PREPARATION AND *IN VITRO* EVALUATION OF IBUPROFEN SPHERICAL AGGLOMERATES

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ABSTRACT

Ibuprofen, an anti inflammatory drug, is characterized by poor water solubility which limits the pharmacological effects. The present work is aimed to the study the effect of agglomeration on micromeritic properties and dissolution. Ibuprofen agglomerates were prepared by solvent change method using water, dichloromethane and DMSO as poor solvent, bridging liquid and good solvent respectively in the ratio of 57.5:12.5:30. Process variables such as amount of bridging liquid, mode of addition, temperature and stirring rate were optimized. SEM studies indicate that agglomerates produced were spherical and exhibit irregular shape. X-Ray Powder Diffraction spectra revealed the absence of polymorphism. DSC spectra showed no change in melting point indicating absence of crystal modification. The agglomerates exhibited improved solubility, dissolution rate and micromeritic properties compared to pure drug. Anti-inflammatory studies were conducted in Wistar strain male albino rats and ibuprofen agglomerates showed more significant activity than the pure drug which may be due to better absorption. Ulcerogenic potential study was carried out for pure ibuprofen and agglomerates. Better ulcerogenic potential was observed in ibuprofen agglomerates treated rats.

Key words: - Ibuprofen, Spherical agglomeration, anti-inflammatory activity, ulcerogenic potential.

IBUPROFEN KÜRESEL AGGLOMERATLARININ HAZIRLANMASI VE VİTRO DEĞERLENDİRMESİNDE

Soyut

Bir anti-inflamatuvar ilaç olan Ibuprofen, zayıf su çözünürlüğü ile karakterize olup, farmakolojik etkileri sınırlar. Bu çalışmanın amacı aglomerasyonun mikromeritik özellikler ve çözünme üzerindeki etkisini incelemektir. İbuprofen aglomeraları, su, diklorometan ve zayıf solvent olarak DMSO kullanılarak 57.5: 12.5: 30 oranında sırasıyla köprülü sıvı ve iyi çözücü kullanan çözücü değiştirme yöntemi ile hazırlandı. Köprü sıvısı miktarı, ilave modu, sıcaklık ve karıştırma oranı gibi proses değişkenleri optimize edildi. SEM çalışmaları, üretilen aglomeraların küresel olduğunu ve düzensiz şekli olduğunu göstermektedir. X-Işın Pudrası Difraksiyon spektrumu polimorfizmin yokluğunu ortaya koydu. DSC spektrumu erime noktasında kristal modifikasyonun olmadığını gösteren herhangi bir değişiklik göstermedi. Aglomerler, saf ilaca kıyasla gelişmiş çözünürlük, çözünme hızı ve mikromeritik özellikler sergiledi. Anti-inflamatuar çalışmalar Wistar suşu erkek albino sıçanlarında yürütülmüş ve ibuprofen aglomera'ları saf ilacın daha iyi emilimine bağlı olabileceğinden daha önemli bir aktivite gösterdi. Saf ibuprofen ve aglomera için ülserojenik potansiyel çalışması gerçekleştirildi. İbuprofen aglomeratları ile tedavi edilen sıçanlarda daha iyi ülserojenik potansiyel gözlenmiştir.

Anahtar kelimeler: - Ibuprofen, Küresel toplanma, anti-inflamatuar aktivite, ülserojenik potansiyel.

Introduction

Tablets are known to be the most popular dosage form of all pharmaceutical preparations for oral route of administration because of easy administration by patient, least content variability and great precision. Apart from these advantages, formulation and manufacturing of tablets is most convenient and easy process. One of important factors which influence the success of tablet formation is flowability and compressibility of materials. Direct compressibility is one of the best and economical techniques for manufacturing of tablets. This facilitates processing without the need of moisture, heat and involves small number of processing steps. But the technique depends on the flowability, particle size, the particle size distribution, bulk density and the compressibility of the crystalline drug substances (1-3). Most of the drugs like NSAIDs exhibiting poor compressibility and flowability and are not suitable for direct compression. For enhancing the flow properties and compressibility of drugs several methods have been introduced by researchers. In addition to the increasing the efficiency of the manufacturing process it is also important to increase bioavailability of the drug by improving the solubility of the bulk drug powder (4-6).

Spherical crystallization/agglomeration is a novel method to increase the bioavailability of the drug that inherently has poor aqueous solubility. It is a multiple unit process in which crystallization, agglomeration and spheronization can be carried out simultaneously in one step. The resultant crystals have characteristic shape, so the micromeritic properties such as flowability, packability and compressibility of the resultant crystals are dramatically improved so that direct tabletting or coating is possible without further processing steps like mixing, agglomeration and sieving (7-9).

