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Physicochemical and formulation studies of paracetamol suspension using *Cissus populnea* Guill. & Perr. (Vitaceae) and *Grewia mollis* Juss. (Malvaceae) gum as suspending agents

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Abstract
In the present investigation, an attempt has been made for formulation and evaluation of paracetamol suspension using *Cissus populnea* (CPP) and Grewia gum (GG) as suspending agents in comparison with acacia a standard natural gum. This study investigated compatibility profile of CPP and GG with paracetamol as well as the suspending efficacy of *Cissus populnea and* Grewia gum in paracetamol suspensions. The physicochemical properties of the gums were characterized and their effects on the suspensions' rheological behaviour, sedimentation volume, viscosity, and particle size and drug release were evaluated. The results showed that both CPP and GG exhibited good suspending properties at 1.0 to 1.5% w/v concentrations, but CPP demonstrated superior performance in terms of suspension stability. The Sedimentation volume of CPP was highest compared to GG and acacia; the ranking is CPP>GG>acacia. In terms of viscosity, CPP and GG showed more stability at 1.5% w/v concentration whereas acacia was more stable at 2.0% w/v. The optimized formulations were characterized by acceptable physicochemical properties indicating their potential for use in pharmaceutical suspensions. This study highlights the potential of *Cissus populnea* and Grewia gum as alternative suspending agents in pharmaceutical formulations.

Keywords: *Cissus populnea;* Formulation studies; Grewia gum; Suspending agents

INTRODUCTION

Suspensions are biphasic heterogeneous coarse dispersions containing essentially the insoluble particulate matter or drug suspended with the help of a suspending agent(s) in a liquid medium. The continuous or external phase is generally a liquid or semisolid. The liquid phase may be aqueous or in some instances may be organic or oily liquid for non-oral use. The dispersed or internal phase is the insoluble particulate matter dispersed throughout the continuous or external phase. These are thermodynamically unstable; almost all suspension systems separate on standing. Because some products occasionally are prepared in a dry form to be placed in suspension at the time of dispensing by the addition of an appropriate liquid vehicle, this definition is extended to include these products [1].

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Suspending agents are generally added to the dispersion medium in order to maintain uniform suspendability or to prevent caking of the suspended particles during shelf-life. Pharmaceutical suspension is usually defined as a coarse dispersion of insoluble particles in a liquid medium [2].

Mucilages are polysaccharide macro molecules that dissolve more or less upon contact with water and form colloidal solutions. In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse applications in pharmacy in the formulation of both solid and liquid dosage forms as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers [3].

Stability study of suspensions is a very important aspect to enable the patient receives the intended amount of the drug(s) in the dose administered. Physicochemical stability of suspensions is important for maintaining the quality of the product. Physical stability of suspensions may be defined as the condition in which the particles do not aggregate and in which they remain uniformly distributed throughout the dispersion. Because this ideal situation is seldom realized, it is appropriate to add that if the particles do settle, they should be easily re-suspended by a moderate amount of agitation [4]. Paracetamol is N- acetyl-aminophenol, 4-hydroxyacetanilide, [5-6]. It is a major metabolite of phenacetin (p-ethoxy acetanilide) and analgesic agent with mechanism similar to that of the salicylates. It produces antipyresis by acting on the hypothalamic heat-regulating centre and analgesia by elevating the pain threshold. It is useful particularly as an analgesic-antipyretic in patients who experience untoward reactions to aspirin. Unlike aspirin acetaminophen does not antagonize the effects of uricosuric agents [7-8]. The slight solubility of paracetamol in aqueous medium has made it a candidate for suspension which will require the inclusion of a suspending agent preferably, a natural suspending agent. The growing role and application of natural suspending agents in the pharmaceutical industry may be attributable not only to the fact that they are biodegradable and toxicologically harmless raw materials of low cost and relative abundance compared to their synthetic and semi-synthetic counterparts (9-10), but also because natural resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw material (11). Paracetamol is the most widely used and readily available antipyretic [12]

EXPERIMENTAL METHODS

Materials: Paracetamol (BDH chemicals Ltd, Poole England) obtained from our Laboratory, Acacia (BDH chemicals Ltd, Poole England) obtained from our Laboratory, both polymers (*Cissus populnea* and Grewia gum were harvested from the same place in Apir, Makurdi Local Government Area of Benue state, Nigeria and extracted in our Laboratory. All other chemicals and reagents used in the study were of analytical grade.

Sample collection. The Sample plants were collected from Rukuba, in Bassa local government area of Plateau state, Nigeria. After identifying the aerial and underground parts in the herbarium of the Federal College of Forestry Jos, the *Cissus populnea* and *Grewia mollis* plants were issued voucher numbers FCF-H 00467 and FCF-H 00468 respectively.

