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Evidence for Complex Formation of Aceclofenac with Go-Ghrita by FT-IR., In-vitro Release, DSC, X-RD, NMR and SEM.

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Research Article

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ABSTRACT

Present investigation had undergone the evidence for complex formation of Aceclofenac (Ace) with Go-Ghrita (GG). Aceclofenac binary mixture in (1:0.5), (1:1), (1:2), (1:3) w/w proportions were prepared by adding to molten Go-Ghrita kept over a water bath at 65-70^{o}C with continuous stirring. Organoleptic and Physico-chemical properties of GG (procured from Magan Sangrahalaya, Wardha) were as per the specifications given in Ayurvedic Pharmacopoeia (AP) and Indian Pharmacopoeia (IP). All proportions were subjected for FT-IR and *in-vitro* release behaviour (SGF, P^{H} 1.2 for 2 Hrs and SIF, P^{H} 6.8 for 7 Hrs) and on basis of it, optimized (1:1) w/w proportions were further analyzed and characterized for Differential Scanning Calorimetry, X-ray diffraction, Scanning Electron Microscopy. Carbonyl stretching of oxyacetic acid group to slight lower frequency 1.41 % cm^{-1} (decrease) as well as (–C=O) linked (-OH) band stretching to slight lower frequency 0.67 cm^{-1} (decrease) shown in FT-IR spectra, *In-vitro* release of Aceclofenac 99.92± 0.11 followed by zero order release kinetics with 0.9933 R^2 value from (1:1) w/w in sustenance form than remainings. Exclusion of polymorphic modification in X-RD, Slight lowering of 3^{o}C Tm and enthalpy change of 4.05 % (loss) in DSC, deviation in chemical shift (ranging from -0.044 to +0.186) as well as selective broadening of signals in NMR and entrapment of discrete and irregular shape of Aceclofenac crystals in Ace-GG binary mixture shown in SEM photographs, reflecting possible inclusion type complexation between Aceclofenac and GG.

INTRODUCTION

A sanskrit Indian word, Go-Ghrita (GG) usually be the common name of cow ghee. GG, along with other substances, composed of numerous saturated fatty acids like myristic, stearic, lauric, butyric, capric, caprylic and unsaturated fatty acids like linoleic, linolenic, vaccenic and arachidonic acids [1], leads to difficulty in proposing any single chemical structure of it. Among these fatty acids, palmitic acid, a 16:0 saturated fatty acid, constitutes 29.95%, while oleic acid, which is 18: 1 monounsaturated acid with a double bond between 9-10 carbon atoms, is present to the extent of 27.42% [2]. *GG* has been shown to

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exhibit excellent wound healing property [3] as well as substantial anticonvulsant action [4]. A formulation containing some herbs and GG has been shown to exert remarkable memory enhancing activity [5] and patented in U.S. as an ointment base [6]. Literature repleting with reports on use of GG in designing the sustenance release formulation [7], as well as few of its interaction study with NSAIDs like Acetaminophen [8,9,10] and Diclofenac sodium [11] with use of several sophisticated analytical techniques.

Keeping in mind all such few and rare interactions study of NSAIDs with GG, attempt has been made to examine the nature, type of interaction and complex formation of non reported Aceclofenac NSAID with GG (composing saturated and unsaturated fatty acids). To investigate such phenomenon one or more sophisticated analytical techniques like FT-IR, cumulative % Aceclofenac release from binary mixtures (1:0.5), (1:1), (1:2), (1:3) w/w proportions, DSC, X-rd, NMR and SEM were studied. In addition to this preliminary analysis of GG were carried out prior its used to confirm purity in binary mixture.

Aceclofenac [12] – BCS *class* II drug, having anti-inflammatory, analgesic properties, and is widely used over-the-counter due to its proven efficacy and low cost in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Chemically, Aceclofenac (Fig. 1) is 2-[2-[2-(2, 6-Dichlorophenyl) aminophenyl] acetyl] oxyacetic acid.

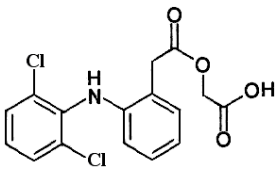

Figure 1: Aceclofenac

MATERIALS AND METHODS

Aceclofenac as a gift sample was kindly supplied by Zim Laboratories Ltd., Kalmeshwar, Nagpur. Go-Ghrita was purchased from Magan Sangrahalay, Wardha, India. All other chemicals and reagents used were of analytical grade and were procured.

