Evaluation Of Analgesic Activity Of Solid State Forms Of Analgin

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Abstract:

Prevalence and importance of polymorphism occurring in pharmaceutical compounds are well recognized. It is of great importance to prepare and select the right form from the beginning during drug discovery and development. The main objective of the present study is to investigate the polymorphic behavior of Analgin under different preparative conditions and to evaluate the solid forms.

Analgin is amipyrone sulphonate, analgesic, antispasmodic, and antipyretic which is most commonly given orally or parenterally to prevent and treat pain related to surgery or for the treatment of acute pain. No polymorphic forms were reported so far. The solid-state forms of Analgin were developed under different preparative conditions like solvent addition, neat grinding, solvent assisted grinding, cooling, slurrying, etc. Which were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRPD), Scanning electron microscopy (SEM), and Differential scanning calorimetry (DSC).

On slurrying of anlagin by employing methanol and water solvent combination, the input material of analgin containing Form I was partially transformed into Form II, a new solid form. The appearance of new peaks at 4.0 (100), 3.3 (15), 25.5 (10), 29.3 (11), 32.5 (11) and the other peaks at 26.6 (37), 19.6(26), 28.8 (22), and 21.5 (19) corresponding to polymorph 1 indicate that under slurrying conditions the input material of analgin was partially transformed into a new polymorph.

Key words:

Analgin, DSC, PXRD, SEM, Dissolution, Analgesic activity.

1. INTRODUCTION

Polymorphism in solids is a common phenomenon in drugs, which can lead to compromised quality due to changes in their physicochemical properties, particularly solubility, and, therefore, reduce bioavailability. The intensive knowledge on the selection of a suitable solid form/ polymorphic form to build out a commercial form with desired characteristics, as different solid forms may different physicochemical properties[1]. Since, the differences in physicochemical properties may impact directly on pharmaceutical processing, therapeutic efficacy, bioavailability, the performance of the drug finally on the quality of the drug[2]. This can be important to the quality of a given product. In the pharmaceutical field, an active substance may exhibit different activities and shelf life depending on the polymorph. Properties such as solubility, dissolution rate, density, physical stability, and melting point change depending on the type of crystalline forms. Therefore, the various polymorphs of a given pharmaceutical compound will exhibit different drug release characteristics and biological activity[3]. Rigorous screening methods practiced today by crystallization techniques are increasingly identifying new solid forms even for the old drugs. Hence, there is a growing demand for new and efficient screening techniques to be able to legally secure all possible drug forms[4]. Polymorph screening is

diligently conducted by the pharmaceutical industry to discover the appropriate crystal form for downstream development. Although traditional approaches such as cooling crystallization from solution, antisolvent addition, evaporation, and slurry experiments are commonly applied during polymorph screening, there is no industry- standard experimental conditions[5]. Polymorphs are solid crystalline phases of a drug compound, resulting from at least two different molecular arrangements of the compound in the solid-state[6].

Analgin, also known as metamizole, dipyrone, is a common phenylpyrazolone analgesic, antispasmodic and antipyretic used for the treatment of arthtalgia, neuralgia, myositis, mild to moderate pain, high fever, headache, etc. It is a white or almost white, crystalline powder with a scarcely perceptible yellowish tinge. It inhibits the production of prostaglandins(MOA). It is soluble in water, ethanol, and insoluble in ether, benzene, acetone and chloroform. Its chemical designation is sodium [N-(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl)-N-methylamino] methanesulphonate monohydrate [7-9]. No polymorphic forms of Analgin have been reported so far.

2. MATERIALS & METHODS

Analgin was procured from Mylan Yarrow Chem products, Mumbai, India. Ethanol, ether, methanol, etc were procured from Merck, Mumbai, India.

Methodology

Different solid forms of analgin were prepared by the following methods [10-11]. The solvents used for the preparation of solid forms were chosen based on the solubility of the samples in a wide range of solvents.

Antisolvent Method

To the solution of 0.5g of analgin in 50ml of water, 10ml of ether was added dropwise, which acts as an antisolvent and kept at 4^0C for 20hrs in a beaker. The precipitated crystals produced were filtered, dried at room temperature for 24h and designated as Solid form A.

Solvent Assisted Grinding Method

0.5g of analgin was ground in mortar and pestle for about one hour in a clockwise direction incorporating two drops of methanol. The obtained product was collected and labeled as Solid form B.

Cooling Method

0.5g of analgin was dissolved in 10ml of ethanol and the solution was heated on a boiling water bath for 15min. Then it was placed immediately on an ice bath till the formation of crystals. The solid obtained was filtered, dried, and designated as Solid form C.

Neat Grinding Method

0.5g of analgin was ground in mortar and pestle for about one hour in a clockwise direction. After one hour, the product was collected and designated as Solid form F.

Slurry Method

Slurry was prepared by the addition of 5ml of methanol and 5ml of water to 0.5g of analgin under continuous stirring on a boiling water bath for 1 hr. Then it was cooled to room temperature filtered, dried, and was designated as Solid form G.

SOLID STATE ANALYSIS OF ANALGIN

The prepared solid forms were characterized by using FTIR, DSC, PXRD, *In-vitro* dissolution studies. The pharmacological activity of solid forms was also performed.

