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Formulation and Evaluation of Paracetamol Tablet to Assess Binding Property of *Limonia acidissima* Pectin

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Abstract

Plants acts as a major source of medicines and are used to formulate various pharmaceutical preparations. Apart from this they act as excellent pharmaceutical aids and excipients. *Limonia acidissima* is nutritious, easily available, cheap but underutilized fruit. The aim of present research is to isolate the pectin from dried fruit pulp of *Limonia acidissima* and to assess its binding property. Pectin was extracted by boiling dried fruit pulp powder with water for 7 hrs at pH 3.2 adjusted with citric acid and using ethanol as precipitating agent. Binding property of pectin was assessed using paracetamol as a model drug. Paracetamol granules were prepared by wet granulation method. Granules were prepared using starch as disintegrant, calcium carbonate as filler, talc as glidant, Magnesium stearate as lubricant and varying concentration of pectin as binder and were compressed into respective tablets. A reference batch of starch as binder was prepared to carry out the comparative study and to assess the binding property of pectin. Total four batches of formulation were prepared using different concentrations of pectin. For each formulation pre compression and post compression studies were performed and compared to range as per pharmacopoeias. Tablets were evaluated based on Weight uniformity, Tablet Hardness, Friability, Disintegration and In vitro drug release. The results obtained for all pre-compression and post compression parameters were found within acceptable range of pharmacopoeias. *Limonia acidissima* pectin can act as excellent binder in dosage forms. In vitro dissolution studies were revealed that batch F3 showed 99.64 % of drug release. In pharmaceutical dosage form like tablet, *Limonia acidissima* pectin acts as an excellent binder since it is of natural origin and *Limonia acidissima* fruits were available easily at very low cost and proved to be better alternative for synthetic binder.

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Keywords: Pectin, *Limonia acidissima*, Binding Property, Water based extraction.

Introduction

The objective of any formulation is to deliver drug to the patient in the definite amount at the required rate as well as to maintain the stability of drug over the product's shelf life. To produce a drug formulation in a final dosage form, a number of pharmaceutical excipients were required. (Sanjib bahadur *et al.*, 2014). Plant derived polymers are gaining interest day by day as they are inexpensive, readily available, biocompatible and are superior over synthetic polymers due to safe, non pollutant, non-irritant property and low cost. This enables them to compete with the currently available commercial synthetic binder. Mucilage and gums are natural polymers researched for their binding activity in tablet. However, pectin is also one of the natural polymer possesses binding activity (F.W.A. Owusu *et al.*;2021).

As compared with other routes, oral route of drug administration is the most popular and has been successfully used for conventional drug delivery. It is considered convenient, most natural, uncomplicated, safe means of administering drugs, greater flexibility in dosage form design, ease of production and low cost. Drugs are administered by the oral route in a variety of pharmaceutical dosage forms. One of the most popular forms is tablets (Ayush *et al.*, 2015).

The development of new excipients for potential use as binder in tablet formulation continues to be of interest. Binder possesses both cohesive and adhesive properties and polymeric in nature. Because binders are added to granules mixture to improve agglomerate formation, granule flowability and compression. Binders impart plasticity which ultimately increases interparticulate bonding strength within the tablet and drug release properties of different pharmaceutical interest (Debnath *et al.*;2019).

Pectic substances or Pectin are polysaccharides present in cell wall of plants and are contributed in the complex physiological processes like cell differentiation and cell growth thus determining rigidity and integrity of plant tissue. Pectin is probably the most complex macromolecule in nature, as it can be composed out of as many as 17 different monosaccharides containing more than 20 different linkages. As it is constituent of plant and anionic in nature pectin polysaccharides are considered to be involved in the porosity of cell wall, regulation of ion transport and thus controlling the cell walls permeability for enzymes (Voragen *et al.*, 2009). Pectins occurs as light brown to white powder or granular and has slightly characteristic odour or odourless. Pectin is a natural polymer and easy to isolate and is biocompatible and non-toxic. Pectin is widely used in food industry but also in pharmaceutical industry as binder, thickening and suspending agents (Nilesh R. Khule *et al.*; 2012). Tablet is the most popular conventional dosage form and paracetamol was selected as model drug. *Limonia acidissima* is an underutilized, easily available and cheap fruit selected for isolation of pectin.