Extraction of Cissus gum. Cissus gum was extracted by following the method of Ibrahim et al. [13]. A 1 kg portion of sliced stem of *Cissus polpunea* was weighed and soaked in 2 litres of distilled water for 24 hours, followed by filtration of the viscous solution with muslin bag. The filtered viscous solution was dried at 60°C for 24 hours and pulverized in an Osterizer blender to produce gum powder. The powder obtained was stored in an airtight bottle.

Extraction of Grewia gum. Grewia gum was extracted by following the method of Nep et al. [14]. *Grewia mollis* inner stem bark that had been dried and ground up was mixed with 0.1% w/v.

Sodium metabisulphite solution and left to hydrate for 48 hours. After that, the mixture was agitated for 2 hours and then run through a muslin bag to get rid of any leftover particles. After treating the filtrate with 0.1M NaOH, it was centrifuged for ten minutes at 3,000 rpm. After adding acidified ethanol containing 0.1 M HCl to the supernatant, it was centrifuged once more in accordance with the earlier instructions. Absolute ethanol was added to the supernatant, and the resulting precipitate was repeatedly washed until only clear ethanol remained. After wet milling the precipitate, extra ethanol was filtered out using muslin and the result was allowed to air dry. After that, the product was air-dried and dry-milled before being further dried. There upon the air-dried product was dry milled before it was further dried at 50℃ in an oven for 24 hours. The dried product was passed through a 1.0 mm sieve, weighed and stored in air-tight containers.

Determination of pH. A 1% w/v dispersion of the samples in water was shaken for 5 min and the pH determined using a pH meter (WA Saffron Walden, England).

FT-IR protocol. FT-IR technique was used to evaluate compatibility studies in this research. A disc is made by grinding 1mg of the solid sample with 250 µg of potassium bromide powder using a mortar and pestle. The sample and the potassium bromide were weighed with the aid of a sensitive electronic weighing balance. The very fine powder is then placed into a circular die, and placed under a mechanical pressure of 1-7×108 Nm-2 (15000 – 100000 pounds per square inch) under a vacuum. The pressure was maintained for up to six minutes. The resulting transparent disc was used to record the spectrum which was scanned on a Mattson Galaxy 3020 FT-IR spectrophotometer (Unicam, England).

DSC protocol. DSC (Diamond DSC Perkin Elmer, USA) was used to study the interaction of Cissus gum with API (PCM) or with Grewia gum. About 3 mg of the pure drug or excipient was accurately weighed into the aluminium (4 mg) sample PCM which was then sealed before scanning from 20ºC up to 200ºC at a rate of 10ºC/min under nitrogen atmosphere. Physical mixture of the drug and excipient (1:1) was also transferred into the sample pan, sealed, and scanned as detailed previously.

Physicochemical characterization of the drug-polymer mixtures. Fourier transforms infrared radiation measurement (FT-IR) and differential scanning calorimetry (DSC): compatibility studies were carried out to investigate the incompatibilities between paracetamol and the suspending agents (*Cissus populnea* and Grewia gum) by using FT-IR and DSC spectroscopy. Sample preparation: Drug to excipient ratio of 1:1 provides maximum possibilities of interaction between the drug and suspending agent thus enabling easy detection of any incompatibility. Therefore, homogeneous 1:1 physical mixture of paracetamol and suspending agents were prepared by trituration in a clean and dry glass mortar and pestle [13]. These mixtures were stored in glass vials in a stability chamber at 25 ± 20ºC for four weeks after which they were subjected to FT-IR and DSC studies using model IR Affinity-I, Shimadzu Corporation, Japan and DSC-4000 respectively.

Preparation of paracetamol suspensions. Paracetamol suspensions were prepared with five different concentrations of the suspending agents as described hereunder. The suspending agents were used in five concentrations at 0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/v as shown in Table 1.

Table 1: Formulation of paracetamol suspension using various strengths of suspending agents

Formulation*	Code	Conc. (g/mL)	Paracetamol (g)	Sodium benzoate (g)	Suspending agent (g)
Acacia	AC1	0.5	5	1	0.5
	AC2	1.0	5	1	1.0
	AC3	1.5	5	1	1.5
	AC4	2.0	5	1	2.0
	AC5	2.5	5	1	2.5 t
CPP	CP1	0.5	5	1	0.5
	CP2	1.0	5	1	1.0
	CP3	1.5	5	1	1.5
	CP4	2.0	5	1	2.0
	CP5	2.5	5	1	2.5
GG	GG1	0.5	5	1	0.5
	GG2	1.0	5	1	1.0
	GG3	1.5	5	1	1.5
	GG4	2.0	5	1	2.0
	GG5	2.5	5	1	2.5

CPP – *Cissus populnea* polymer, GG – Grewia Gum; *Purified water was added to make up 100 mL final volume

The suspending agents were kept in contact with about 90 mL of water containing 100 mg of sodium benzoate for 12 hours to allow swelling of the suspending agents [14]. The dispersions were thoroughly mixed with a laboratory stirrer (REMI) for 30 minutes at an average speed of 200 rpm to get a uniform dispersion. Paracetamol was then added to the dispersions under stirring and stirring continued for another 30 minutes and made up to 100 mL volume.