Melting Point Determination

A pure, non-ionic, crystalline organic compound usually has a sharp and characteristic melting point (usually 0.5-1.0°C range). A mixture of very small amounts of miscible impurities will produce a depression of the melting point and an increase in the melting point range. Consequently, the melting point of a compound is a criterion for purity as well as for identification. Melting points were determined using a Stuart (SMP_3) melting point apparatus.

FT- IR Spectroscopy

This technique is useful to determine the chemical nature of the compound and to determine the molecular state, which is based on a chemical substance that shows marked selected absorption in the infrared region. The FT-IR spectra were obtained on a BRUKER model 65 with OPUS software at room temperature from 4000-400 cm^{-1}. Dry KBr (50 mg) was finely ground in mortar and samples of input material or prepared solid forms (1-2 mg) were subsequently added and gently mixed to avoid trituration of the crystals and pressed with 10ton.

DSC Analysis

The thermal behavior of samples was measured on differential scanning calorimeter (DSC) with Mettler Toledo 821^e DSC equipped with a $DSC821^e$ Module for thermal analysis, on 6-7mg (Mettler M3 Microbalance) weighed samples of each solid form were placed in crimped 40µL aluminum pans. Each sample was heated from 40 to 500 at a ramp rate of 10 °C/min. The instrument was preventively calibrated with Indium as standard reference (M Bartolomei et al., year). A purge gas of nitrogen was passed over the pans with a flow rate of 20mL/min. The temperature and enthalpies readings were calculated by the software (Mettler, Switzerland) by integrating the transition areas associated and normalizing the weight of each sample.

Powder X-Ray Diffraction Analysis

Powder X-ray diffraction patterns of prepared solid forms were performed on a Shimadzu 7000 diffractometer. The divergence and scattering slits were set at 1.0mm, and the receiving slit was set at 0.3 mm. Diffraction patterns within the 2θ range of 10-80° were recorded at room temperature using CuKα radiation at the following conditions: tube voltage if 40 kV, tube current of 30 mA, step-scan mode with the step size of 0.02°, 2θ and counting time 0.6 s/step. Powder samples were gently consolidated in an aluminum holder. Diffraction grams were analyzed with Shimadzu software.

TGA- Analysis

The thermogravimetric analysis of solid forms was carried out by using a Perkin Elmer thermogravimetric module Pyris 1 TGA. TGA and DTG curves were obtained under a dynamic atmosphere of synthetic air (flow rate of $35mL^{-1}$) heating ate of 10 °C min^{-1} from 0 to 600 °C with a sample mass of 7-8 mg in a ceramic pan (50 µL)[12].

Scanning Electron Microscopy

Morphological studies were performed using a Scanning Electron Microscope (SEM) with Energy – Dispersive X-ray Analysis (EDAX) System (model CARL- ZEISS EVO MA 15). The samples were observed at 10,000 X magnification with an accelerating voltage of 20kV. The samples were mounted on a metal stub with double adhesive tape and under the pressure of 0.7 torr before observation. Powder samples of PGH were mounted onto aluminum stubs using double-sided adhesive tape and sputter-coated with a thin layer of gold at 10Torr vacuum before the examination. The specimens were scanned with an electron beam of acceleration potential of 20kV and the images were collected as secondary electron mode.

DISSOLUTION PROFILE

The *in vitro* dissolution studies were determined according to the USP XXV type II (paddle) method (Electro Lab, TDT-08L, Dissolution tester, USP) to detect the differences in drug release profiles for solid forms obtained by different methods. The dissolution was studied using 900ml of KCl buffer with pH 2 (pH meter- Digital pH meter 802 SYSTRONICS). The temperature was maintained at 37 ± 0.5°C. The test was performed with a paddle rotation speed of 75 rpm. 10 mg of the sample of each solid form was added to the dissolution apparatus, and the medium was withdrawn for every 15 min up to 2hrs and was replaced with an equal volume of fresh dissolution medium. Samples collected were filtered through a 0.45 µm membrane filter, suitably diluted, and analyzed for analgin content using a UV spectrophotometer (UV-Double beam spectrophotometer, UV- 1800 model, Shimadzu) at a λ_{max} of 267 nm. The results were expressed as the mean % of drug released (±SD) at the given sampling time. All the dissolution experiments were conducted in triplicate.

PHARMACOLOGICAL STUDIES

Materials

The chemicals used in the studies were procured from Merck, HIMEDIA, Mumbai.

Animals used

Swiss albino mice weighing 25-30g were procured from Sri Venkateswara enterprises, Banglore **(CPCSEA Regd. No: 237/99/ CPCSEA).** The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions. The animals were maintained under control conditions of temperature and light. They were provided standard mouse feed/rat feed and water *ad libitum.*
Animals were fasted before dosing (e.g. with the mice, food but not water was withheld over-night, with the mouse, food but not water was withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test substance was administered. After the substance has been administered, food was withheld for a further 1-2 hours in mice. Suspensions of the solid forms and standard drugs were prepared shortly before the administration. Oral administration was done in animals carefully using a feeding tube.