Spherical crystallization/agglomeration is a process of formation of crystal aggregates held together by liquid bridges. The agglomerates are formed by agitating the crystals in a liquid suspension in presence of bridging liquid. The bridging liquid should be immiscible in the suspending medium, but capable of cementing the particles to be agglomerated (10). This technique can also be exploited to increase solubility, dissolution and hence bioavailability of poorly soluble drugs (11). These modifications allow for the practice of more efficient manufacturing methods that could save time and reduces economic risk.

Materials and methods

Materials

Ibuprofen was obtained as a gift sample from Granules India, Hyderabad, India. DMSO and Dichloromethane were procured from Qualigens fine chemicals, Chennai, India and SRL chemicals, Mumbai, India respectively. All chemicals and buffers used were of analytical grade.

Methods

Selection of liquid proportions for spherical agglomeration

A typical spherical agglomeration process required a good solvent, a poor solvent for a drug and a bridging liquid (3). The selection of these solvent depends on the miscibility of the solvents and solubility of the ibuprofen in individual solvents. Ternary phase diagram of DMSO, dichloromethane and water was constructed to select a suitable zone with appropriate ratio of three solvents for the preparation of spherical agglomerates.

Effect of amount of bridging liquid on the agglomeration

Bridging liquid used to cause the spherical agglomeration. It should be capable, not only for wetting the particle surface so as to form liquid bridges, but also for dissolving the sample particles

(12). Hence bridging liquid exerts marked influence on the yield and rate of agglomeration as well as on the strength of the resulting agglomerates. The rate determining step, in the spherical agglomeration, is when the bridging liquid is squeezed out of the pores of the initial flocs, later transformed into small aggregates or spherical crystals. Hence the amount of bridging liquid used is one of the critical operating variables (12). The amounts of solvents selected from ternary diagram were further modified and studied for influence of bridging liquid on the process and product. The effect of type of bridging liquid on the agglomeration was seen using dichloromethane, chloroform and cyclohexane as bridging liquids.

Effect of mode of addition of bridging liquid on the agglomeration

To investigate the effect mode of addition of bridging liquid, the bridging liquid was added drop wise and whole amount at a time separately (12).

Effect of agitation speed of the system on the agglomeration

The impact of agitation speed 300±25, 500±25 and 700±25 rpm was observed on the preparation of spherical agglomeration of ibuprofen (12).

Effect of agitation time of the system on the agglomeration

The impact of agitation time 20 min, 30 min and 60 min was observed on the preparation of spherical agglomeration of ibuprofen (12).

Effect of temperature on the agglomeration

The effect of different temperatures on formation of spherical agglomerates of ibuprofen was observed at 20±5°C, 40±5°C and 60±5°C (12).

Preparation of ibuprofen agglomerates

Ibuprofen (4 g) was dissolved in 30 ml of DMSO at 40°C and the solution was added to 57.5 ml of water which was maintained at 20°C under continuous stirring at 500 rpm with a propeller in mechanical stirrer. When fine crystals of ibuprofen began to precipitate, 12.5 ml of dichloromethane (bridging liquid) was added slowly drop wise. After 30 minutes of stirring, agglomerates thus obtained were separated by filtration and dried. The dried agglomerates were then stored in screw capped jar in a desiccator (13, 14).

Determination of drug content

Spherical agglomerates (50 mg) were triturated and dissolved in 250 ml of phosphate buffer pH 7.2. The solution was then filtered. After suitable dilution with phosphate buffer pH 7.2, solution was analysed spectrophotometrically (1601 A, Shimadzu corporation, Kyoto, Japan) at 221 nm (15).

Determination of melting point

Melting point of ibuprofen agglomerates were determined by placing the drug filled capillary tubes in digital melting point apparatus (CDMP-300, Contech Instruments Ltd., Mumbai, India) and melting point was noted (triplicates) and compared with the pure drug (16).

Scanning electron microscopic studies

The morphology of agglomerates was examined using Scanning Electron Microscopy (LEO 440I, Cambridge, England) operating at 15 KV (16).

Fourier Transform Infrared Spectroscopy

The FTIR spectral measurements were taken at ambient temperature using a Perkin Elmer Model 1600 (Minneapolis, Minnesota, USA). Samples were dispersed in KBr powder and the pellets were made by applying 5 ton pressure. FTIR spectra were obtained by powder diffuse reflectance on FTIR spectrophotometer (17, 18).