Preliminary analysis of Go-Ghrita

Organoleptic analysis

Colour, Odour, Taste and Texture of GG sample was evaluated as described in AP [13].

Physical characterization

Moisture content and Refractive index (reading at 40^{0}C) of GG sample was determined by the method descrAceed in AP [13].

Chemical analysis

Acid and Saponification values

Acid and Saponification values of GG were determined as per the method described in AP [13].

Iodine and Peroxide values

Iodine and Peroxide values of GG were determined by pyridine bromide method and titration method as described in AP [13].

Ester value of GG

Ester value, difference between Saponification value and Acid value was determined as described in AP [13].

Baudouin test for GG

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Sample response for this test is checked to verify purity and adulterant present in it, as described in AP [13].

Free fatty acids (% oleic acid) and Unsaponifiable matter in GG

Free fatty acids levels of GG sample was determined by the method as described in AP [13] and IP [14].

Preparation of sample

To the molten GG kept over a water bath at 65-70^{0}C an amount of Aceclofenac was added and uniformly dispersed by continuous stirring to prepare (1:0.5), (1:1), (1:2), (1:3) w/w proportions. The 1:0.5 to 1:3 w/w proportions were selected for observing minimize to maximize the interaction (if any) [15] involved in it. The fused mixtures were homogenized and allowed to cool slowly to room temperature with stirring.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a rapid analytical technique that measures vibrations of bonds within functional groups. FTIR spectral studies were carried out using an FTIR spectrometer (Perkin Elmer Spectrum 2000, Norwalk, CT). Aceclofenac, GG and their binary mixtures (1:0.5), (1:1), (1:2), (1:3) w/w proportions were smeared onto KBr windows and the spectra were recorded from 500 to 3500/cm.

In-Vitro Aceclofenac Release Study

The % cumulative Aceclofenac release from the binary mixtures (1:0.5), (1:1), (1:2), (1:3) w/w proportions were studied in 900ml Simulated Gastric Intestinal fluid (SGF), P^H 1.2 without pepsin for first 2 Hrs and subsequent 7 Hrs in Simulated Intestinal fluid (SIF), P^H 6.8 Phosphate buffer, stirred at 50 rpm, 37°C ± 0.5°C by USP - I (Rotating Paddle type) method, VIII stations Dissolution Test Apparatus, Electrolab, Mumbai. Scanning of Aceclofenac was carried out in both SGF and SIF between 200-400 nm and λ_{max} was reported to be at 272.5 nm and 274 nm respectively. Absorbance of standard calibration curve of Aceclofenac in SGF and SIF were analyzed, after adequate dilutions, at λ_{max} 272.5 nm and 274 nm respectively on UV Spectrophotometer (UV-1700; Pharmaspec, Shimadzu, Japan) equipped with UV probe software (2.01 version). Data was depicted in Microsoft excel and had correlation coefficient (R^2) 0.999, 0.998 and equation of regression lines Y = 0.004X - 0.002 and Y = 0.025X - 0.001 respectively.

Drug content

The percent drug content of each binary mixture was determined. Weighed accurately about 50 mg of Aceclofenac binary mixtures (1:0.5), (1:1), (1:2), (1:3) w/w proportions and dissolved in 20 ml of alcohol using the magnetic stirrer for 20 min. To the solution obtained, simulated gastric fluid or simulated intestinal fluid was added and volume was made upto 100 ml. It was then filtered through Whatman filter paper no. 42 and required dilutions were made and absorbance was taken.

Differential Scanning Calorimetry (DSC)

5-10 mg of Aceclofenac sample, GG and their 1:1 w/w binary mixture was weighed into pin holed platinum pans (TG/DTA instruments) and heated under dry nitrogen in 0 - 340^{0}C scanning range at a rate of 10^{0}C/min. An empty pan was used as reference. Experiments were carried out in duplicate.

X-Ray Diffraction

X-ray diffraction of Aceclofenac, GG and their 1:1 w/w binary mixture was carried out on a Rigaku Rotating Anode Diffractometer RUH3R (Tokyo, Japan). Measurement conditions were 40 kV voltage, 30 mA current, at a scanning speed of 2^0/min, step size 0.02 and scanning range from 10–80^0 2Theta.

Nuclear Magnetic Resonance Spectroscopy

e-ISSN: 2320-1215
p-ISSN: 2322-0112

NMR spectra (using Bruker DRX-300 MHz) for Aceclofenac, GG and their 1:1 w/w binary mixture were recorded in the solvent (CD_3OD: $CDCl_3$ 3:1, v/v), using tetramethylsilane (TMS) as an internal standard. Samples (in solution form) were equilAcerated in the probe 5 min prior each run.