Acetic Acid-Induced Writhing Assay

Analgesic activity of input material of analgin and its solid form was determined by the chemical method namely acetic acid-induced writhing. The injected chemical produces a pain reaction which is characterized as a writhing response. The test consists of injecting 0.7% acetic acid solution intraperitoneally and then observing the animal for the specific contraction of the body referred to as 'Writhing'. If the sample possesses analgesic activity, the animal that received the sample will give a lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing[13].

Experimental Protocol:

Swiss albino mice weighing 25-30g were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 6 mice in each group. Group-I received 0.5% sodium CMC, group-II received solid form G, and group-III received analgin respectively at the dose of 150mg/kg. Each mouse was weighed properly and the dose of the samples and control materials were adjusted accordingly. The suspensions of the test samples were prepared in 0.5% sodium CMC and administered orally using a feeding tube at the dose of 150mg/kg body wt. A 60min interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%, 15ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of 5min, which was given for absorption of acetic acid, the number of squirms (writhing), was counted for 30min according to the following formula[14].

$$\text{Inhibition (\%)} = \frac{\text{Number of Writhes (Control)} - \text{Number of Writhes (treatment)}}{\text{Number of Writhes (Control)}} \text{ X } 100$$

Preparation Of Acetic Acid Solution:
For the preparation of 0.7% acetic acid solution, 0.7ml glacial acetic acid was mixed with distilled water to 100ml.

Tabulation Of Writhing
Each mouse of all groups was observed to count the number of writhing that they had made in 30min. Two half writhing was counted as a full writhing.

Statistical Analysis

All the data were represented as means ± SEM. Statistical analysis was performed by a two-way analysis of variance (ANOVA) test for multiple comparisons followed by the Turkey-Kramer test. The statistical significance was set accordingly.

3. RESULTS AND DISCUSSION

To investigate the solid-state behavior of analgin, samples recrystallized from various solvents and processing conditions were screened using FTIR, DSC, XRPD, SEM analysis, and dissolution studies. As no polymorph was reported so far, the input material of analgin was designated as polymorph I.

Input Material Of Analgin

FTIR is used to investigate the intermolecular interactions within the crystalline structure of the drug. The FTIR spectrum of input material displayed a characteristic absorption band at 3478 cm^{-1} due to N-H amine stretching, a strong absorption band was ascribed at 3034 cm^{-1} due to aryl or vinyl stretching, C-H stretching vibration was observed at 2902 cm^{-1}, a sulfone group was noticed at 1344 cm^{-1} and phenyl group was observed at 755 cm^{-1} [15].

Powder X-ray diffraction patterns of input material of analgin displayed characteristic 2θ values at 26.3 (100), 15.6 (97), 10.5 (84), 18.7 (64), 19.2 (48), 21.3 (45), 28.9 (38), 28.6 (36), 23.7 (30), 19.9 (30), 25.0 (25), 24.1 (23), 14.1 (21), 32.3 (21), 23.0 (16), 31.8 (16).

DSC thermogram of input material of analgin showed a single sharp melting endotherm at 234.9 °C (ΔH 44.8 j/g) with the onset and endset temperatures at 228.9°C and 246.3°C respectively.

Conditions Favouring Transformation Of Analgin

A solid-state analysis of the solid forms of analgin by FTIR, PXRD and DSC revealed the following polymorphic transformations.

Solid Form G

The FTIR spectrum of solid form G obtained from slurrying of anlagin by employing methanol and water as solvents displayed significant absorption bands at N-H amine stretch 3523 cm^{-1}, aryl, or vinyl stretch 3034, C-H stretch 2905, sulfone group 1344, benzene 1495, and phenyl group at 755 cm^{-1}.

The PXRD spectrum showed sharp peaks with straight baselines indicating the crystallinity of solid form G when compared to input material. Diffraction lines recorded for solid form G5showed prominent 2θ values at 4.0 (100), 3.3 (15), 25.5 (10), 29.3 (11), 32.5 (11), 26.6 (37), 19.6(26), 28.8 (22) and 21.5 (10). The peaks at 26.6 (37), 19.6(26), 28.8 (22), and 21.5 (19) correspond to polymorph 1, however, the appearance of new peaks at 4.0 (100), 3.3 (15), 25.5 (10), 29.3 (11), 32.5 (11) indicate that under slurrying conditions the input material of analgin was partially transformed into a new polymorph, designated as form II. The PXRD data of solid form G suggested that a new polymorph was formed with a small amount of form I being present.

DSC profile of solid form G presented a single melting endotherm at 235.0 °C (ΔH 24.8 j/g) with the onset and endset temperatures at 230.7°C and 245.5°C respectively which is similar to input material.

Conditions Which Retained The Polymorphic Nature Of Analgin

A solid-state analysis of the solid forms of analgin by FTIR, PXRD, and DSC that the following conditions retained the nature of analgin.

Solid Form A

The IR spectral characteristic bands of solid form A_5, obtained from antisolvent addition method using was water as solvent and ether as an antisolvent, the band at 3478cm^{-1} being shifted to 3481 cm^{-1} (N-H amine stretching vibration), 3034 cm^{-1} (aryl or vinyl stretch), 2905 cm^{-1} (C-H stretch), 1345 cm^{-1} (sulfone group), 1494 cm^{-1} (benzene) and 756 cm^{-1} phenyl group.