Differential scanning calorimetric studies

Differential scanning calorimetry (DSC-60, Shimadzu Corporation, Kyoto, Japan) studies were carried out to authenticate the formation of the spherical crystals or agglomerates. DSC was used after calibration with Indium and lead standards, samples of the crystals (3-5 mg) were heated (range 30-200°C) at 10°C/min in crimped aluminium pans under a nitrogen atmosphere. The enthalpy of fusion and melting point were automatically calculated (17, 18).

X-ray powder diffraction studies

X-ray powder diffraction patterns were obtained at room temperature using a Philips X' pert MPD diffractometer, with Cu as anode material and graphite monochromator, operated at a voltage of 40 Ma, 45 Kv. The process parameters used were set as scan step size of 0.0170 (2θ) (17, 18).

Micromeritic properties

Determination of angle of repose

Flow properties of the powder were evaluated by determining the static angle of repose. This was measured according to the fixed funnel and free standing cone method (19). A funnel with the end of the stem cut perpendicular to the axis of symmetry was secured with its tip 2.5 cm height, above graph paper placed on a flat horizontal surface. The powder was carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel (h). The mean diameter (D=2r) of the powder cone was determined and the tangent of the angle of repose was given by:

$\theta = \tan^{-1}(h/r)$ Where, θ is the repose angle; h is the height; r is the radius (19).

Measurement of compressibility index

Flowability of pure and agglomerated samples prepared was also assessed by Carr's Index (CI). The CI was calculated from the bulk density and tapped densities. Tapped density was determined by tapping the samples by using digital bulk density apparatus (Veego, Mumbai, India). The CI was calculated according to the following equation (20)

$$CI\ (\%) = [(\rho_b - \rho_t)/ \rho_b] \times 100$$

Where, ρ_t is tapped density and ρ_b is bulk density

In vitro dissolution Studies

The dissolution of ibuprofen pure drug and agglomerates were determined using USP dissolution apparatus type II (Electrolab, Mumbai, India). The dissolution medium used was 900 ml of gastric simulating fluid without enzymes (pH 1.2). Five ml of sample solutions were withdrawn at predetermined time intervals (10, 20, 30, 40, 50 and 60 min) and then filtered through whattman filter paper No.40 and same amount was replaced in the dissolution flask to maintain sink conditions. The amount of dissolved ibuprofen was analyzed spectrophotometrically at 221 nm (15). *In vitro* dissolution data was statistically analysed by one way ANOVA followed by turkey post hoc test for multiple comparison using graph pad prism. Differences were considered to be significant at a level of $p<0.05$.

In vivo evaluation of agglomerates

Evaluation of anti-inflammatory activity

Healthy male albino rats weighing between 150-200 gm were used for the study and individually maintained under standard conditions (12 hrs light and dark cycles, 25±2^{0}C, and 35-60 % humidity). Food and water were available, except that animals were fasted overnight before experiment although they continued to have free water. The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 557/02/c/ CPCSEA) was obtained. The animals were divided into three groups each containing 6 animals (21).

Group I: Inflammation induced and vehicle treated control.
Group II: Inflammation induced and ibuprofen suspension (pure ibuprofen 20 mg in 1% CMC) administered animals.
Group III: Inflammation induced and ibuprofen suspension (Ibuprofen agglomerates 20 mg in 1% CMC) administered animals.

Pure ibuprofen suspension (in 1% CMC) and ibuprofen agglomerates suspension (in 1% CMC) were given orally. All the animals were treated as per treatment schedule. After one hour paw edema was induced by injecting 50 µl of 1% w/v carrageenan into the sub planar region of the left hind paw. Paw volume was determined after one hour in all groups. Using digital plethysmometer (PLM-01 plus, Orchid Scientifics, India) percentage inhibition of edema was calculated after by using the following equation (22, 23).

% inhibition= (paw volume of control - paw volume of treated) × 100/ paw volume of control.

Anti inflammatory activity of ibuprofen and ibuprofen spherical agglomerates was statistically analysed by one way ANOVA followed by turkey post hoc test for multiple comparison using graph pad prism. Differences were considered to be significant at a level of $p<0.05$.

Assessment of ulcerogenic potential of ibuprofen agglomerates

Healthy male albino rats weighing between 150-200 gm were used for the study and individually maintained under standard conditions (12 hrs light and dark cycles, 25±2^{0}C, and 35-60 % humidity). The rats were fed with animal fed pellets and were given water (24, 25 and 26). The rats were divided into three groups each containing 6 animals that are controlled (group I), standard (group II) and test group (group III).