Scanning Electron Microscopy

Scanning electron microscopy of Aceclofenac, GG and their 1:1 w/w binary mixture mounted on scanning electron microscope stubs with double-sided carbon tape and observed under 370701-14, S-3700, Scanning Electron Microscope.

Statistical analysis

The t-test was performed on all collected mean data obtained from physiological evaluation as well as dissolution studies. Significance was accepted at $p \leq 0.05$ [16].

RESULT AND DISCUSSION

Physico-chemical analysis of GG

Physico-chemical properties of GG given in Table 1 revealed the purity and adulterant free GG. All the tested parameter of GG passes the standards and limit given in Ayurvedic Pharmacopoeia [13] and Indian Pharmacopoeia [14] respectively.

Table 1: Physico-chemical analysis of GG

Sr. No	Physiological parameters of GG	Observations (Mean ± S.D.)	A.P. standards
1.	Moisture content	0.087% ± 0.0290	NMT 0.5%
2.	Refractive index	42 ± 0.0090	40 – 45
3.	Acid value	0.22 ± 0.0190	NMT 0.15 - 0.25%
4.	Saponification value	190.74 ± 0.0210	NMT 225
5.	Ester value	189.79 ±0.0030	NMT 225
6.	Iodine value	25.88 ± 0.0199	NMT 35
7.	Free fatty acids (% oleic acid)	2.73 ± 0.0171	NMT 3%
8.	Unsaponifiable matter (%)	0.4 % w/w ± 0.0025	NMT 1.5% w/w
9.	Baudouin test	No pink color formation	No pink colour
10.	Peroxide value	0.00	Less than 0.5

All the determinations are carried out three times with significance ($p \leq 0.05$)

Fourier Transform Infrared Spectroscopy

FT-IR spectra of Aceclofenac showing in Fig. 2 (A), GG (B) and binary mixture of Ace with GG in different w/w proportions (C – F), reveling retention of characteristics bands as reported in literature [17, 18]. The ability of the GG to form a complex with Aceclofenac depends on the nature of the open lattice matrix or cage like spherical shape crystals of GG (composed of saturated and unsaturated fatty acids), electrostatic interactions between the GG and the Ace, and the ability of the Ace to form a conjugate with the GG through chemical bonding. One might expect that the Ace with the carboxylic group may form a complex with surface unsaturated -C=C- fatty acids group (may exists as dimer involving hydrogen bonding) [19] of GG and may physically encapsulate the Ace.

In pure Aceclofenac (Fig. 2A), shows OH peak at 2920.92 cm^{-1}, NH peak in diethanolamine at 3320.10 cm^{-1}, strong carbonyl band absorbance at 1751.94 cm^{-1}, which corresponds to the carboxyl acid group (COOH) where as other smaller peaks (corresponds to O-H in plane bending, C-N aromatic amine, O-H bending out of plane, Out plane for N-H bending and Skeleton vibration of aromatic C-C stretching for N-H) in the region 1500–500 cm^{-1} are contributions from the benzene ring [16]. FTIR spectrum of all binary mixture (Fig. 2C - F) 1:0.5, 1:1, 1:2, 1:3 w/w shows disappearance of strong carbonyl band at 1740.43 cm^{-1} of Aceclofenac and shifted to slight lower frequency 1715.94 cm^{-1} (i.e. 1.41 cm^{-1} decrease), as well as (–C=O) linked (-OH) band stretching to slight lower frequency 2920.92 cm^{-1} (i.e. 0.67 cm^{-1} decrease) band due to observed band respective at 1742.93 cm^{-1} and 2921.49 cm^{-1} (Fig. 2B) of carboxylic and OH group of saturated or unsaturated fatty acid [8, 9] present in GG.