The diffraction lines of solid form A_5 displayed prominent 2θ values at 10.8 (100), 10.7(86), 21.5 (35), 40.3 (42), 26.5(31), 28.8 (26), 15.8 (24), 14.3 (23), 19.0 (18), 32.5 (15), 17.9 (14), 18.8 (14), 25.2 (13), 29.1 (13), 23.8 (12), 24.2 (11) which are in agreement with input material indicating that addition of antisolvent did not induce any polymorphic changes in solid form A. The presence of more intense peaks in solid form A indicated the higher crystallinity of the solid form. DSC trace of the solid form A depicts a single melting endotherm at 230.6 °C (ΔH 29.0 j/g) with the onset and endset temperatures at 224.0°C and 247.5°C respectively. The melting temperature of solid form A was slightly reduced compared to the melting temperature of the input material of analgin.

Solid Form B

The IR spectrum recorded for solid form B obtained from solvent assisted grinding using methanol as solvent, exhibited band at 3478 cm^{-1} being shifted to 3467 cm^{-1} (N-H amine stretch), 3034 cm^{-1} (aryl or vinyl stretch), 2905 cm^{-1} (C-H stretch), 1344 cm^{-1} (sulfone group), 1496 cm^{-1} (benzene) and 755 cm^{-1} (phenyl group).

Powder diffraction spectrum of solid form B displayed signature 2θ values at3.9 (100), 15.9 (55), 26.6 (30), 18.9 (27), 19.5(27), 10.8 (13), 20.1 (13), 24.2 (11), 29.1 (10) and 20.2 (10) corresponding to polymorph I which are in agreement with input material indicating that on solvent drop grinding, analgin does not undergo any polymorphic change. But, the crystallinity was reduced when compared to input material which may be due to grinding in presence of a solvent.

The DSC behavior of solid form B furnished a single melting endotherm at 231.9 °C (ΔH 44.8 j/g) with the onset and endset temperatures at 229.3°C and 234.7 °C respectively. The broadening of melting endotherm of solid form B was noticed when compared to input material which may be due to reduced crystallinity, which is also supported by PXRD data.

Solid Form C

The FTIR spectrum of solid form C, obtained from cooling crystallization technique using ethanol as a solvent, displayed characteristic signals at 3467 cm^{-1} due to N-H amine stretch, 3034 cm^{-1} due to aryl or vinyl stretch, 2905 cm^{-1} due to C-H stretch, 1344 cm^{-1} due to sulfone group, 1493 cm^{-1} due to benzene and 755 cm^{-1}due to a phenyl group.

PXRD patterns of solid form C exhibited significant characteristic 2θ values at 10.7 (100), 15.8 (86), 26.6 (48), 19.5 (41), 21.5(40), 18.9 (34), 14.3 (28), 20.1 (26), 17.9 (26), 28.8 (26), 24.3 (24), 32.5 (18) corresponding to polymorph I which are in agreement with input material indicating that under cooling conditions, input material did not undergo any polymorphic change. A single short melting endotherm depicts at 233.8 °C (ΔH 40.7 j/g) with the onset and end set temperatures at 228.8°C and 247.2°C respectively.

Solid Form F

The FTIR spectrum of solid form F obtained from neat grinding displayed significant absorption bands at N-H amine stretch 3523 cm^{-1}, aryl or vinyl stretch 3034 cm^{-1}, C-H stretch 2905 cm^{-1}, sulfone group 1344 cm^{-1}, benzene 1495 $cm^{-1}$and phenyl group at 755 cm^{-1}.

Diffraction lines recorded for solid form F_5 showed prominent 2θ values at 3.9 (100), 16.0 (46), 26.7 (24), 19.5(23), 19.0 (22), 24.3 (11), 20.2 (10), 10.9 (10) correspond to polymorph I indicating that under neat grinding conditions, input material does not undergo any polymorphic change. But, the crystallinity was significantly reduced when compared to input material.

DSC profile of solid form F presented a single sharp melting endotherm at 234.4 °C (ΔH 24.8 j/g) with the onset and endset temperatures at 231.0°C and 238.4 °C indicating that the melting endotherm is similar to that of input material.

SEM Analysis:

During the crystallization of a substance, external factors like crystal size, shape, crystal lattice etc can induce the formation of a particular crystalline habit. The crystal morphology plays an important role in pharmaceutical processing and the development of solid dosage forms. Differences in the crystal habit may strongly influence the particle orientation and modify the flowability, packing, compaction, compressibility and dissolution characteristics[16].

The influence of crystallization solvent on habit modification of analgin polymorphs was clearly shown. The interaction of solvent to preferential adsorption at selected faces of the solute that influences the habit of crystallizing solid[17].

The SEM images of the solid forms of analgin confirmed the differences in the crystalline habit, morphology and particle nature. In case of solid forms B & F which are obtained under grinding conditions, analgin transformed more into amorphous form. Solid form C showed prismatic crystals where as solid form G, exhibited plated crystals.

***In-Vitro* Dissolution Studies**

The solid forms of Analgin were evaluated by dissolution studies & were compared with the input material. The profiles were significantly varied from one another. It is well known that the bioavailability of the drug and drug products not only depends on the only solubility of the active pharmaceutical ingredient but also on the dissolution of the drug from the dosage form [18]. The difference in crystal faces affects the nature of each crystal habit which influences the dissolution of a drug. The variation in drug habit by crystallization using different solvents, additives, and crystallization conditions[19,20].