Evaluation of suspension. Organoleptic Properties of suspensions containing Cissus gum and Grewia gum - which include colour, odour, texture, form and taste were noted.

Sedimentation volume. Sedimentation volume (F) is a ratio of the final volume of sediment (V_U) to the original volume of sediment (V_O) before settling. 50 mL of each suspension were transferred to 50 mL measuring cylinders and the volume of sediment formed was noted at every 24 hours for 7 days. The sedimentation volume F (%) was calculated using the formula:
$F = 100\ V_U/V_O$.

Viscosity measurement. The viscosity of the samples was determined at 25°C using the Brookfield Synchro- lectic viscometer, model LVF (Brookfield Laboratories, Massachusetts) at 30 rpm (spindle ≠ 4).

Particle size measurement. The particle size of suspended particles in the prepared suspensions was measured by optical microscopy using a trinocular microscope (Olympus, Olympus Corporation, Tokyo, Japan) at 100x (10 x 10) magnification. The size of 100 particles was measured and the average particle size was determined.

Drug release. The release studies were carried out at 37 ± 0.5°C by using a beaker method rotating cellophane membrane apparatus [11]. A 1000 mL volume of 0.1 N HCl of the release medium was used. The cellophane membrane containing 5.00 mL of suspension of the paracetamol was placed inside the vessel at time zero. Release of the paracetamol from the cellophane into the aqueous sink condition was studied from the suspensions formed in situ in the cellophane membrane cell. This was done for every concentration (Table 1). Samples were withdrawn after time intervals of 5, 10, 15, 30, 45, 60, 75, 90, 105, 120 and 135 min. maintaining sink condition. The withdrawn solutions were further diluted with 0.1 N HCl and absorbance measured at 243 nm in double beam UV-spectrophotometer (Shimadzu, UV-1700 and Shimadzu corporation [15-19].

RESULTS AND DISCUSSION

Organoleptic properties of Cissus gum and Grewia gum suspensions. The results of organoleptic properties of Cissus gum and Grewia gum are shown in Table 2. All are fine powders and taste is bland for both gums, while Cissus gum appear to be dark brown, Grewia gum is ordinary brownish in colour. Cissus gum has a pleasant odour while Grewia gum is odourless.

Compatibility testing of drug with polymers Fourier transforms infrared radiation measurement (FT-IR). Compatibility of paracetamol with suspending agents (CPP and GG) was studied using FT-IR spectroscopy. Interactions in the samples were derived or deduced by FT-IR studies from changes in the characteristic peaks. However, some broadening of peaks due to hydrogen bonding was expected while using the excipients from natural origin and also due to moisture without indicating any significant interaction [14]. If all the characteristic peaks are retained and there is no significant change in the peak position, compatibility can be expected.

Major functional groups present in *Cissus populnea* and *Grewia mollis* show characteristic peaks in the IR spectrum. Figures 2 and 3 show FT-IR peaks observed at different wave numbers and the functional groups associated with these peaks for drug (API) and drug polymer mix for CPP and GG respectively. The major peaks are identical to functional groups of both CPP and GG. Hence, it was confirmed that there was no incompatibility between drug and various polymers [14].

Differential scanning calorimetry (DSC). Results of DSC shown in figures 4 and 5 describe thermal behaviour of paracetamol with CPP and GG. DSC thermograms of paracetamol and the suspending agents (CPP and GG) and their physical mixtures were studied on the samples stored at 25±2°C. DSC thermograms showed that there was no any major difference in onset temperature and peak temperature, when compared with pure drug thermogram. In majority of cases, melting endotherm of drug was well preserved with slight changes in terms of broadening or shifting towards the lower temperature. It has been reported that the quantity of material used, [20] especially in drug-excipients mixtures, affects the peak, shape, and enthalpy. Thus, the minor changes in the melting endotherm of drug could be due to the mixing of drug and excipients, which lower the purity of each component in the mixture and may not necessarily, indicate potential incompatibility [14, 20]. Hence, it was confirmed that there was no incompatibility between drug and various polymers.

Sedimentation volume. Table 3 shows the sedimentation volume of paracetamol suspension at 0.5 to 2.5% w/v suspending agents for 3 months. The internal phase settled rapidly within the first 24 hours of preparation of suspensions containing acacia and settled constantly over the next 3 months. Paracetamol suspension formulated with CPP exhibited the highest sedimentation volume while suspensions containing acacia had the lowest sedimentation volume. High sedimentation volume is an indication that although the internal phase particles have settled as would be expected with suspensions, the inter-particulate attraction and bonding were loose and not strong enough to form hard cakes during study period [21]. The result suggested that differences in the sedimentation profiles was probably due more to the suspending agents used than properties of the internal phase. The rank order of suspending ability of the suspendants as evaluated by sedimentation volume was CPP>GG>acacia.