e-ISSN: 2320-1215
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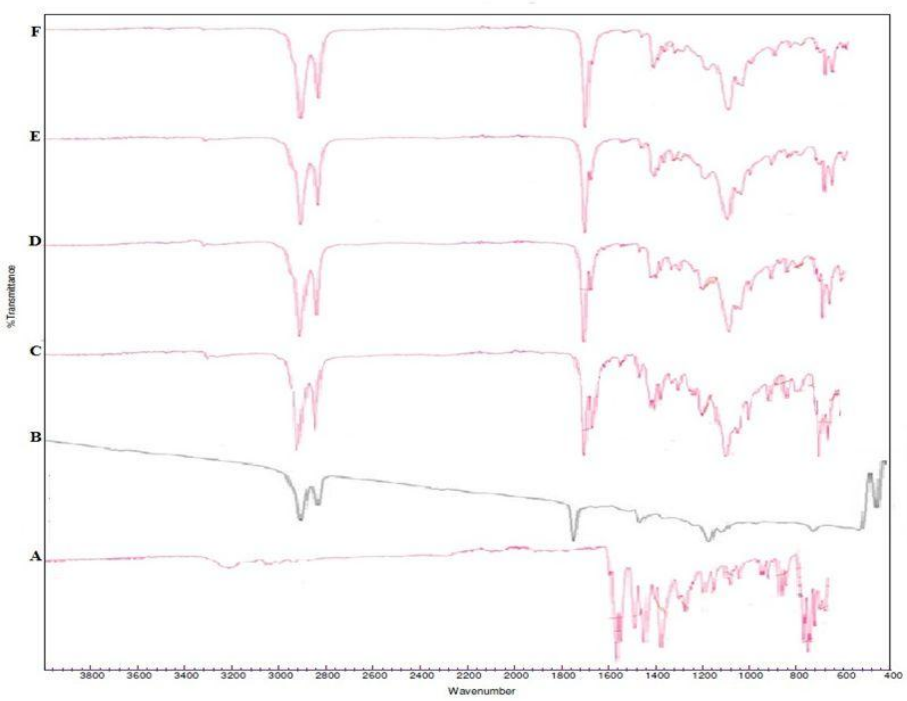

Figure 2: FT-IR Spectra of A) Aceclofenac, B) GG, C) Binary mixture of Ace-GG 1:0.5 w/w, D) 1:1 w/w, E) 1:2 w/w, F) 1:3 w/w proportion.

Table 2: FTIR Spectrum Interpretation

Characteristic peaks	Ace	1:0.5	1:1	1:2	1:3	% Stretching
NH stretching	3319.98	3317.53	3320.10	3318.47	3319.24	0.004 cm^{-1} (Increase)
O-H stretching	2940.48	2921.16	2920.92	2920.99	2920.87	0.67 cm^{-1} (Decrease)
C-O stretching	1740.43	1715.33	1715.94	1716.46	1742.46	1.41 cm^{-1} (Decrease)
Skeleton vibration of aromatic C-C stretching for N-H	1574.97	1575.27	1577.32	1578.24	1579.02	0.15 cm^{-1} (Increase)
O-H in plane bending	1376.18	1342.92	1343.50	1376.48	1376.39	2.37 cm^{-1} (Decrease)
C-N aromatic amine	1272.52	1245.89	1245.68	1245.49	1244.55	2.11 cm^{-1} (Decrease)
O-H bending out of plane	952.23	951.36	964.24	964.56	964.60	1.26 cm^{-1} (Increase)
Out plane for N-H bending	744.04	748.06	748.56	748.98	748.78	0.61 cm^{-1} (Increase)

Drug content and % Aceclofenac Release

Binary mixtures of various w/w proportions were subjected for Aceclofenac determination and 1:1 w/w found to be highest 98.95± 0.09 in SGF, 98.99± 0.17 in SIF observed slightly less (Table 3) in 1:0.5, 1:2, 1:3. The strength and stability of the Ace-GG complex was examined using in vitro release study. Fig. 3 represents more uniform and sustenance zero order release 99.33±0.11 (Table 4) of Aceclofenac with R^2 value 0.9931 (Table 5) from 1:1 binary complex in both SGF and SIF, represents the efficacy, integrity and entrapment of Ace in Ace-GG binary mixture. The order of % cumulative Aceclofenac release is 1:1 > 1:0.5 > 1:2 > 1:3.

e-ISSN: 2320-1215
p-ISSN: 2322-0112

Table 3: Percent Aceclofenac content from binary mixtures

Sr. No.	Binary mixtures w/w proportion	Drug content* (%)	
		SGF	SIF
1.	1:0.5	95.30± 0.20	96.62± 0.12
2.	1:1	98.95± 0.09	98.99± 0.17
3.	1:2	96.65± 0.26	97.14± 0.24
4.	1:3	97.56± 0.06	96.09± 0.19

(* Represents mean ± S. D.) (n=3)