The solid forms obtained from different processing conditions were subjected to dissolution studies and were compared with the input material. The results demonstrated that the dissolution profiles were significantly different. There were observed marked differences in the dissolution behavior of the solid forms and input material of analgin. The dissolution rates were found to be increased to 96.6±1.6 for solid form G at 120 min when compared to input material (90.3±1.4). The dissolution rate was comparatively low for the solid form B (82.6 ±1.9).

The other solid forms of analgin showed dissolution rates in the order of 80.3 ±0.4, 85.2±0.3, and 88.6±1.4 for solid forms A, C, and F respectively. This difference in dissolution may be due to crystal habit and closely related properties such as particle size, anisotropy defects, etc. The increased dissolution rate observed for solid form G when compared to input material may be due to partial transformation of Form I into Form II.

Analgesic Activity

Analgesic activity of solid form G and input material of Analgin evaluated at a dose of 150mg/kg body weight in Swiss albino mice indicted in table 1. Solid form G evaluated showed slightly increased activity (69%) when compared to input material (56%). The increase in activity may be due to the partial phase transition of form I into form II, in the case of solid form G.

5. CONCLUSION

As no polymorphic forms were reported so far, the input material of analgin was considered as polymorph I. Analgin when subjected to under different preparative conditions like antisolvent, grinding, etc, the input material retained its original polymorphic nature in most of the conditions without any transitions. However, under slurring conditions (solid form G) partial transformation of analgin was observed. Some amount of input material was retained as such along with a new solid-state form. SEM analysis revealed that solid form G showed different crystal morphology when compared to input material. The dissolution of solid form G was also improved when compared to input material. Similarly, the analgesic activity was also improved for solid form G, suggesting that it is interesting to further explore the complete polymorphic transformation of analgin.

Conflicts of interest: None declared

Ethical Clearance:

The study protocol was approved by the Institutional Animal ethics committee,Sri Padmavati Mahila Viswavidyalayam, Tirupati (Regd No: 1677/PO/a/12/IAEC/6 dated 24/02/2014).