Viscosity measurement. Table 4 presents the viscosity measurement in 3 months. Formulation using acacia 0.5 and 1.0% w/v was not pourable from 1 to 3 months, so the viscosity could not be measured. With increased concentration of suspending agent from 1.5 to 2.0% w/v, a slight increase in

viscosity was found. Both CPP and GG were not pourable at concentration of 2.0 to 2.5% w/v. Their viscosities could be measured at concentration 0.5 to 1.5% w/v, with concentration 1.5% w/v being more stable in the case of CPP and GG. Acacia however, is indicated to be more stable at concentration 2% w/v. Generally, viscosity was found to increase with increase in concentration of suspending agent.

Particle size analysis. In acacia suspending agent, 0.5 – 1.0% w/v particle size slightly change over a period of 2 weeks, but after 1 month due to formation of non-pourable mass, particle size measurement was not possible. In formulation 1.5 – 2.0% w/v, there was slightly decrease in the particle size (Table 4). As for CPP and GG, particle size measurement could not be possible at concentration 2.0 – 2.5% w/v.

pH. The pH of acacia 0.5% w/v was found to be 7.12, when kept for long time, formulation exhibited a non-pourable mass so that determination of pH was not possible. Other formulations 1.5 – 2.0% w/v showed more or less constant pH value. This indicates that there is no chemical change when kept for long time. The same applies to the CPP and GG at concentration 0.5 – 1.5% w/v.

Drug release. All the formulations showed acceptable properties as shown in Figures 1a-c. The results for acacia indicated that concentration 2.0% w/v release maximum drug in the media. In the case of CPP and GG, maximum drug release was observed at concentration 1.0% w/v.

Conclusion. The results of the study indicated that on increasing the concentration of acacia powder in the suspensions, there is improved physical stability. The same applies to CPP and GG. Among the formulated suspensions, acacia showed better in vitro drug release as well as better physical stability at concentration 2.0% w/v compared to other formulated suspensions. CPP and GG showed the same at concentration of 1.0 – 1.5% w/v.

Table 2: Organoleptic properties of the suspensions containing sample gums

Physical character	Cissus Gum	Grewia Gum
Colour	Dark brown	Brown
Odor	Pleasant	Odourless
Texture	Fine powder	Fine powder
Form	Powder	Powder
Taste	Bland	Bland

Table 3. Values of sedimentation volume (%) suspension using different concentration of suspending agents

	Conc. (g/mL)	24 h.	1 week	2 weeks	1 month	2 months	3 months
	0.5	20	19	17	17	15	14
	1.0	28	24	23	23	21	21
Acacia	1.5	30	28	26	26	24	23
	2.0	29	29	27	27	25	25
	2.5	33	33	31	31	29	29
	0.5	37	36	34	34	31	30
	1.0	60	59	57	57	54	53
CPP	1.5	95	94	94	93	91	90
	2.0	100	100	100	99	99	98
	2.5	100	100	100	100	99	98
	0.5	48	47	45	45	43	42
	1.0	67	66	64	63	60	60
GG	1.5	89	89	87	86	86	85
	2.0	100	100	99	96	89	95
	2.5	100	100	100	99	98	97

Table 4. Viscosity values of different formulations for 3-month period

	Conc. (g/mL)	Viscosity (25 rpm.) in cps					
		24 h.	1 week	2 weeks	1 month	2 months	3 months
Acacia	0.5	6.02	7.24	6.63	NP	NP	NP
	1.0	6.01	7.83	6.17	NP	NP	NP
	1.5	8.02	8.16	7.54	6.38	5.59	4.62
	2.0	8.01	8.62	7.37	7.72	6.43	5.61
	2.5	0.01	NP	NP	NP	NP	NP
CPP	0.5	6.72	6.81	6.18	5.47	5.26	5.01
	1.0	8.37	8.43	7.62	6.29	6.02	5.48
	1.5	20.03	21.23	20.41	18.78	18.21	18.02
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP
GG	0.5	6.51	6.78	6.62	5.98	5.47	5.28
	1.0	7.68	7.91	7.82	7.53	7.12	6.73
	1.5	18.11	19.13	18.22	17.64	17.22	17.24
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP

NP = Not pourable

Table 5. Particle size determination values of different formulations for 3-month period