Group I: Vehicle treated control.
Group II: Inflammation induced and ibuprofen suspension (pure ibuprofen 20 mg in 1% CMC) administered animals.
Group III: Inflammation induced and ibuprofen suspension (Ibuprofen agglomerates 20 mg in 1% CMC) administered animals.

The animals were kept on fasting for 48 h before test, water was given. Pure ibuprofen suspension (in 1% CMC) and ibuprofen agglomerates suspension (in 1% CMC) were given orally. All the animals were treated as per treatment schedule. Then animals were sacrificed after 6 hr (25, 26). The stomachs were isolated and opened along the greater curvature, the mucosa was washed under slow running tap water and the number and size of ulceration was scored as per the method of Rao et al (24). pH of the stomach contents was determined by using broad range pH paper (27).

Severity Score:
0 = Normal colored stomach
0.5 = Red coloration of stomach
1 = Spot ulcer
1.5 = Hemorrhagic streaks
2 = Ulcers ≥ 3 but ≤5
3 = Ulcers > 5.

Results and Discussion

Selection of solvent proportions of spherical agglomeration

Spherical agglomerates of ibuprofen were prepared by simple agglomeration technique using a three solvent system (16, 18). A typical spherical agglomeration system involved a good solvent, a poor solvent for a drug and a bridging liquid. The selection of these solvents depends on the miscibility of the solvents and solubility of the drug in individual solvents. Accordingly acetone, DMSO and octanol are chosen as good solvents for ibuprofen. Water acts as non solvent. Dichloromethane, cyclohexane and chloroform are chosen as bridging liquid. Different combinations were tried by ternary phase diagram to produce optimum, solvent system. Similar trials were conducted by Saritha D et al (16) and Maghsoodi M et al (18). The drug solubility and mutual miscibility of solvent systems are examined and DMSO, dichloromethane and water selected as good solvent, bridging liquid and poor solvent respectively for the preparation of ibuprofen agglomerates.

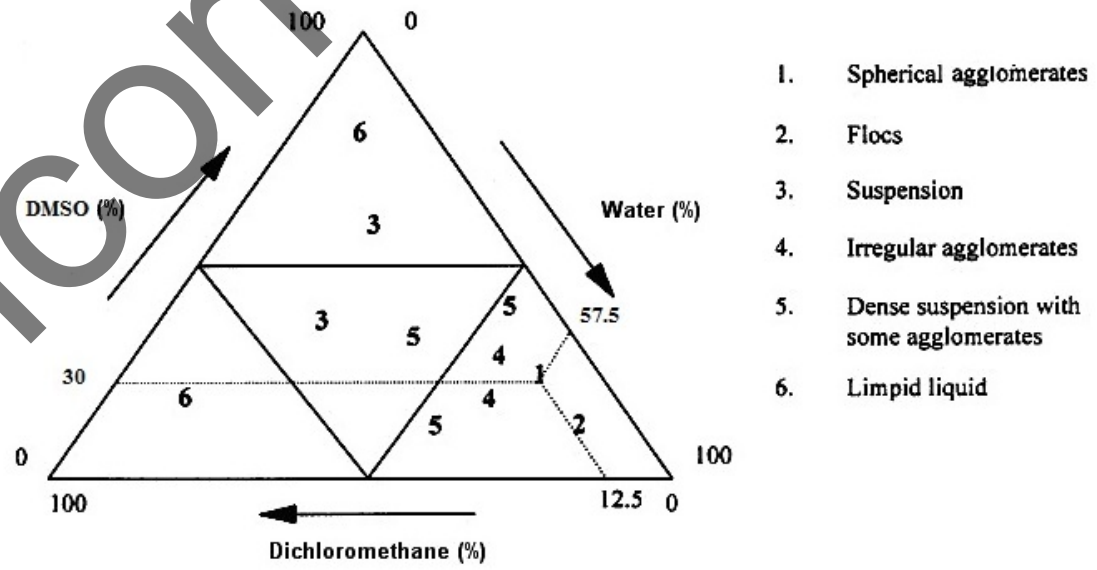


Figure 1: Ternary phase diagram of ibuprofen- DMSO/water/DCM

Table 1: Amount of solvents selected from the phase diagram used to prepare agglomerates of ibuprofen

DMSO (ml)	Dichloromethane (ml)	Water (ml)	% of bridging liquid
30	12.5	57.5	12.5
30	30	40	30
30	15	55	15
40	10	50	10

Preparation of spherical agglomerates

To select the best solvent composition, a ternary diagram was envisaged and DMSO: Dichloromethane: Water (30:12.5:57.5) were chosen for study (Table 1) and (Figure 1).