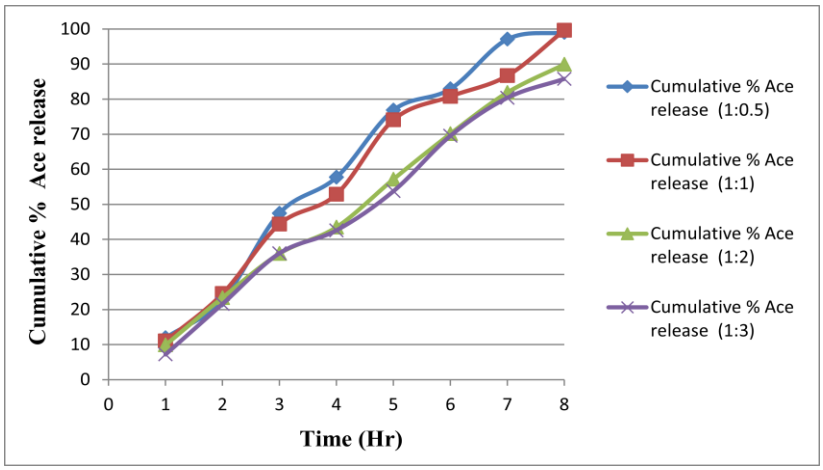

Figure 3: Cumulative % Aceclofenac release from (1:0.5), (1:1), (1:2), (1:3) w/w proportions.

Table 4: Cumulative % Aceclofenac release of various binary mixtures

Medium	Time (Hr)	Cumulative % Aceclofenac release*			
		1:0.5	1:1	1:2	1:3
0.1N HCl, PH 1.2	1	11.93± 0.12	11.03± 0.17	9.90± 0.23	7.20± 0.25
	2	22.74± 0.21	24.54± 0.26	23.41± 0.11	21.61± 0.17
Phosphate Buffer, PH 6.8	3	47.52± 0.16	44.39± 0.19	36.00± 0.13	36.10± 0.23
	4	57.80± 0.17	52.97± 0.08	43.56± 0.01	42.66± 0.26
	5	77.02± 0.24	74.24± 0.09	57.29± 0.17	53.87± 0.10
	6	83.15± 0.31	81.01± 0.30	71.00± 0.37	69.73± 0.18
	7	97.42± 0.14	87.01± 0.27	82.17± 0.25	80.64± 0.33
	8	99.48± 0.07	99.92± 0.11	90.29± 0.08	86.13± 0.09

(*Represents mean ± S.D.) (n=3)

Table 5: Release kinetics of various binary mixtures

Release Model	1:0.5	1:1	1:2	1:3
	R^2	R^2	R^2	R^2
Zero Order	0.990473928	0.993344437	0.998926769	0.997154503
First Order	0.83452192	0.82700786	0.83665117	0.84368086
Higuchi Release	0.96629489	1.037181316	0.96187244	0.95962797
Corse Mayer Release	0.113914291	0.090443856	0.034162572	0.042615011
Hixson Crowell Model	0.925252471	0.915858264	0.927257325	0.928950589

Differential Scanning Calorimetry

The DSC thermograms of Aceclofenac, GG and Ace-GG 1:1 w/w proportions are presented in Fig. 4. The following general results can be derived from Table 6, 7; the melting temperature of binary systems (Fig.4C) is lower than those of single Aceclofenac (Fig.4A). The thermogram of Aceclofenac-GG 1:1 w/w records sharp endothermic peaks corresponding to their melting point with onset at 151.6^{0}C along with

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approximately 4.00 % loss in enthalpy ($\Delta H_{observed}$) in comparison to ($\Delta H_{calculated}$) values of prepared binary system, suggesting the possibility of interaction [20,21].