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Figures & Tables

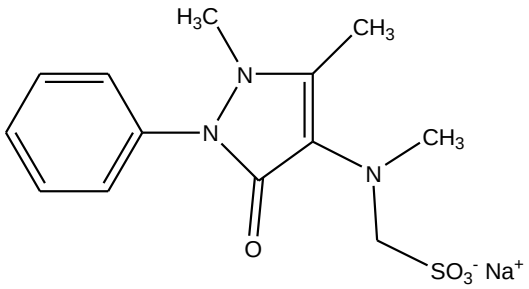


Fig.1. Structure of Analgin

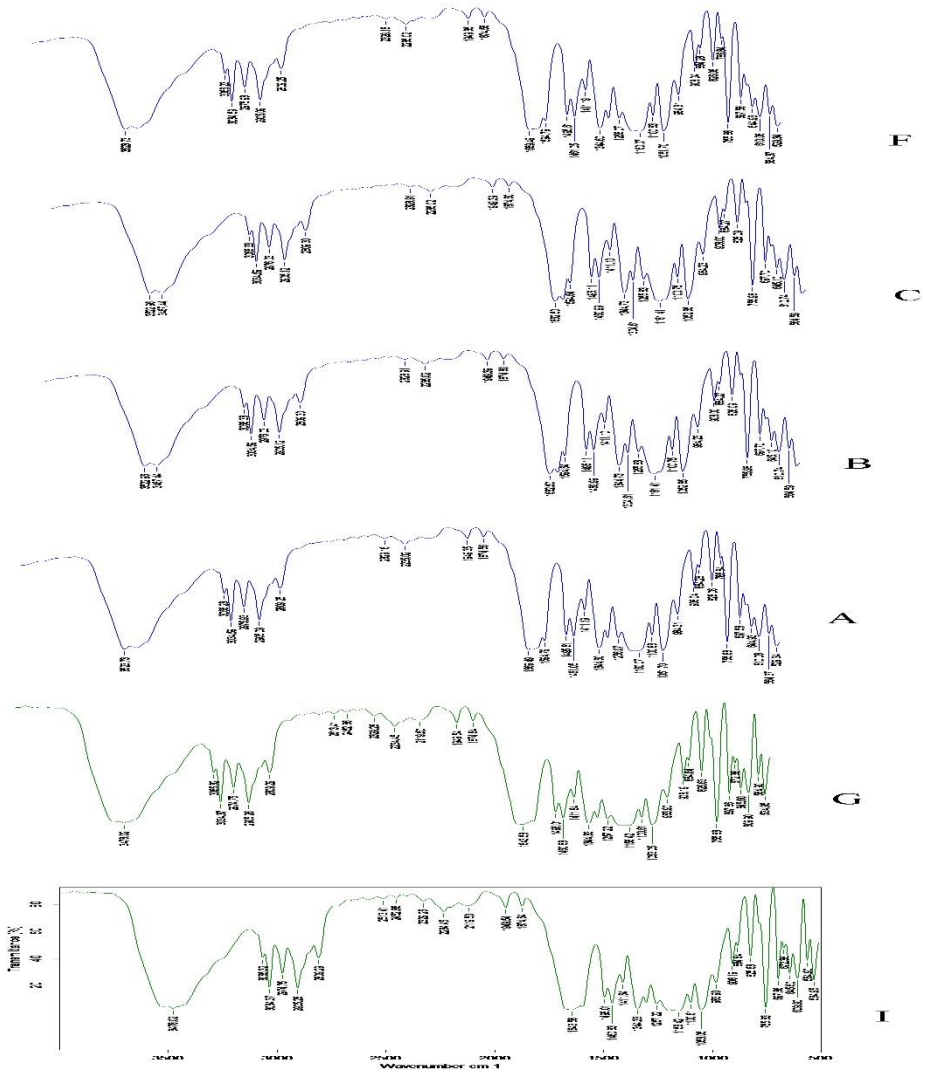


Fig.2. FTIR spectra of Input material (I) & other solid forms (A-G) of Analgin