To optimize ibuprofen spherical agglomeration by DMSO: Dichloromethane: Water system, several parameters were considered: among these temperature difference between drug solution in DMSO and water, stirring time, stirring speed and amount of dichloromethane (bridging liquid). Bridging liquid used to cause the spherical agglomeration. And same was stated by Saritha D et al (16) and Maghsoodi M et al (18, 28). It should be capable, not only of wetting the particle surface so as to form liquid bridges, but also of dissolving the sample particles and same phenomenon was explained by Xia D et al (29). Hence bridging liquid exerts influence on the yield and rate of agglomeration as well as strength of the formed agglomerates. Same mechanism was explained by Maghsoodi D et al (30).

Agglomerates were formed by agitating the crystals in a liquid suspension and adding a bridging liquid, which preferentially wets the crystal surface to cause binding. The addition of bridging liquid (dichloromethane) promotes the formation of liquid bridges between the drug crystals to form agglomerates. To optimize ibuprofen spherical agglomeration by DMSO/dichloromethane/water system, other process parameters were considered such as amount and mode of addition of bridging liquid, stirring speed and time, temperature and similar condition was followed by Saritha D et al (16). The diameter of agglomerates was found to increase with increasing amount of bridging liquid in the medium due to excessive bridging liquid on the surface for coalescence. Size of agglomerates is very much dependent on the degree of agitation and same was reported by Saritha D et al (16). Due to agitation droplets will be formed in the agglomeration medium and it induces movement of droplets from in to out. The intensity of this internal circulation depends on the speed. At lower stirring rate (300 rpm) reduces the possibility of obtaining agglomerates due to slow circulation of the droplets in the medium and slight collision between the droplets, similar procedure followed by Kulakarni PK et al and Subhash Chandra Bose et al (31, 32). At optimum speed (500 rpm) more compact and dense agglomerates were obtained (Table 2). Higher speed (700 rpm) induces agglomerate destruction due to more impact energy for collision due to increased turbulence resulting in formation of agglomerates with irregular shape and same results were observed by Subhash Chandra Bose et al (32). So, 500 rpm was selected for preparation of ibuprofen agglomerates, similar condition was followed by Saritha D et al and Kulakarni PK et al (16, 31). The temperature of solvent system was found to have pronounced effect on the process of agglomeration. Agglomeration was not observed when the process was carried out at 20±5°C. It could be due to reduced solubility of drug in the solvent system. When the temperature was increased to 60±5°C very large agglomerates were produced due to enhanced solubility (Saturation of the drug in the medium). Optimum agglomeration was achieved at 40±5°C due to optimum solubility of the drug. So, 40±5°C was selected for preparation of ibuprofen agglomerates, similar procedure followed by

Kulakarni PK et al and Subhash Chandra Bose et al (31, 32). Addition of bridging liquid plays vital role in formation off agglomerates. When the bridging liquid was added at a time agglomerates were of irregular geometry which may be due to its localization and hence its unavailability for efficient agglomeration, same theory was reported by Subhash Chandra Bose et al (32). Dropwise addition with continuous agitation resulted in agglomerates of regular geometry which can be attributed to uniform distribution of bridging liquid, same theory was reported by Subhash Chandra Bose et al (32). So, dropwise addition selected for preparation of ibuprofen agglomerates.

Table 2: Optimization of process variables for ibuprofen spherical agglomeration

S. No	Parameter	Variables	Observations
1	Concentration of bridging liquid (dichloromethane)	10%*	No agglomeration
		12.5%*	Agglomeration
		15%*	No agglomeration
2	Agitation time	20 min*	No agglomeration
		30 min*	Agglomeration
		1hr min*	Fine particles are formed

***At 40°C±5°C, 500±25 rpm and drop wise addition of bridging liquid**

From the above study ibuprofen agglomerates were prepared by using following conditions

- ✓ DMSO: Dichloromethane: Water : 30:12.5:57.5
- ✓ Mode of addition of DCM : Dropwise
- ✓ Agitation speed : 500±25 rpm
- ✓ Agitation time : 30 min
- ✓ Temperature : 40°C±5°C

Drug content

The drug content was determined in triplicates and was found to be in the range of 98.94±1.73-99±0.93%.

