angle of repose or

the critical angle of flow, the experimental procedure was conducted as follows: the fixed- funnel method was used to determine angle of repose.^[10] The granules were carefully poured through affixed funnel until the apex of the conical pile just touched the tip of the funnel. The height (h) of the pile of the granules and the radius (r) of it is conical base were measured and applied to compute the angle of repose (θ) as in the following equation:

$$\theta = \tan^{-1} h/r \dots\dots\dots \text{Eq 9}$$

Where h= 2cm.

Table 2: Carr's classification of flow ability of a powder based on angle of repose.^[11]

Description	angle of repose
High flow	<30°
Free flowing	30-38°
Fair to passable flow	38-45°
Cohesive	45-55°
Very cohesive (non-flowing)	>50°

2.2.2- Carr's index and Housner ratio

Carr's index (the compressibility index) and Hausner ratio are measurement of the propensity of a powder to be compressed. Carr's index quantified the flow ability utilizing the previously calculated bulk density and the tapped density. Equation 4 expresses the calculation of Carr's index.

$$\text{Carr's index} = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \dots\dots\dots \text{Eq (10)}$$

The quantification of the Carr's index was used to indicate powder flow as indicated in table 10.^[12]

Table 3: Indication of powder flow by mean of the cars index.

Carr's index(%)	flow ability
5-15	Excellent
12-16	Good
18-21	Fair to passable
23-35	Poor
33-38	Very poor
>40	Extremely poor

Hausner ratio: an additional method that was applied to quantify the flow proprieties of the granules was by mean of Hoasner ratio. This parameter was calculated as the ratio of tap density to bulk density of the granules according to the following equation:

$$\text{Hausner ratio} = \frac{\rho_T}{\rho_B} \dots\dots\dots \text{Eq. 11}$$

Where ρ_T = tapped density, ρ_B = bulk density.

The mean tapped density and bulk density values where substituted in to equation 11 to calculate the Hausner ratio.

4-Drug content uniformity

A sample equivalent to 50mg of ibuprofen of physical mixtures of formulation A1-A4 was separately taken and added to 25 ml of methanol in conical flasks. The sealed flasks were agitated on a rotary shaker for 24 hours. The solution was filtered and diluted with methanol and was assayed spectrophotometrically for drug content at 264 nm.^[13]

5-Fourier Ttransform Infrared Spectroscopy(FTIR)

FTIR is commonly used to identify compounds in pharmacy, it is used to identify drugs, excipients and polymorphism. FTIR data is commonly utilized for the confirmation of the presence of interactions and identification of the change in the functional groups. Infrared (IR) Spectroscopy studies of ibuprofen and its formulation (A1-A4) were recorded in FTIR spectrophotometer, potassium bromide pellet method was employed and background spectrum was collected under identical conditions.

Preparation of the samples

The samples of the four formulation (A1-A4) where thoroughly powdered and dried before milling with anhydrous potassium bromide (KBr) powder using a mortar and pestle. The mixture is then placed between a punch and die and compressed into translucent disc using a mechanical die press to make the disk.^[14]

1-Solubility study of the Ibuprofen in phosphate buffer at PH 7.4

An excess weighed amount of Ibuprofen (model drug) was introduced in to 20 ml stopper conical flask containing 20ml phosphate buffer pH 7.4. The sealed flask was stirred thoroughly using magnetic stirrer at 50rpm for 24 hours at room temperature, and then the supernatant solution was filtered through 0.45 μm membrane filter. The filtrate was suitably diluted and

analyzed on a UV visible spectrophotometer at 221 nm. Determination was carried out in triplicate.^[13]

2-Determination of Chitosan density

Density of Chitosan was measured using specific gravity method, the weight of a clean and dry specific gravity bottle (SGB) was determined. The SGB was filled with water and weighed again. Chitosan solution (prepared by dissolving 0.1g of Chitosan in 1%acetic acid solution) was filled in the bottle and weight was noted.^[15] The density was calculated as follow:

$$\rho = \frac{wt\ of\ SGB\ and\ chitosan - wt\ of\ SGB}{wt\ of\ SGB\ and\ water - weight\ of\ SGB} \dots\dots\dots E.q\ 12$$