Materials and Methods

Paracetamol was obtained as gift sample from Blue Cross Pvt Ltd Nashik. Starch used as disintegrant, Calcium Carbonate as filler, Talc as glidant, Magnesium stearate as lubricant. All the chemicals and reagents were used are of analytical grade.

Raw Material

Limonia acidissima fresh fruits were collected from Satana, Nashik. The hard shell was broken by hammer and the pulp of fruit was collected. Fruit pulp was dried under sunlight and grinded in mixer to get fine powder.

Extraction of Pectin

The pectin extraction was carried out following Patra & Basak, 2020.

Dried powder of fruit pulp (200g) was weighed and blended with distilled water. The pH was set to 3.2 using 0.1 N citric acid. Mixture of powdered fruit pulp and acidified water was stirred properly for blending and heated at 90°C for about 7 hrs with continuous stirring. After the heating period is over the mixture was passed through two folded muslin cloth and cooled at room temperature. After cooling the solution was incubated for 24hrs at room temperature.

Isolation of Pectin

Ethanol was used for precipitation of pectin. For pectin isolation twice the quantity of ethanol was added to filtered solution followed by continuous stirring for 15 minutes. Pectin was precipitated and floated on the surface was kept for 24 hrs in dark condition at 25°C. Precipitated pectin was filtered through muslin cloth. Dropwise acetone was added to remove unwanted colour of pectin. The resulted pectin was washed thoroughly using 70% ethanol. The pectin was dried at 50°C in hot air oven until constant weight was achieved and then kept in desiccator for further use.

$$\text{Pectin Yield (\%)} = \frac{\text{Pectin obtained (gm)}}{\text{Weight of sample}} \times 100$$

Preparation of Tablets

4 Different batches of tablets were prepared by wet granulation method. Formula for single tablet per formulation is given in table no 1. Calculated amount of filler, disintegrant, lubricant and 500mg of drug paracetamol was mixed uniformly. Add slowly sufficient amount of water as granulating agent and wet mass was prepared. Granules were prepared by sieving method using sieve 20# and dried in hot air oven at 35-45°C for 1 hour. Until tablet compression, dried granules were stored in a desiccator. Granules were subjected to micromeritics studies and flow properties prior to tablet compression. In reference batch starch in paste form is used as binder.

Table 1: Composition of Tablet Containing *Limonia acidissima* fruit pulp Pectin as Binder

Ingredients	Formulation Code				
	F1(2%)	F2(4%)	F3(6%)	F4(8%)	Reference (4%)
Paracetamol(mg)	500 mg	500 mg	500 mg	500mg	500 mg
Binder (Pectin) (mg)	23	46	69	92	-
Starch (mg)	-	-	-	-	46
Starch (mg)	50	50	50	50	50
Calcium Carbonate(mg)	477	454	431	408	454
Magnesium stearate(mg)	50	50	50	50	50
Talc(mg)	50	50	50	50	50
Weight of each Tablet	1150	1150	1150	1150	1150

Weight of each tablet = 1150 mg

In the formula weight of one tablet (i.e. 1150mg) is mentioned, but each batch was calculated and taken for 100 tablets.

Evaluation of Granules (Pranati Srivastav et al;2010, Bansal et al;2013, Kulkarni et al;2022, Ofori Kwayke et al;2010)

Granule evaluation was carried out by using all pre formulation parameters like tapped density, Hausner's ratio, bulk density, angle of repose and compressibility index. The evaluation was carried out using the methods specified in pharmacopoeias. All the determinations were carried three times and averages reported (IP,1996).

Evaluation of Granules (Bansal et al; 2013, Ofori Kwayke et al;2010)

Bulk density

Bulk density is ratio of mass of powder to volume of bulk. The bulk density depends on the cohesiveness and shape of particles and particle size distribution. Accurately weighed quantity of powder was carefully poured

in to a 10ml graduated measuring cylinder through large funnel and volume was measured, which is called initial bulk volume. It is expressed in gm/ml and is given by the formula;

Bulk density= M/V_0 Where,

M = mass of the powder

V_0 = bulk volume of the powder.