Melting Point

Melting point of the ibuprofen and spherical agglomerates of ibuprofen are 72°C and 71°C respectively. It was observed that no change in melting points of pure and prepared agglomerates. Thus, formation of polymorphs was overruled as crystal habit will not change the melting point. Similar findings are reported by Kumar S et al (33).

Scanning electron microscopic studies

The crystals habits and surface features were examined by using scanning electron microscopic studies. The pure ibuprofen was in the form of rod like particles and agglomerates were having round shaped particles of irregular shape (Figure 2A & 2B). Upon high magnification it was further observed that surfaces are not smooth and no liquid bridges found in between two particles.

Thus the produced clusters may be spherical agglomerates. Similar observations were reported by Viswanathan et al (34) and Kulakarni et al (35) spherical agglomerates of ibuprofen prepared by neutralization method.

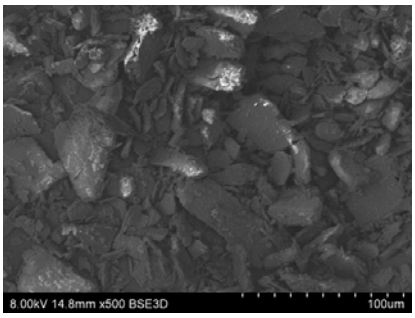


Figure 2A: SEM of pure ibuprofen

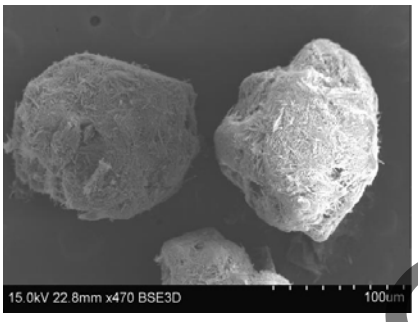


Figure 2B: SEM of agglomerates

Fourier Transform Infrared Spectroscopy studies

FTIR spectra (Figure 3) showed the characteristic absorption peaks of ibuprofen (According to Klaus Florey 36) at 1260 (CH_3 stretching vibration), 3000 (CO=OH stretch), 1710-1665 (C=O stretch) and 1600-1585 (C-C stretch) wave numbers which indicates the presence of alkane, carboxylic, ketone group and ring structure respectively in both pure and agglomerates of ibuprofen similar findings were observed by Kachrimanis K et al (37). From the spectral data it can be concluded that there is not much significant difference in spectra and absence of polymorphs. Similar observations were reported by Kulakarni PK et al (38).

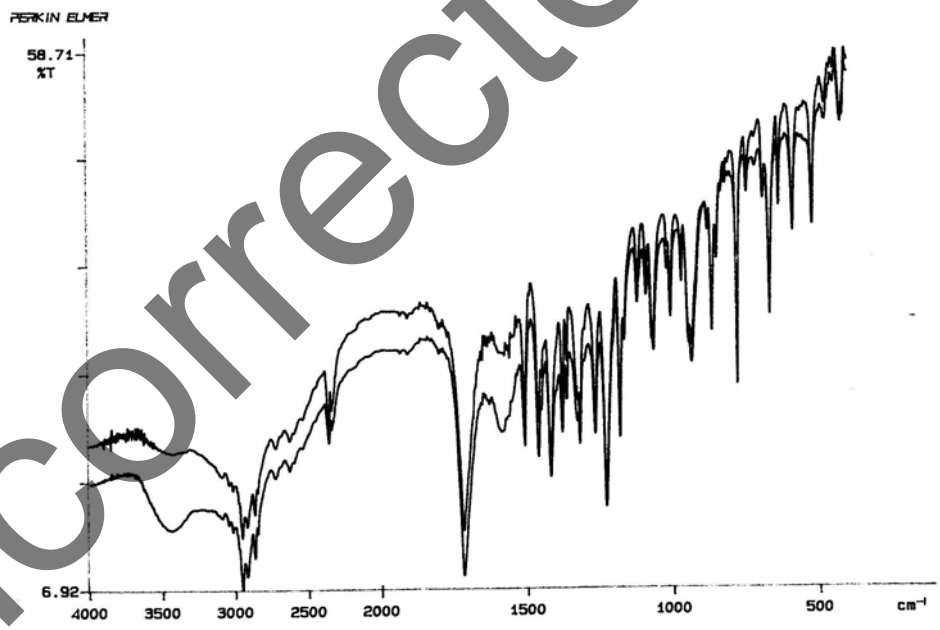


Figure 3: FTIR spectra of ibuprofen and its agglomerates

Differential scanning calorimetric studies

DSC thermograms of pure and ibuprofen crystals are illustrated in figure 4. The DSC pattern of pure ibuprofen and agglomerates showed a sharp endothermic peak at 77.51ºC and 77.80ºC respectively corresponding to its melting point. Sharp melting point with flat base line, which

indicated that the material was not affected by hydration, solvation, polymorphic transition and in addition there was no interaction of drug with solvents. Similar observations were reported by Kulakarni et al (35, 38), Kachrimanis K et al (37) and Atmaram P. Pawar et al (39).