	Conc. (g/mL)	24 h.	1 week	2 weeks	1 month	2 months	3 months
Acacia	0.5	59.34	58.25	57.14	NP	NP	NP
	1.0	52.12	51.38	52.64	NP	NP	NP
	1.5	47.75	43.15	44.53	43.62	43.37	42.62
	2.0	42.54	38.27	33.18	33.49	32.48	32.15
	2.5	48.67	NP	NP	NP	NP	NP
CPP	0.5	60.34	59.33	59.52	59.58	59.38	58.78
	1.0	59.22	59.68	58.74	58.55	58.43	57.98
	1.5	52.13	51.34	51.75	51.33	51.29	50.96
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP
GG	0.5	59.43	59.12	59.77	58.64	58.33	57.68
	1.0	52.23	51.48	53.13	52.52	52.21	51.72
	1.5	48.78	49.82	49.44	48.43	48.45	47.94
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP

NP = Not pourable

Table 6. pH values of different formulations for 3-month period

	Conc. (g/mL)	24 h.	1 week	2 weeks	1 month	2 months	3 months
Acacia	0.5	7.12	7.12	7.08	NP	NP	NP
	1.0	6.24	6.36	6.28	NP	NP	NP
	1.5	6.53	6.41	6.74	6.12	6.24	6.09
	2.0	6.75	6.84	6.57	6.72	6.68	6.75
	2.5	6.15	NP	NP	NP	NP	NP
CPP	0.5	7.63	7.85	7.54	7.71	7.15	7.12
	1.0	7.24	7.15	7.56	7.28	7.23	7.13
	1.5	7.67	7.63	7.64	7.43	7.24	7.22
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP
GG	0.5	5.24	5.51	5.67	5.66	5.74	5.64
	1.0	5.36	5.32	5.33	5.37	5.42	5.38
	1.5	5.67	5.66	5.69	5.43	5.41	5.40
	2.0	NP	NP	NP	NP	NP	NP
	2.5	NP	NP	NP	NP	NP	NP