The mean of three determinations was recorded.

Tapped density

Ten gram of powder was introduced into a clean, dry 100 ml measuring cylinder. The cylinder was then tapped 100 times from a constant height and the tapped volume was read. It is expressed in gm/ml and is a known weight of granulates for a fixed time period. The tapped density was obtained by dividing the weight of granulate by the minimum volume of granulate attained after tapping. The mean of three determinations was recorded.

Tapped density= M/V_t

Where, M = Mass of the powder V_t = Final Tapping Volume of the powder.

Hausner's ratio

Hausner's ratio is used to predict the ease of flow of granules or powders means flowability of the powders. Hausner's ratio ~ 1.2 portrays good granulate flowability and low interparticle friction while values >1.6 signifies poor granulate flowability and cohesive properties.

This method is similar to compressibility index. Hausner's ratio can be represented by equation

$$\text{Hausner's ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Carr's Index (Compressibility index)

Compressibility index is used as an important parameter to determine the flow behaviour of the powder and compressibility. It is indirectly related to the relative flow property rate, cohesiveness and particle size. It is simple, fast and popular method for predicting flow characteristics. Carr's index can be represented by Equation

The Carr's index (C) is used to predict the compressibility and ease of flow of granulate and was calculated as follows: $C = (\rho_t - \rho_b) / \rho_t * 100$, where ρ_t is tapped density and ρ_b is bulk density.

$$\text{Carr's Index} = X 100 \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}}$$

Angle of repose (θ)

It is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. Angle of repose was determined by using fixed funnel method. A funnel was fixed with its tip at a given height 'h', above a flat horizontal surface to which a graph paper was placed. Powder was carefully poured through a funnel till the apex of the conical pile just touches the tip of the funnel. The radius r of the conical pile was measured and the angle of repose calculated as follows:

$$\theta = \tan^{-1} (h/r)$$

Compression of tablets

Different tablet formulations were prepared by conventional wet granulation technique using different concentrations of pectin. The required amounts of granules were weighed and compressed into tablets using

Accura 8station punching machine having 16mm flat faced punch diameter. The compressed tablets of each batch were stored in air tight container at room temperature for further study. Such method of tablet production has previously been described by several authors who provided reproducible experimental results in terms of in vitro release. For the comparison, controlled tablets were prepared using starch as binding agent instead of isolated pectin.

Evaluation of Compressed Tablets (Chavan et al.,2017, Sandhan et al.,2017, B.V.Basavraj et al., 2011, Vijay J Kumar et al.,2011, Koppam Manjunath et al.,2013)

Hardness

To withstand mechanical shocks of handling in manufacture, packaging and shipping, Tablets require some amount of strength, or hardness and resistance to friability. For each formulation, the hardness of 6 Tablets was determined using the Monsanto hardness tester. The Tablet was held along its oblong axis in between the two jaws of the tester and a zero reading was taken. Then constant force was applied by rotating the knob until the Tablet fractured. The value at this point was noted.

Weight variation

Randomly 20 compressed tablets were selected and weighed individually and average weight was calculated. The tablet passes the test if not more than two tablet fall outside the percentage limit. Weight variation test for the tablet of all the batches were evaluated for weight variation as per IP 1996 (the weight variation limit is $\pm 5\%$) monograph. From each batch twenty tablets were used for evaluation of weight variation and mean and standard deviation was calculated.

Friability

Friability testing was determined using Roche Friabilator with readings in triplicate. Pre-weighed 20 tablets were allowed for 100 revolutions in 4 min and were dedusted and reweighed. The percentage weight loss was calculated by reweighing the tablets [IP]. The percentage friability was then calculated by:

$$\%F = \frac{W_o - W_t}{W_o} \times 100$$

Where, %F= Percent friability, W_o = Initial weight of 20 tablets, W_t = Final weight of 20 tablets

Hardness

To withstand mechanical shock of manufacturing, packaging and shipping, tablets require hardness, strength and resistance to friability. The Monsanto hardness tester was used to determine the tablet hardness. Tablet was held between the two jaws is moving and affixed jaw of tester along its oblong axis. For each formulation 6 tablets were used for hardness test. Scale was adjusted to zero and the constant force was applied was increased gradually until tablet was fractured. The reading value (pressure required to fracture a tablet) at this point was noted. The hardness of the tablet was expressed in kg/cm^2 .