3- Determination of viscosity of Chitosan in 1% Acetic acid

The viscosity of the Chitosan in 1% Acetic acid was measured by Ostwald viscometer at room temperature. The solution of Chitosan was prepared by dissolving 0.1g of Chitosan in 1% acetic acid solution. Distilled water was used to calibrate and wash the viscometer and then the apparatus was washed with acetone and dried in an autoclave. Sufficient amount of distilled water was added to bulb B to fill it up to more than the half. By using a pipette pump sucker to raise the water above the upper mark C. The water was allowed to flow from C to D through the force of its weight. The time required for the water to flow from C to D was determined.

This procedure was repeated three times and the average time was calculated.

The same procedure was repeated with Chitosan solution, the viscosity of Chitosan is calculated by the following equation:

$$\eta = \eta_w \frac{t \times \rho}{t_w \times \rho_w} \dots\dots\dots Eq\ 13$$

where; η = viscosity of Chitosan, η_w =viscosity of distilled water, t, t_w =average time of Chitosan and water respectively in second and ρ, ρ_w are the densities of Chitosan and distilled water respectively in g/cc.

The densities for both water and Chitosan solution in 1% acetic acid were taken at room temperature.

3. RESULTS AND DISCUSSION

3.1. Yield and Beneficial yield percentage (500-1000µm) of the granules

Table 4. shows the calculated Yield percentage for formulations A1-A4, by the aid of equation 16. The Beneficial yield percentage was calculated by the aid of equation 17.

$$\text{Yield Percentage} = \frac{\text{Experimental weight}}{\text{Theoretical weight}} \times 100 \text{ Eq. 16}$$

$$\text{Beneficial Yield Percentage} = \frac{\text{weight of granules (500-1000µm)}}{\text{Experimental weight}} \times 100 \text{ Eq. 17}$$

Table (4): Yield and beneficial yield percentage for formulations A1 -A4.

Formulation code	% of yield of granules	%of beneficial yield (500-1000µm)
A1	99.30 %	84.89 %
A2	82.49 %	87.38 %
A3	60.95 %	86.04 %
A4	75.70 %	83.28 %

These results reveal the fact that the highest Yield percentage was achieved with the formulation with highest concentration of Chitosan (A1).

cars index porosity and hausner ratio) of the prepared granules of formulations A1 – A4.

3.2. Density and flow properties determination of the granules

Table (5) below summarizes the physiochemical properties (bulk density, tapped density, angle of repose,

Table (5): physiochemical properties of Ibuprofen granules.

Formulation code	Bulk density (g/ml)	Tapped density (g/ml)	porosity	Angle of repose(θ)*	Carr's index(%)	Hausner ratio
A1	0.22	0.259	0.15	24.1 $\pm$ (0.277)	15.38	1.18
A2	0.28	0.318	0.13	23.7 $\pm$ (0.152)	12.50	1.14
A3	0.29	0.350	0.17	30.0 $\pm$ (0.248)	17.14	1.20
A4	0.27	0.291	0.07	23.1 $\pm$ (0.276)	6.89	1.07

* All values are expressed as mean $\pm$ SD, n=3.

By reviewing table 5 and according to Carr's classification of flow-ability (table 2), it can be noticed that the angles of repose for the formulations A1, A2 and A4 are 24.1°, 23.7° and 23.31°, respectively, which are considered to be very free flowing granules in contrast to the granules of formulation A3 of which the angle of repose is 30.0°. This formulation has the lowest concentration of Chitosan (10% w/w) which might be related to the low flow-ability of the granules of this formulation. In addition, formulation A3 has the largest bulk and tapped densities which indicate that they had the smallest granules where formulation A1 had the smallest bulk and tapped densities indicating that they have the largest granules. This conclusion is probably attributed to the shape and size of the particles which are

the main factor that determines the density of the material. The number of spherical particles present in the bulk is directly proportional to the density of the material, and the density is inversely proportional to the size of the particles which is consistent with a work reported earlier.^[16] The Housner ratio of all formulation was < 1.25 which is an indicative of good compressibility.