NP = Not pourable

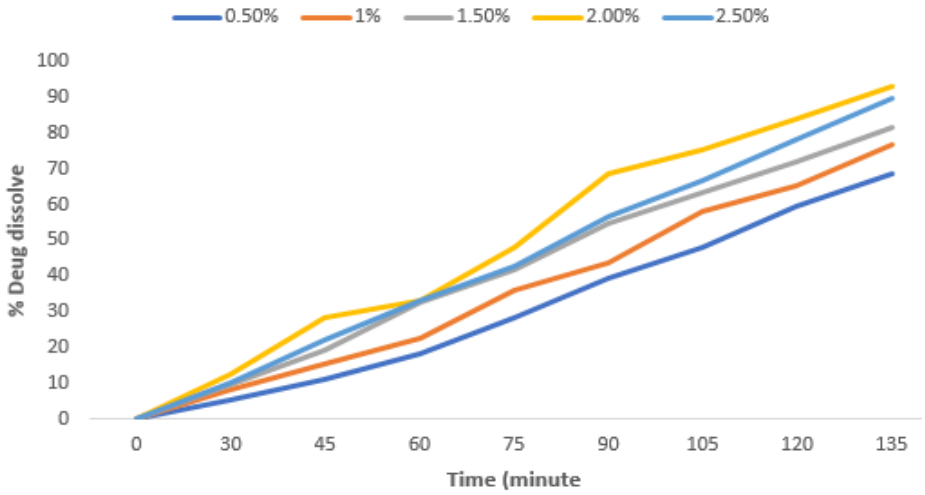

Figure 1a. Dissolution profile of acacia suspension in 0.1 N HCl

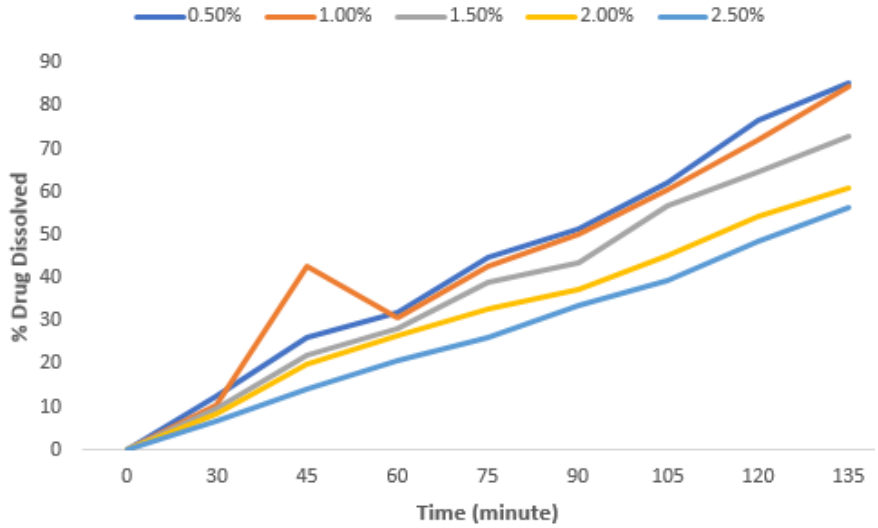

Figure 1b. Dissolution profile of CPP suspension in 0.1 N HCl

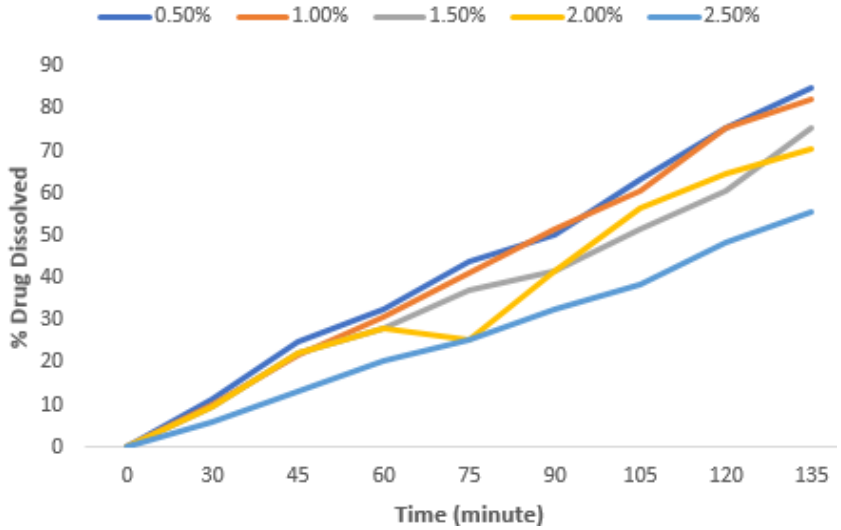

Figure 1c. Dissolution profile of GG suspension in 0.1 N HCl

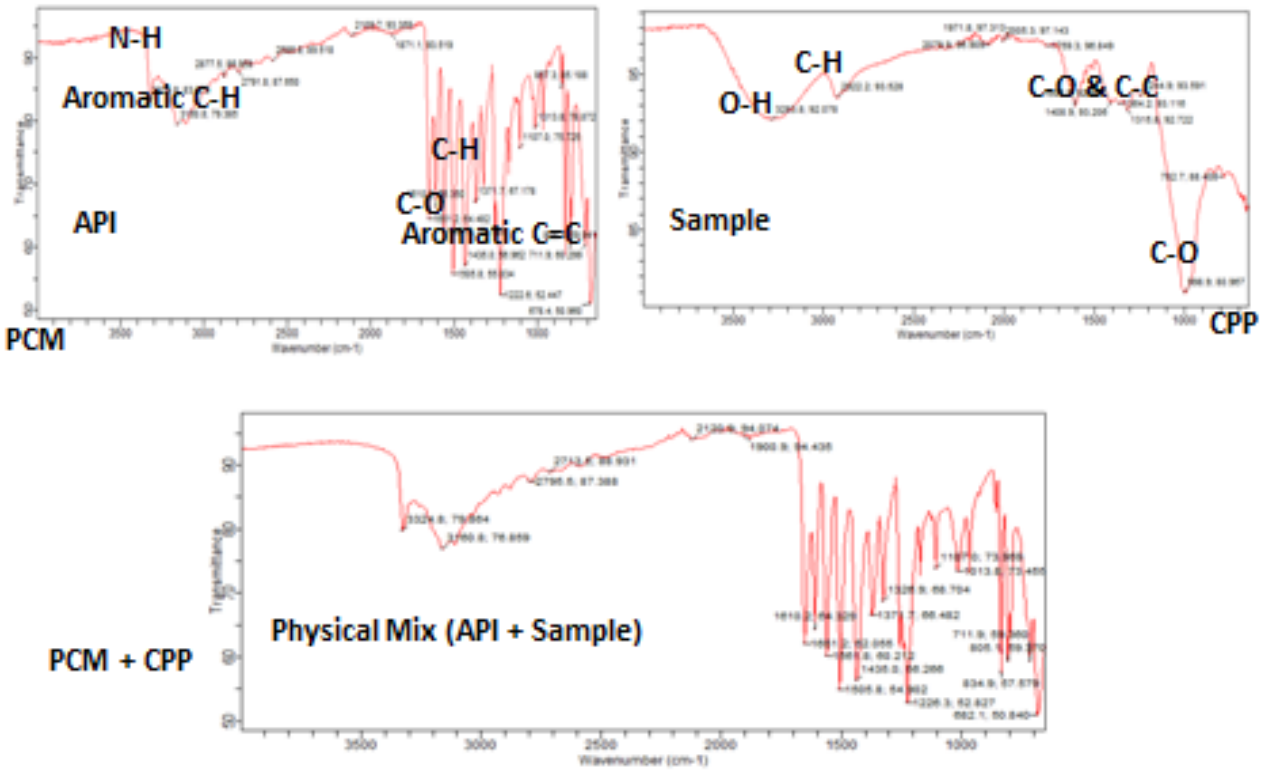

Figure 2. Physical mix of Paracetamol (API) + *Cissus populnea* Polymer (CPP)