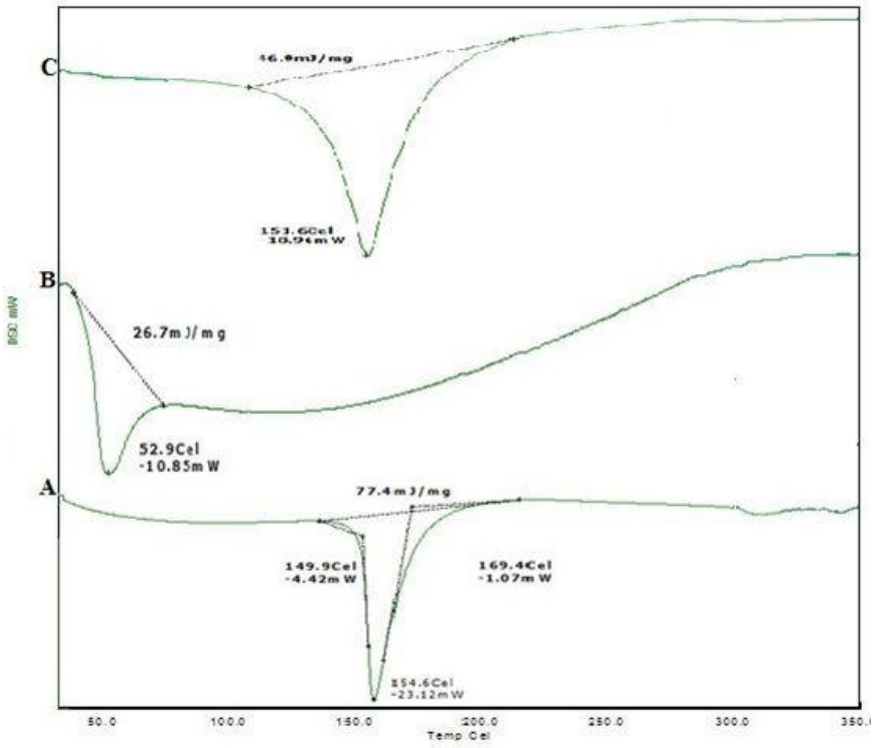

Figure 4: DSC of A) Aceclofenac, B) GG, C) Binary mixture of Ace-GG 1:1 w/w proportion.

Table 6: Thermal parameters of Aceclofenac, GG and binary mixture 1:1 w/w proportions

Sr. No.	Type	T_{peak} (^{0}C)	ΔH (J/g)
1	Aceclofenac	154.6	77.4
2	GG	52.9	26.7
3	Ace-GG 1:1	151.6	46.9

Table 7: Thermal parameters of Ace-GG 1:1 w/w proportions

Binary Mixture	T_{peak} (^{0}C)	ΔH_{cal} (J/g)	ΔH_{obs} (J/g)	ΔH % /Result
Ace-GG 1:1 w/w	151.6	80.5	77.4	4.05 (loss)

X-Ray Diffraction

Almost no change was detected in their diffraction pattern of crystalline nature of Aceclofenac (Fig. 5) as well as GG. In the diffraction pattern of the Ace – GG binary mixture, Aceclofenac and GG retained their respective peaks at their positions.

Nuclear Magnetic Resonance Spectroscopy

NMR spectra of Aceclofenac (Fig. 6A), GG (Fig. 6B) and Ace-GG 1:1 w/w proportion (Fig. 6C) presented with chemical shift records in Table 8. Ace-GG 1:1 w/w proportion (Fig. 6C) showed more pronounced changes in chemical shift of different protons and values ranging from -0.044 to +0.186 as well as selective broadening of 1H, CH in ring and proton involved in -C-OH outside ring, confirming confirmatory evidence of Ace interaction with GG. Interaction between the two substances relies on the observation of selective line broadening and/or chemical shift displacements of H-NMR spectral signals of a substance bonded with other [11, 22, 23].

e-ISSN: 2320-1215
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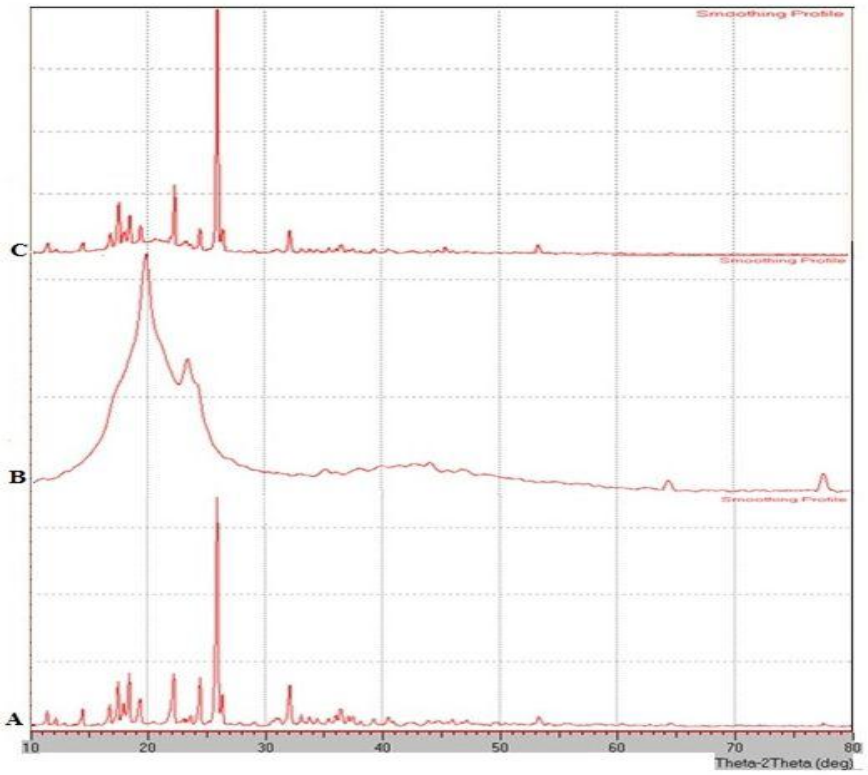