3.3. Drug content uniformity

The drug content uniformity values of formulations A1-A4 were between 102.80% and 110.55% of the theoretical values (Table 6). The observed results of content uniformity indicated that the drug was uniformly distributed throughout the four formulations.

Table 6: The percentage of drug content uniformity of formulations A1-A4.

Formulation	Absorbance	Concentration (mg/ml)*	% of drug content
A1	0.193	2.079±(0.010)	103.95
A2	0.200	2.156±(0.057)	107.80
A3	0.191	2.050±(0.465)	102.80
A4	0.205	2.211±(0.143)	110.55

* All values are expressed as mean ± SD, n=3.

3.4 -Fourier transform infrared spectroscopy (FTIR)

To investigate the possible interactions between the API and the polymers contained in formulations A1-A4, infrared spectroscopy studies were performed. Figures 1-4 show the FTIR spectra for all components of the formulations studied. Spectrum of Ibuprofen (figure 1) shows two characteristic strong absorption bands at 1708 cm⁻¹(stretching vibration C=O) and 2955 cm⁻¹(stretching vibration O-H). In the fingerprint region of 1500 – 550 cm⁻¹ the characteristic vibration bands of the aromatic ring and the isobutyl moiety is noted. In order to detect possible spectral changes due to polymer/Ibuprofen interaction (if any) in selected formulation A3, the resulting spectrum (figure 4) was compared to that of standard Ibuprofen (Figure 1), where no important shift of the vibrational band of the carbonilic moiety (1708 cm⁻¹) was observed. Furthermore, no important changes were recorded in the fingerprint region of Ibuprofen FTIR spectra. Regarding the peak intensity there is a slight change which might be attributed to many factors that are not related to the drug / excipients interaction such as amount of sample tested, concentration variation, air absorption, disk thickness and many other factors.

4. SUMMARY AND CONCLUSIONS

Granules of Chitosan and other polymers were prepared by wet granulation method. The granules were physico-chemically characterized in terms of density, porosity, angle of repose, carr's index and housner ratio and found to be free flowing with good compressibility. Drug content uniformity was established and drug content uniformity values of formulations A1-A4 were found to be between 102.80% and 110.55% of the theoretical values. The experimental drug solubility value was not significantly different from that reported in the literature.

The observed results of content uniformity indicated that the drug was uniformly distributed throughout the four formulations. FTIR spectroscopy was carried out and proved that there was no interaction between the drug and the added polymers.

5. List of Figures

Figure 1: FTIR spectrum for Ibuprofen standard

Figure 2: FTIR spectrum for Chitosan.

Figure 3: FTIR spectrum for HPC.