Thickness and Diameter

The thickness of formulated tablets was determined using Vernier Caliper and the results were expressed as mean values of 10 determinations with SD.

Disintegration test

The USP device to test disintegration was six glass tubes that are 3 long, open at the top and held against 10 screen at the bottom end of the basket rack assembly. Single tablet was placed in each tube and basket rack was positioned in 1 litre beaker of distilled water at $37 \pm 2^\circ\text{C}$, such that the tablets remained below the surface of the liquid on the upward movement and descended not closer than 2.5 cm from the bottom of the beaker.

Suspend the assembly in the beaker containing water and operate the apparatus for 15 min. The assembly was removed from the liquid. The tablets pass the test if all of them have disintegrated.

In Vitro Drug Release Studies

In vitro drug release was studied using USP Dissolution Apparatus. Taking 900ml phosphate buffer pH 6.8 as a dissolution medium maintained at $37 \pm 2^\circ\text{C}$ for 5 hrs at 50 rpm. 5ml of sample was withdrawn at specified time intervals and filtered through Whatman filter paper no.1. From this filtrate 1 ml solution was taken into volumetric flask and diluted up to the mark. After each sample removal an equal volume of fresh phosphate buffer was added to vessel to maintain the constant volume. Collected Samples were analyzed by UV spectrophotometrically at 245 nm (Shimadzu -1800) and the percentage drug release was calculated. The test was performed in triplicate to assure significance of results. Drug release profile was studied using % drug release vs time plot.

Results and Discussion:

Table 2: Precompression Properties of Granules

Properties	Formulation Code				
	F1	F2	F3	F4	Reference
Bulk Density(gm/cm^3)	0.268	0.341	0.385	0.397	0.417
Tapped density (gm/cm^3)	0.281	0.324	0.398	0.410	0.475
Carr's index	17.024	17.011	16.954	17.014	16.541
Hausner's Ratio	1.091	1.073	1.059	1.032	1.031
Angle of repose (Degrees)	28.56	26.93	26.42	26.11	25.144

Table 3: Evaluation Parameters For Tablets

Parameters	Binder Concentration % W/W				
	2%	4%	6%	8%	Reference(4%)
Weight Variation(mg)	1148 ± 1.20	1150 ± 2.15	1149.21 ± 2.32	1150.24 ± 2.00	1150 ± 1.04
Hardness(kg/cm^2)	6.51 ± 1.55	6.86 ± 1.12	7.38 ± 0.36	8.10 ± 1.34	7.15 ± 0.51
Friability (%)	1.68 ± 0.96	1.43 ± 0.84	1.26 ± 0.62	1.05 ± 0.43	1.05 ± 0.30
Disintegration Time	9 min 2 sec	10 min 15 sec	13 min 41 sec	16 min 20 sec	12 min 48 sec
In vitro Cumulative % Drug Release	95.26	97.67	99.64	98.99	99.60

Note: The values having average of triplicate readings, with standard deviation.

Pectin was isolated from pulp of ripe fruits of plant *Limonia acidissima* belonging to family *Rutaceae*. A simple water-based extraction method is efficient for extraction of pectin from wood apple fruit. As per the result of study isolated pectin was used as binder in tablet formulation in a various concentration like 2,4,6 & 8 % concentration. The major governing criteria for to decide whether the herbal pectin is good binder or not is In -vitro release profile of the drug. For a period of 120 min, the release profile for the drug was taken can be best depicted by plotting a graph between % drug release VS time. The release of the active agents from the tablet has been fitted into various equations for the mathematical modelling. This modelling gives an overall idea on the drug release kinetics and possible mechanism of drug release characteristics. The drug release profile obtained from the *In vitro* release from various formulations was mathematically treated with various models to predict how a delivery system might function and gives valuable insight into its *in vivo* behaviour. All the formulations were subjected to *in vitro* release studies. The results obtained of in vitro release studies were attempted to fit into various mathematical models to fit the release studies. Various models such as Zero