Figure 5: X- RD of A) Aceclofenac, B) GG, C) Binary mixture of Ace-GG 1:1 w/w proportion.

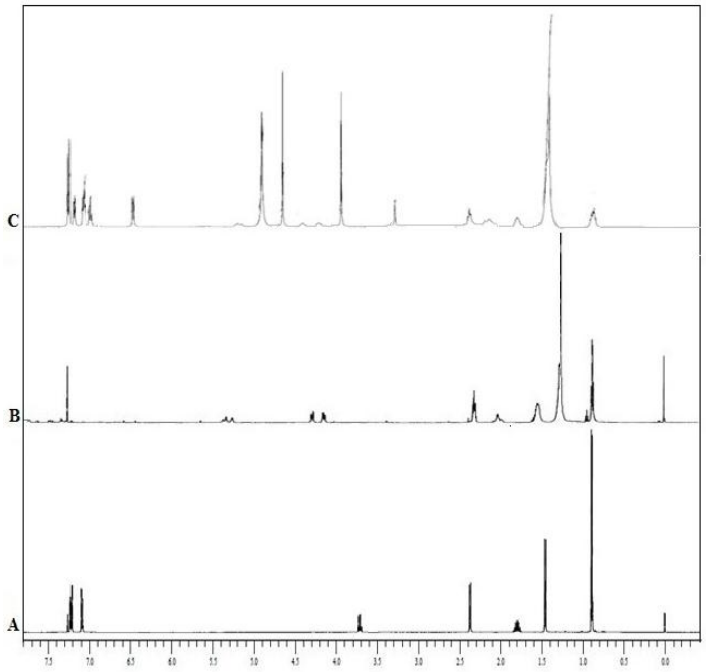

Figure 6: NMR of A) Aceclofenac, B) GG, C) Binary mixture of Ace-GG 1:1 w/w proportion.

Table 8: Chemical shifts (δ) ppm of protons in Aceclofenac and Ace-GG % w/w proportions

H/H	Ppm (δ)		$\Delta\delta = (\delta_1) - (\delta_2)$
	Ace (δ_1)	Ace-GG (δ_2)	
1H, -C-OH	1.501	1.599	+ 0.098
1H, H-C-COOR	2.380	2.336	- 0.044
1H, H-C-Cl	3.733	3.919	+ 0.186
1H, CH in ring	7.298	7.375	+ 0.077

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p-ISSN: 2322-0112

Scanning Electron Microscopy

Scanning electron micrographs of Aceclofenac illustrates Fig. 7 (A) crystals surface appear more discrete and irregular in shaped at 250X magnification. In the admixture of Aceclofenac with GG Fig. 7 (B), the open lattice matrix or cage like spherical shape crystals of GG entrapped discrete and irregular shaped Aceclofenac crystals with them giving a rough outer surface and visible large wrinkles as a sandy appearance Fig. 7 (C) by complete entrapment (inclusion type complex) [21, 24] of Ace (shown by arrow) into interior structure of GG.