Figure 4: FTIR spectrum for formulation A3

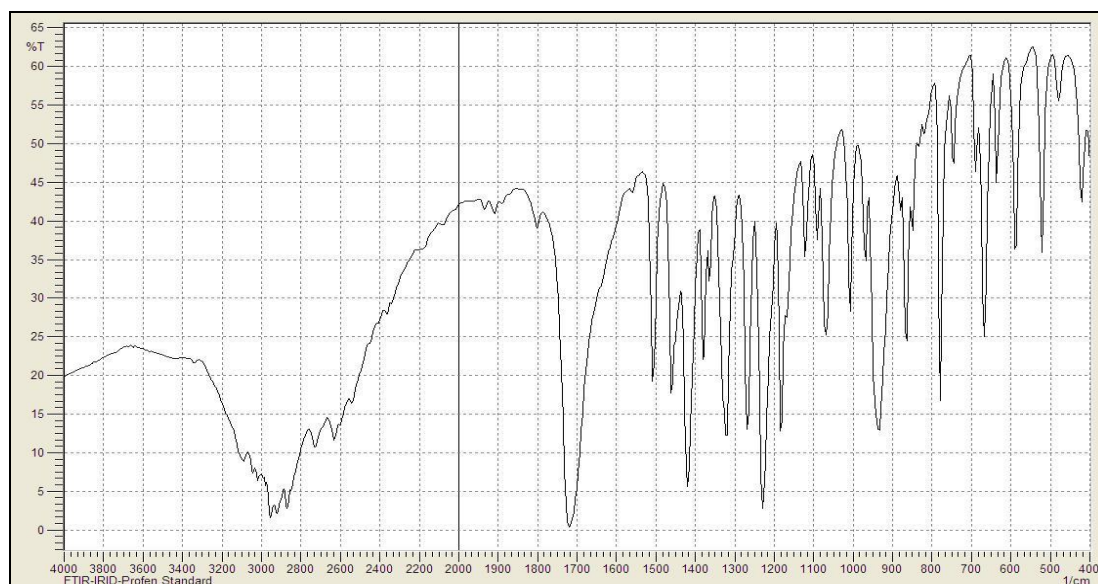


Figure 1

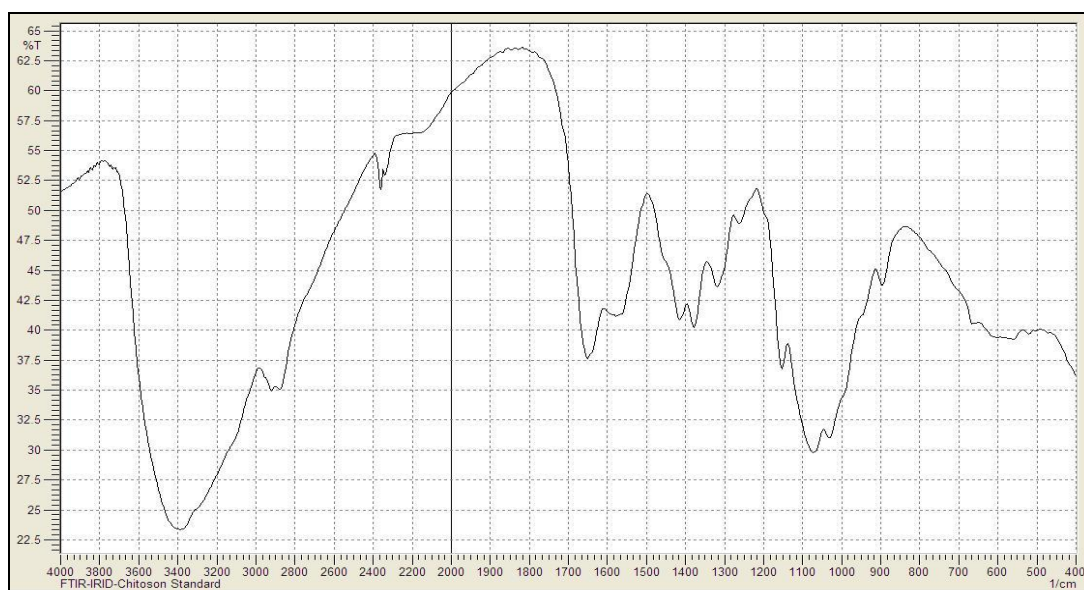


Figure 2