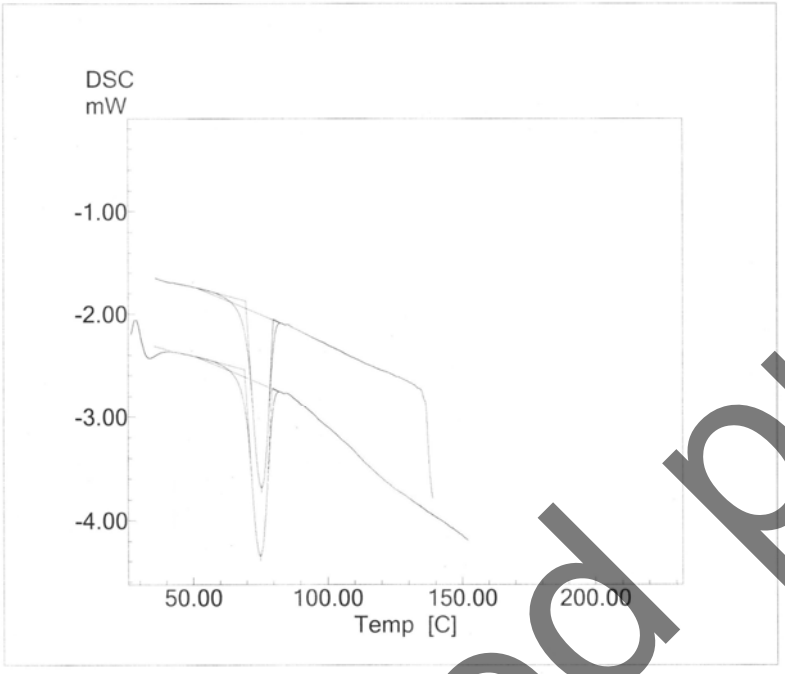


Figure 4: DSC thermograms of ibuprofen and its agglomerates

X-ray powder diffraction studies

XRD spectra of the prepared agglomerates do not show any significant change in crystal structure and crystal habit when compared to pure ibuprofen. The small differences in the relative intensities of their peaks (Figure 5A & 5B) at the respective 2θ values (Table 3) may be attributed to differences in the particle size or crystallinity of the sample. Similar reports observed by Kulakarni et al (35, 38), Atmaram P. Pawar et al (39) and Rasenack et al (40). It indicates that ibuprofen does not undergo any polymorphic changes during the process of spherical agglomeration.

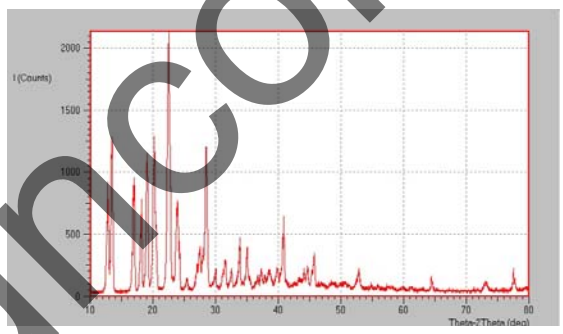


Fig 5A: X-ray diffraction spectra of ibuprofen

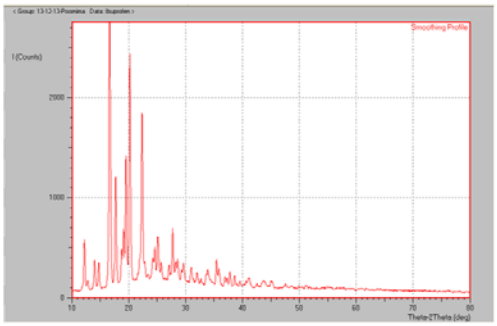


Fig 5B: X –Ray diffraction spectra of spherical agglomerates of ibuprofen

Table 3: Comparative values of 2θ & d values for pure drug and ibuprofen agglomerate