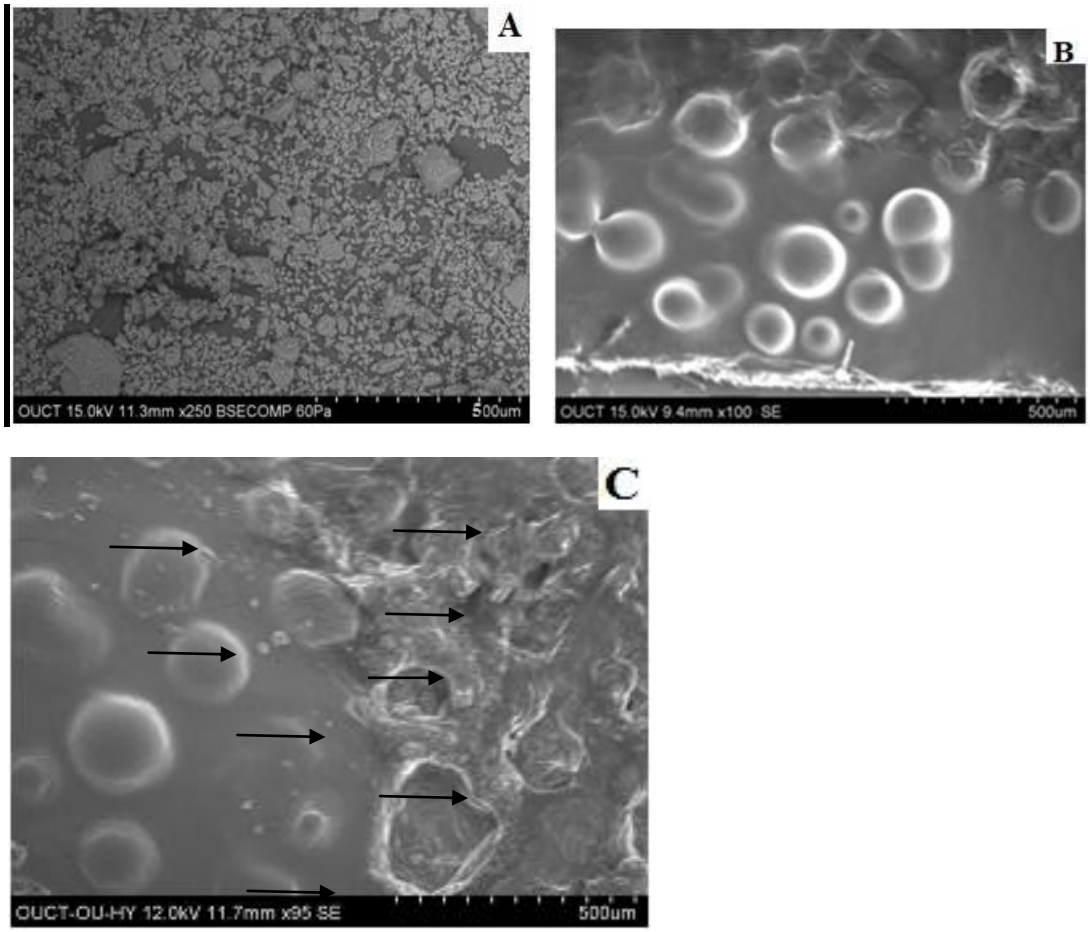

Figure 7: Scanning electron microscopy at a magnification of 250X A) Aceclofenac, B) GG, C) Binary mixture of Aceclofenac with GG (marked arrow showing Aceclofenac crystals get trapped in cage like structures of GG)

CONCLUSION

Present investigation reported complex formation of Aceclofenac with Go-Ghrita. Among various techniques used for determination the nature and type of complex of binary mixtures, the FT-IR spectrum detected characteristics decrease in both strong carbonyl band and (–C=O) linked –OH band. More uniform and sustenance zero order release of 1:1 w/w from *in-vitro* dissolution study (render its selection for optimization), % loss in enthalpy ($\Delta H_{observed}$) in comparison to ($\Delta H_{calculated}$) values of DSC system, suggesting the possibility of interaction. In spite of all above techniques used, Complexation phenomenon can be verified by X-RD, NMR and SEM. Retention of respective peaks at their positions in 1:1 w/w binary mixture, revealed crystalline nature of Aceclofenac minimizes the possibility of polymorphic modification in X-RD. Deviation in chemical shift as a slight displacement of proton as well as selective broadening of signals in 1:1 w/w NMR, confirming possible interaction between Aceclofenac and fatty acids present in GG, substantiated by SEM photographs. SEM elucidated entrapment of discrete and irregular shape of Aceclofenac crystal in open lattice matrix or cage like spherical shape crystals of GG entrapped Ace, suggesting inclusion type complex. Moreover, an interesting part is, such sustenance release inclusion complex of Aceclofenac with GG (or other NSAIDs which may form a complex with GG) may be useful in

increasing the oral bioavailability and its findings or comparing such complex concentration in various biological fluids of experimental animals is going on.

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