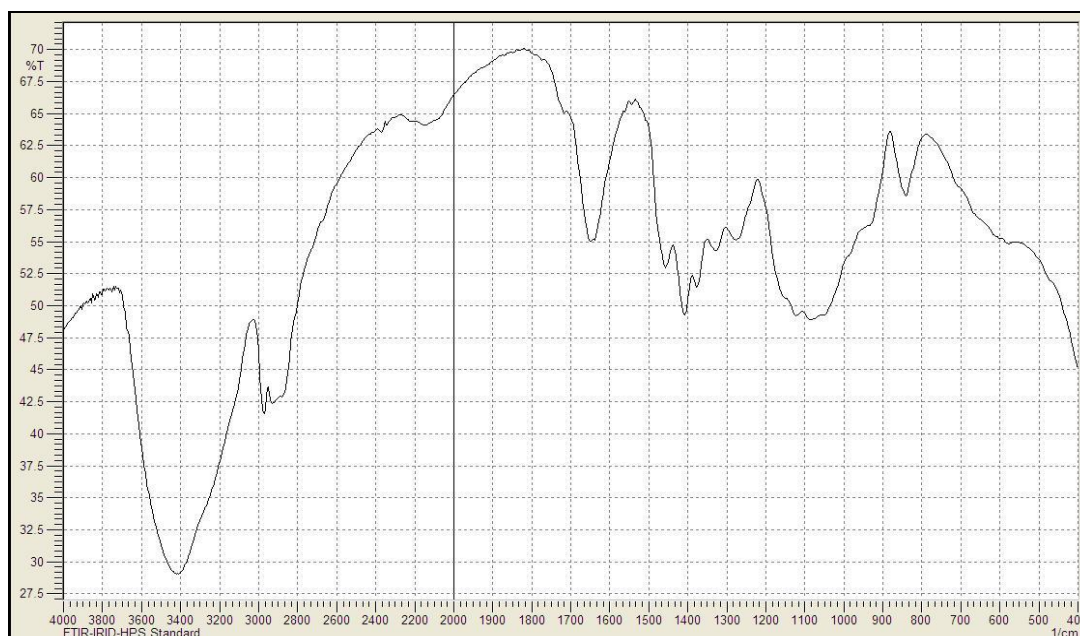


Figure 3

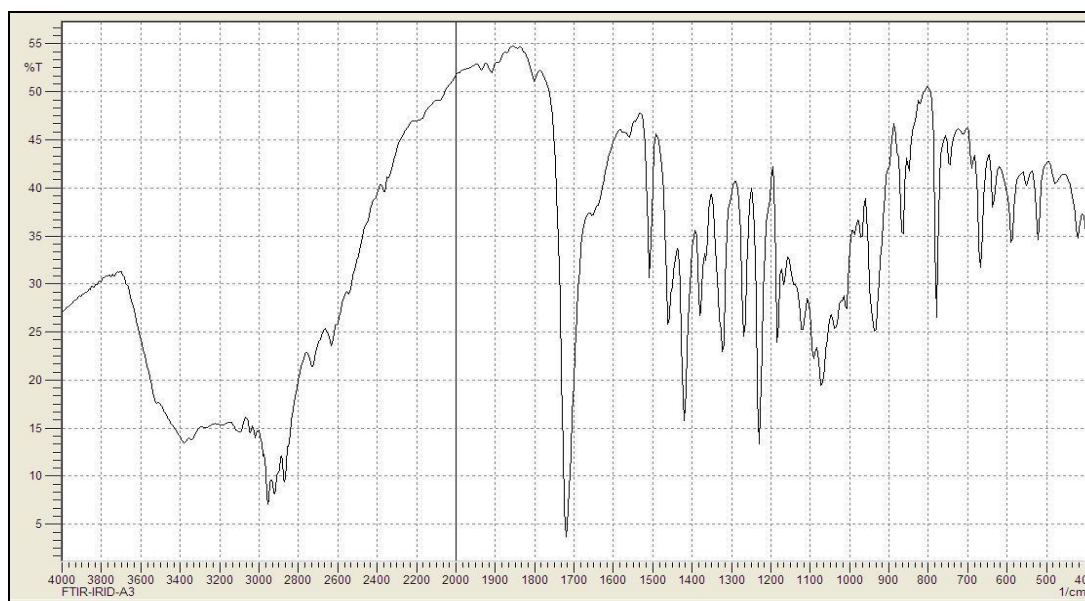


Figure 4

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