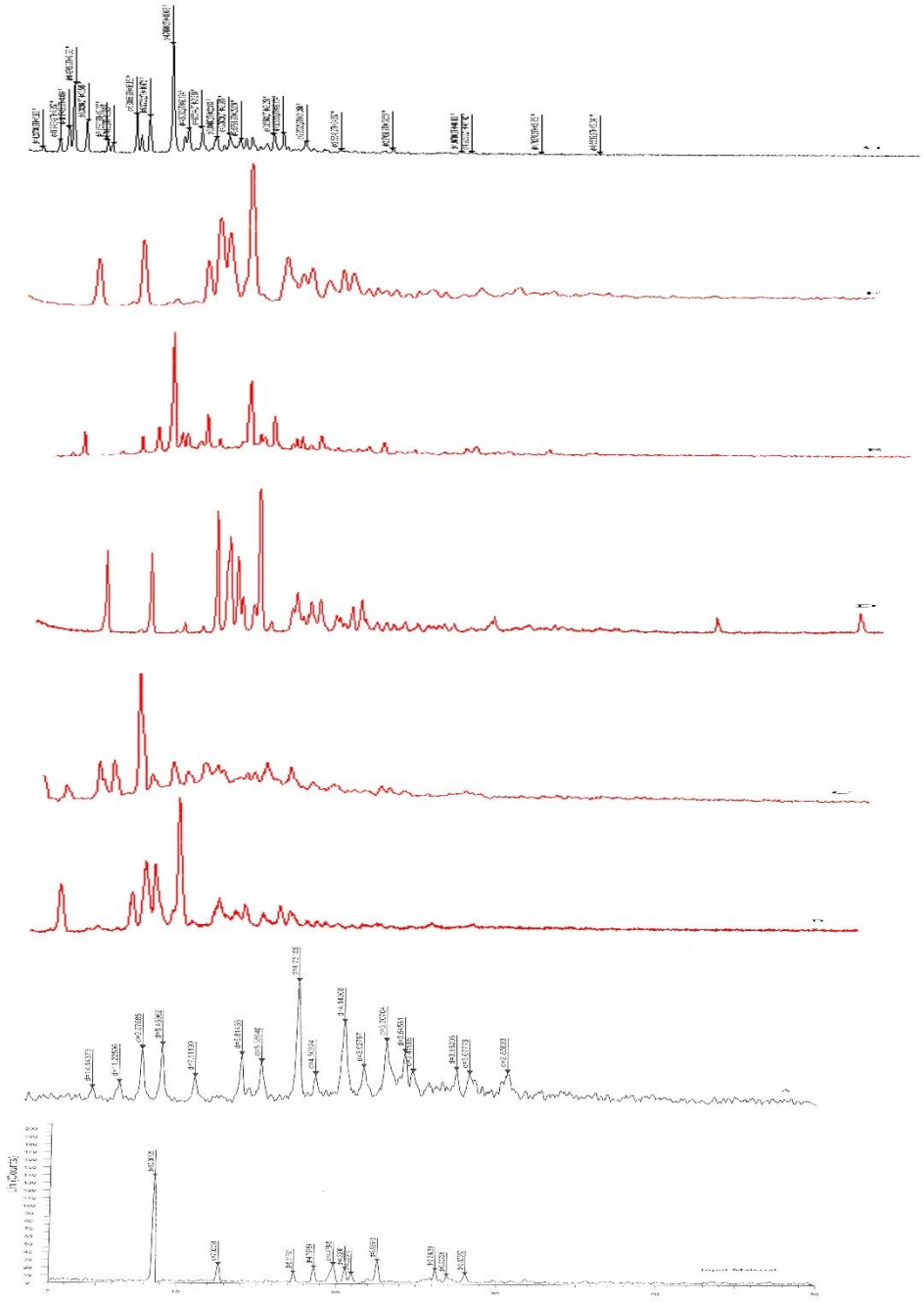


Fig.3. PXRD spectra of Input material (I) & other solid forms (A-G) of Analgin

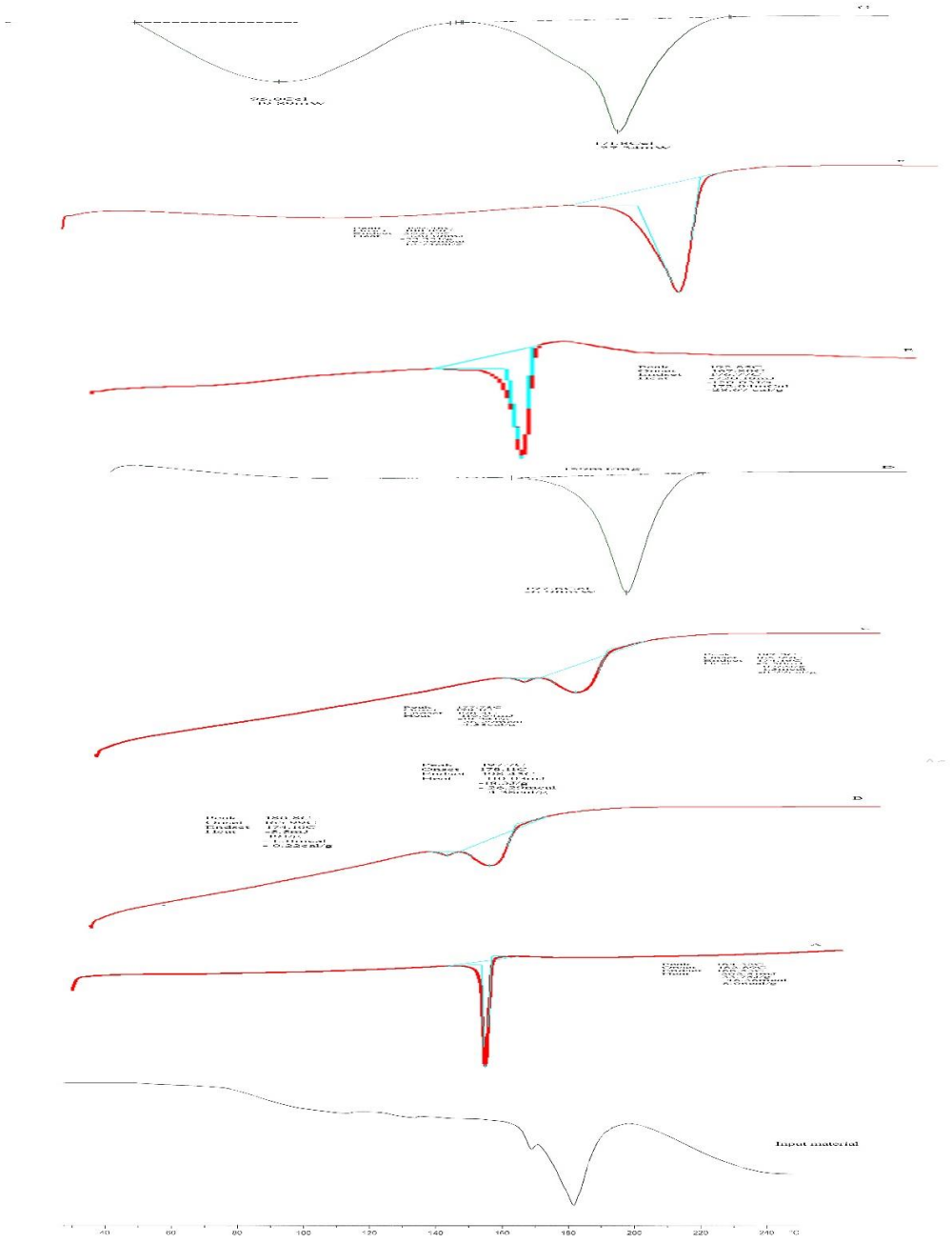
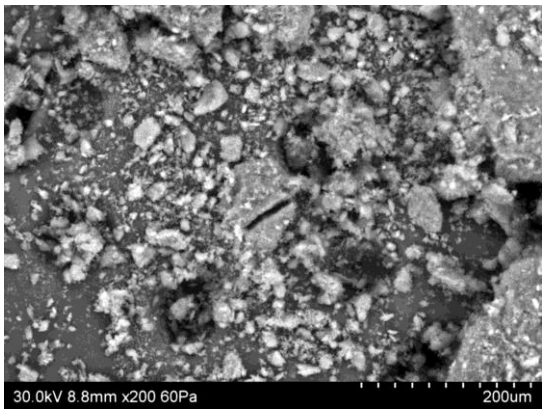
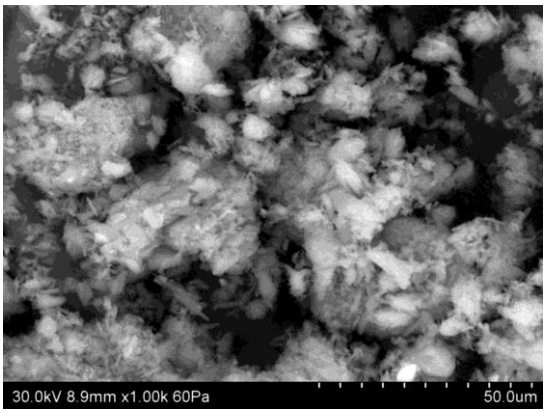