order kinetics (Cumulative percentage amount of drug release versus Time), First order kinetics (Log cumulative percentage of drug remaining to release versus Time), Higuchi (Fraction of drug release, Mt/Mi, versus square root of time), Korsmeyer Peppas (Log fraction of drug released log Mt/Mi, versus log time) and Hixson Crowell Model (Cube root of % Drug remain Verses Time) were applied to assess the kinetics of drug release from prepared tablets. Based on in vitro release profile, it is to predict suitable batch 3rd for formulating drug using *Limonia acidissima* fruit pulp pectin as binder on commercial scale. On the basis of applied kinetics in the present experiment it can be easily seen that varying concentration of the pectin leads to approach to different kinetic theory. Wherein most accepted values of correlation coefficient vary from the reference batch taken as standard and even from batch to batch. Optimized batch F3 follows Higuchi models with correlation values of 0.9963, reference batch with starch as binder follows Korsmeyer Peppas model with correlation values of 0.9967. While Batch F1 follows Hixson Crowell model with correlation values 0.9788, Batch F2 follows both Zero order and Higuchi model with coefficient value 0.9914.

Wherein most accepted values of correlation coefficient differed from batch to batch and even from the reference batch taken as standard. 3rd batch (i.e. 6 % w/w gum binder) followed Higuchi models with correlation values of 0.9963. The values for each batch are given in Table 4.

Kinetic models and its release data of various binder concentration in tablet formulation

Table 4: Correlation Coefficient values for all Formulations

Formulation Code	Zero order Kinetics	First Order Kinetics	Higuchi Kinetics	Korsmeyer Peppas Model	Hixson Crowell Model	Best Fit Model
F1	0.9623	0.7404	0.8845	0.9071	0.9788	Hixson Crowell Model
F2	0.9914	0.7934	0.9914	0.6952	0.9036	First Order & Higuchi Model
F3	0.9879	0.8281	0.9963	0.9252	0.8152	Higuchi Model
F4	0.9818	0.8267	0.9679	0.9919	0.9171	Korsmeyer Peppas Model
Reference	0.941	0.9289	0.893	0.9967	0.883	Korsmeyer Peppas Model