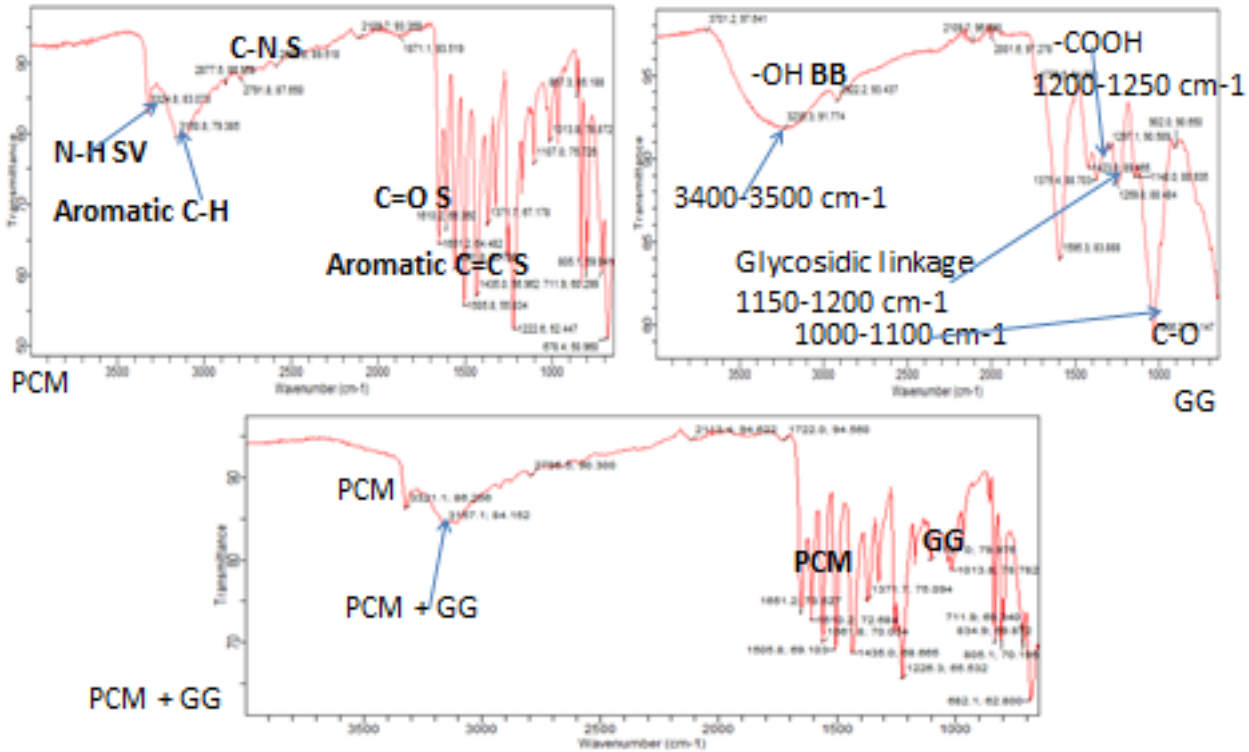

Figure 3. Physical mix of Paracetamol (API) + Grewia Gum (GG)