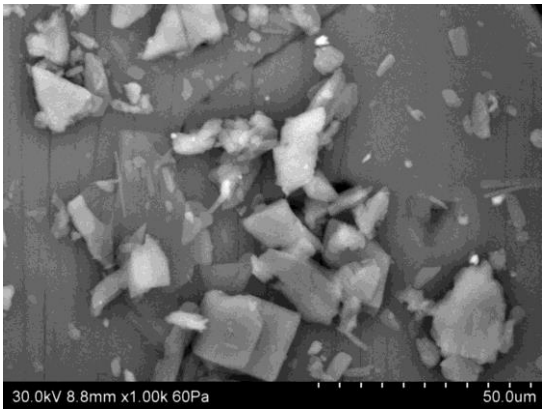
Fig.4. DSC spectra of Input material (I) & other solid forms (A-G) of Analgin



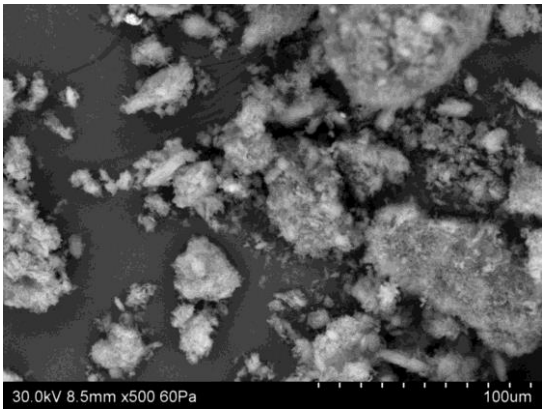
Input material of analgin



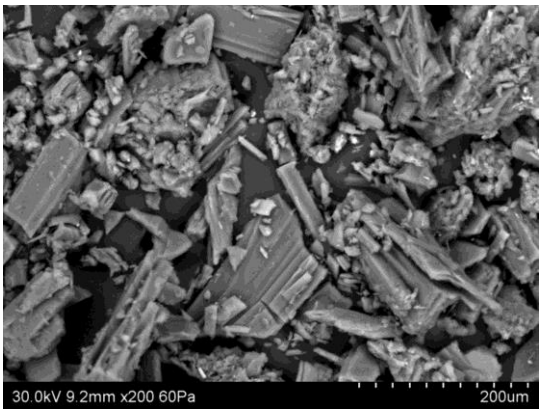
Solid form B



Solid form C



Solid form F



Solid form G

Fig.5. Scanning electron microscopic photographs of Input material (I) & other solid forms (A-G) of Analgin

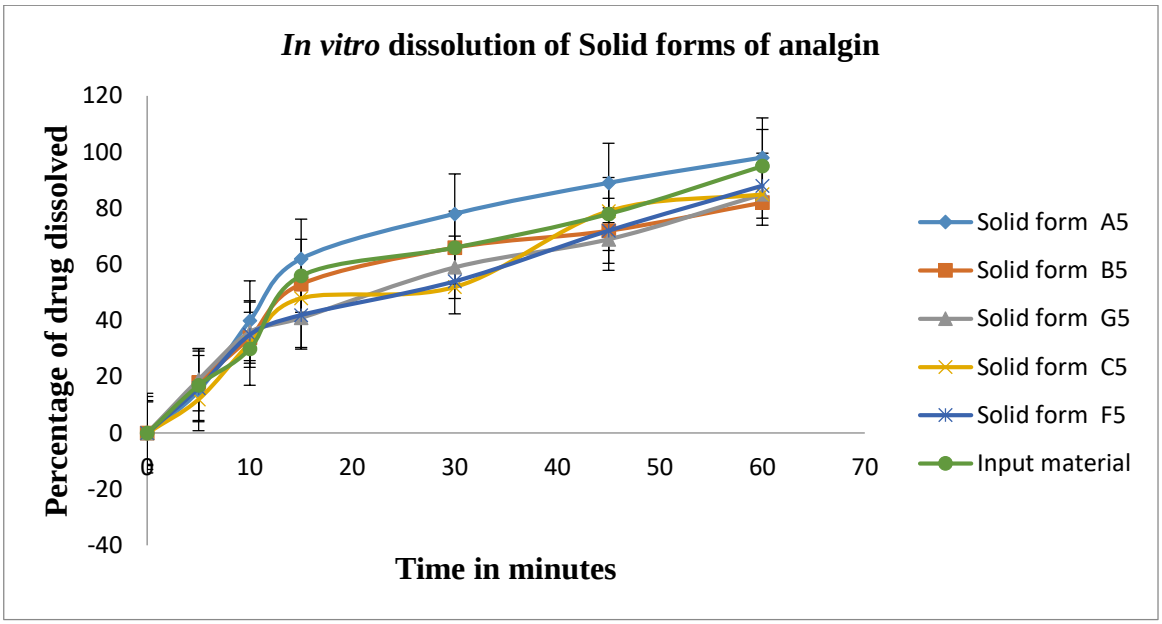


Fig.6. *In-vitro* dissolution of solid forms of Analgin

Table:1 Illustrating analgesic activity of Analgin with its solid state forms

S.No.	**Group**	**No. of Writhes in 30min (mean ± SEM)**	**Inhibition (%)**
1.	Normal	55	--
2.	Input material	38	56
3.	Solid form G	31	69