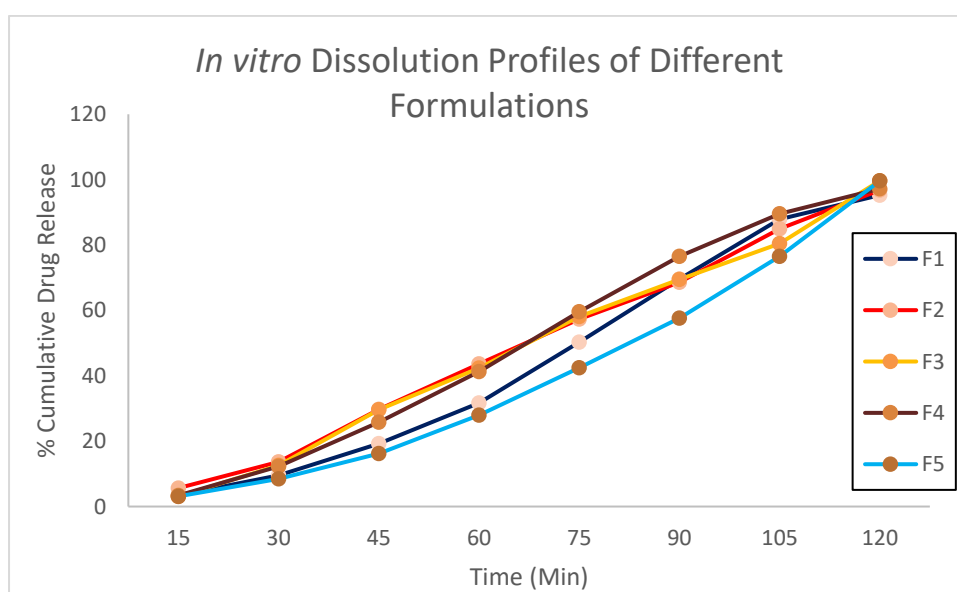


Figure1: In-vitro Dissolution Profile of Different Formulations

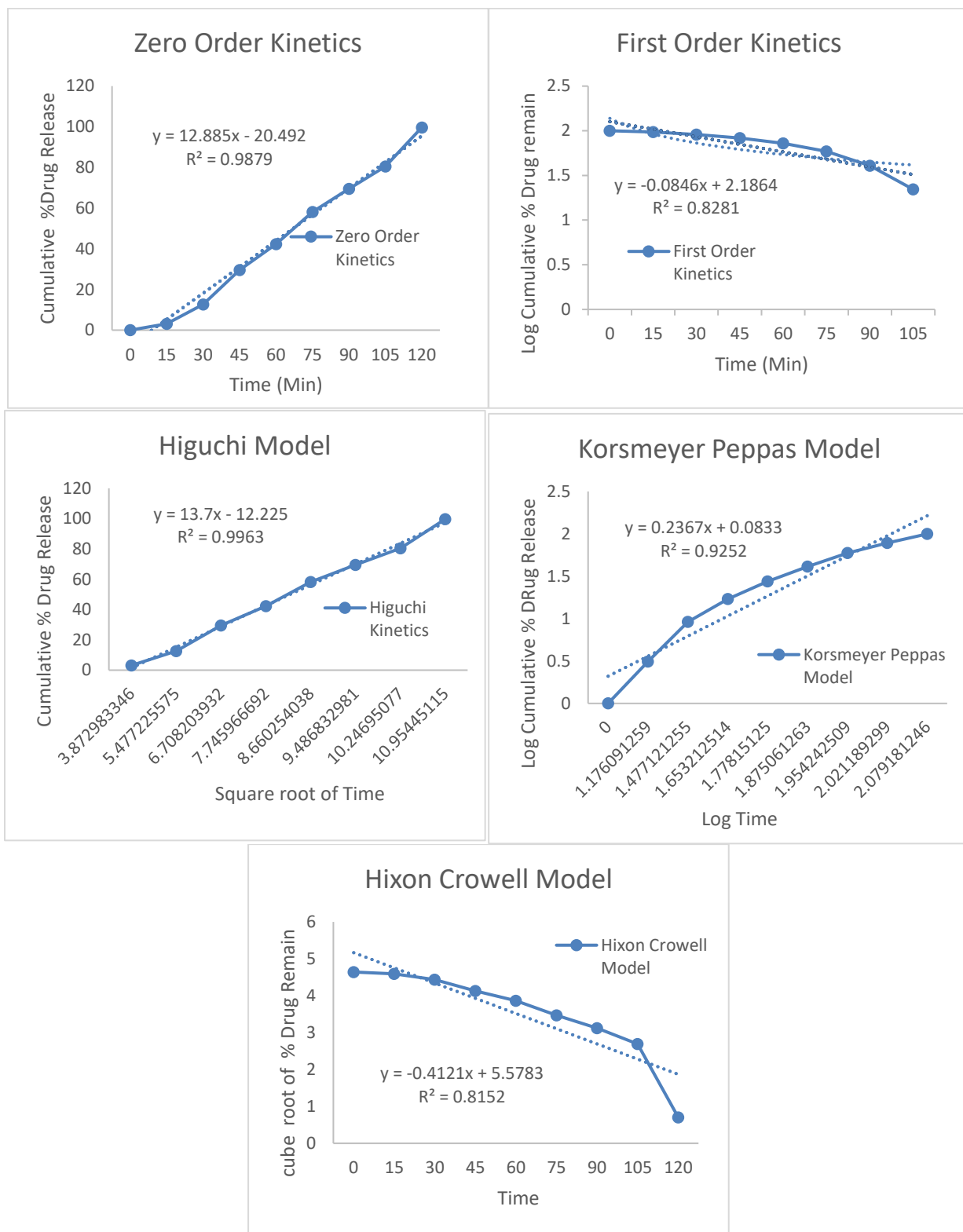


Figure 2: Best fit Release Kinetics data of F3 Formulation

Drug Excipient Interaction

There was no interaction between *Limonia acidissima* fruit pulp pectin binder, other excipients and drug. Also, it is found that there is no interaction between starch as binder, other excipients and drug. This can be predicted on the basis of Infra-Red Spectroscopy as there is no change in peaks.