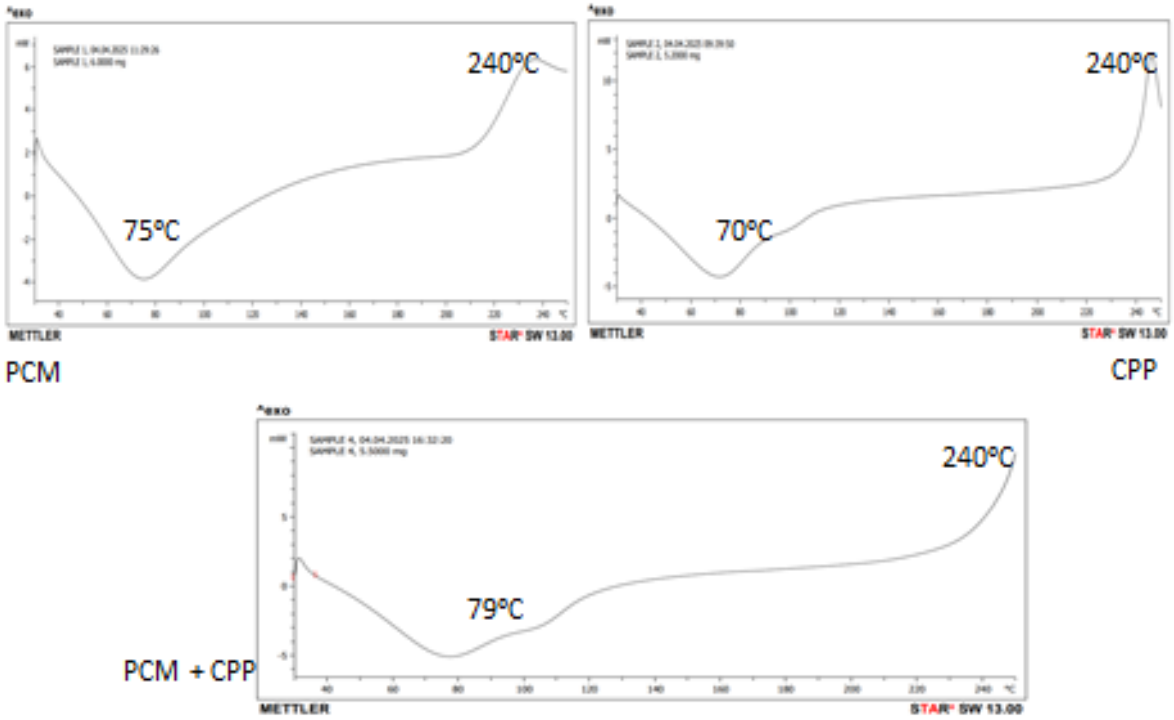

Figure 4. Physical mix of Paracetamol (API) + *Cissus populnea* Polymer (CPP)

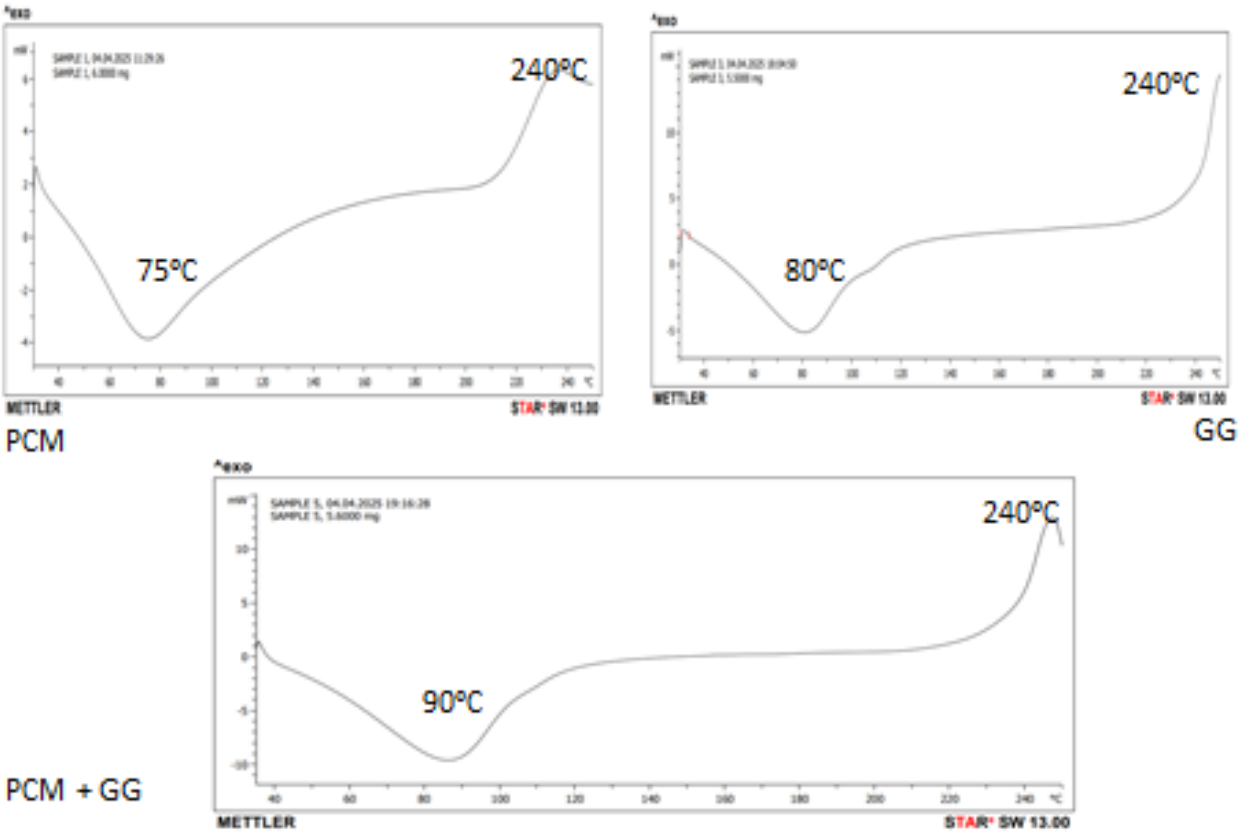

Figure 5. Physical mix of Paracetamol (API) + Grewia Gum (GG)

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