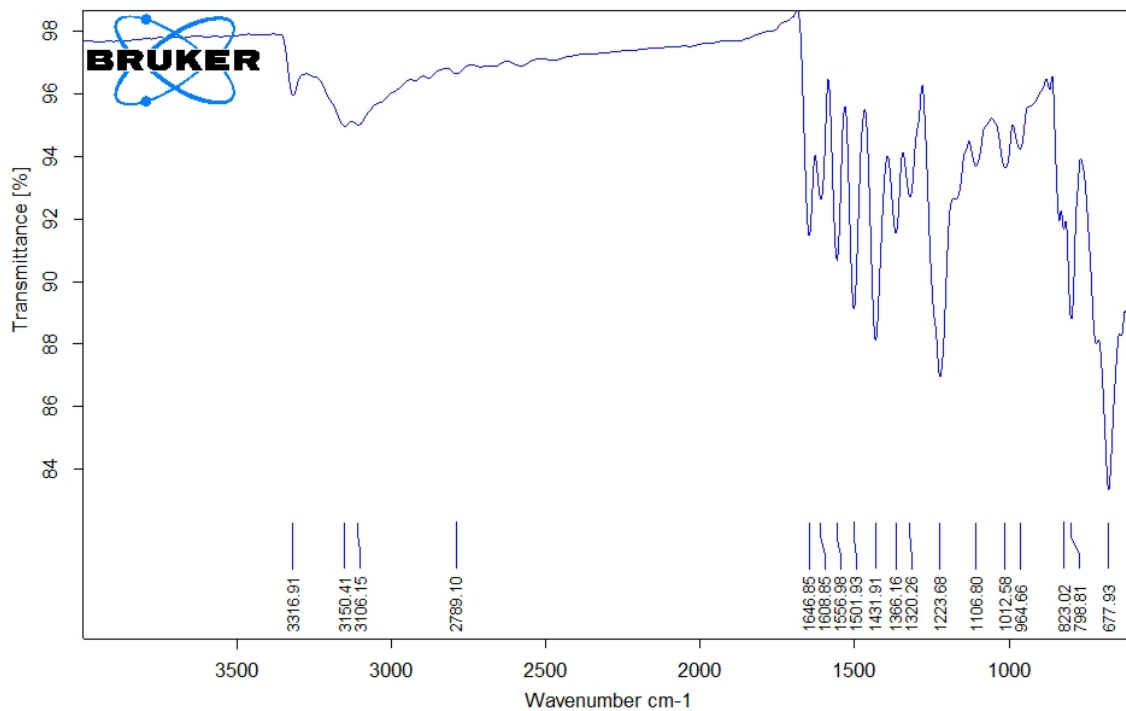


Figure: 3 FTIR of Paracetamol

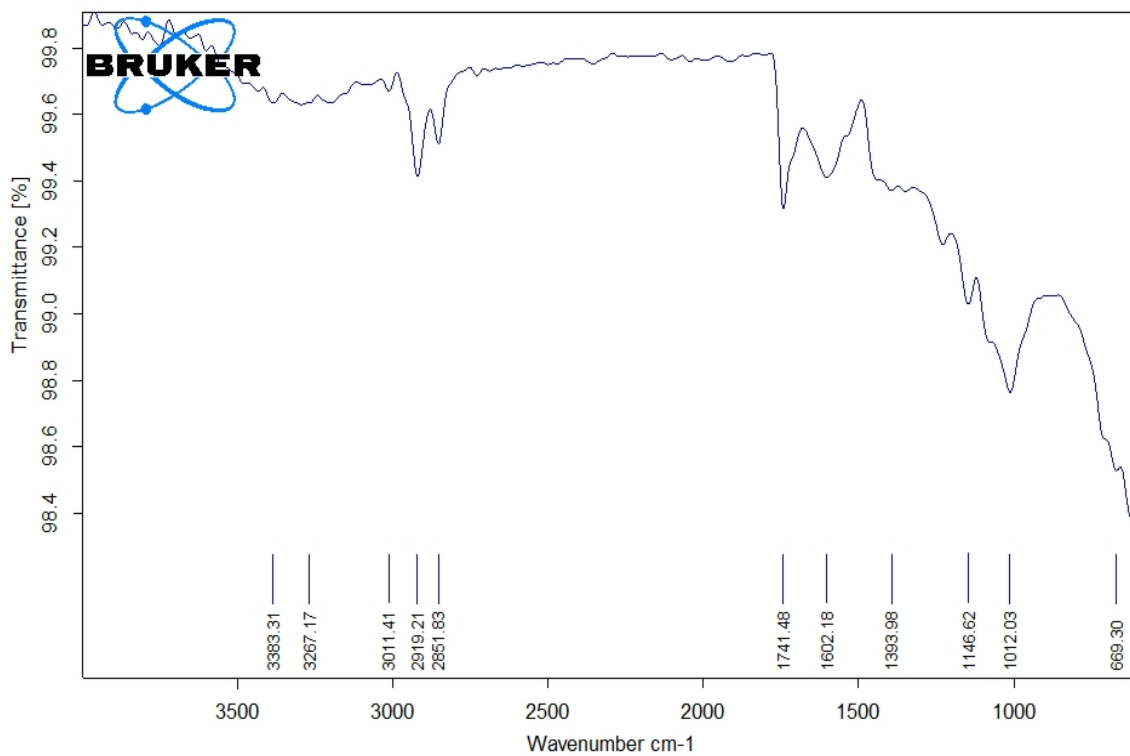


Figure 4: FTIR of Limonia acidissima Fruit Pulp Pectin

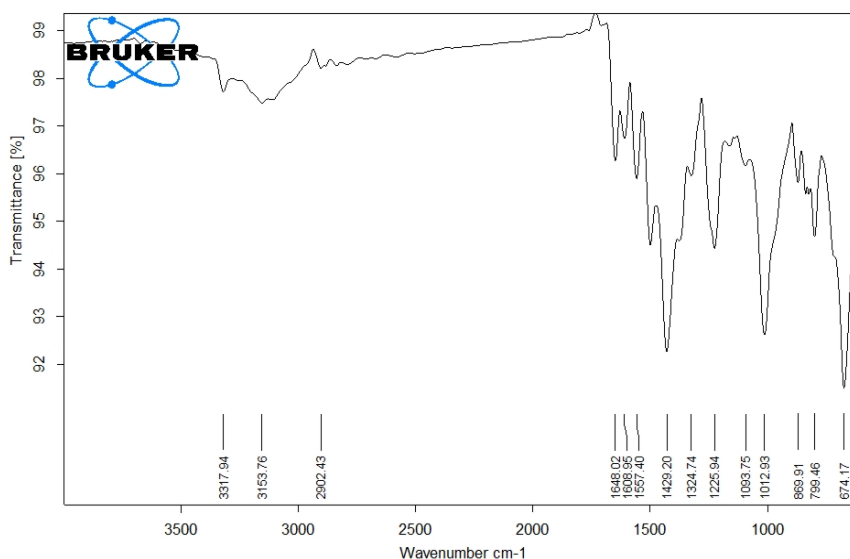


Figure 5: *Limonia acidissima* fruit pulp Pectin and Paracetamol

Physical Properties of Granules

Various parameters were studied like Carr's index, Bulk density, Angle of Repose, Hausner's ratio, Tapped density. As per the results of physical characterization batch from F1 to F4 and especially the reference batch, do not show much difference in granule flow property.

In-Vitro Release Profile

The release profile of the tablets containing pectin as binder showed that the formulated tablets releases drug at different extent depending on concentration of binder. Batch F3 showed the release to the maximum extent, about 99.64%

Conclusion

Limonia acidissima is easily available, underutilized, cheap fruit and is good source of pectin. Tablets prepared with 6 % w/w herbal pectin binder concentration shows more optimum results as tablet binder. The drug release from tablets decreased with increase in binder concentration.

The major conclusion derived from above experiment that *Limonia acidissima* fruit pulp pectin which is polymer of natural origin has immense potential and better alternative for synthetic commercial binders used in tablet dosage forms.

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