S. No	Pure drug		Ibuprofen crystals	
	Angle (2θ°)	D value (A°)	Angle (2θ°)	D value (A°)
1.	12.134	7.2877	12.222	7.23616
2.	14.546	6.0846	14.736	6.00634
3.	16.503	5.3671	16.66	5.31702
4.	17.546	5.0503	17.705	5.00559
5.	18.695	4.724	18.74	4.7313
6.	19.348	4.5838	19.52	4.5439

Micromeritic properties

The micromeritic properties like bulk density, tapped density, angle of repose and Carr's index were determined and shown in table 4. The bulk and tapped densities of the spherical agglomerates were lower than the corresponding value of the pure sample, due to higher particle size and sphericiy. The lower density is likely to be related to the intraparticle porosity and hence reduced in bulk density of the treated samples indicates a greater porosity within the agglomerated particles, similar finding were observed by Maghsoodi M et al and Gupta VR et al (30, 41). Carr's index for agglomerates was found to be lower when compared to pure drug. This may be due to the formation of agglomerates. Fine particles having high surface to mass ratios are more cohesive than coarser particles, hence more influenced by gravitational force and similar finding were observed by Kumar et al and Viswanatha et al (33, 34). Decreased values of Carr's index for agglomerates have better packability indicate that they might be suitable for direct tabletting, similar finding were observed by Maghsoodi M et al and Gupta VR et al (30, 41). Flow properties of the crystals were reflected by angle of repose. It was found that angle of repose of agglomerates were decreased when compared to pure ibuprofen. Such decreased value indicates improvement in flowability, free flow of powder mass in comparison to the pure drug and similar finding were observed by Maghsoodi M et al, Kumar et al, Viswanatha et al and Gupta VR et al (30, 33, 34, 41). Flowability of the powder was found to be decreased due to their formation of agglomerates. Here the value of compressibility and angle of repose represents excellent and good flow of agglomerates respectively when compared to that of pure ibuprofen having very poor flow.

Table 4: Micromeritic properties of pure ibuprofen and agglomerates

S. No	Sample	Bulk density (g/cm^3)	Tapped density (g/cm^3)	CI (%)	Angle of repose (°)
1.	Pure ibuprofen	0.833±2.31	0.55±1.9	33.9	40.6
2.	Ibuprofen Agglomerates	0.641±1.78	0.588±2.01	8.26	25.1

In vitro dissolution studies of agglomerates

The release of pure ibuprofen is less than that of ibuprofen agglomerates. Dissolution profile of agglomerates showed significant ($p<0.05$) difference when compared with pure drug. The reason for faster dissolution may be due to good wettability of the agglomerates, thus it get easily dissolved in the dissolution fluid and same was reported by Jbilou M et al and Di Martino et al (42, 43). Ibuprofen agglomerates showed a better dissolution than the pure drug which may lead to increased absorption rate and bioavailability which is well correlated with the findings of Atmaram et al, Di Jbilou M et al, Martino et al and Sano A et al (39, 42, 43, 44).

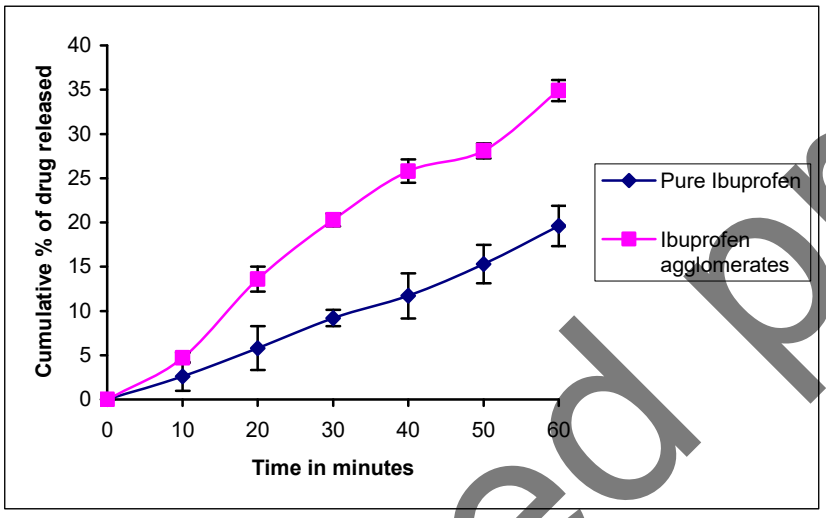


Figure 6: *In vitro* release profile of ibuprofen and its agglomerates

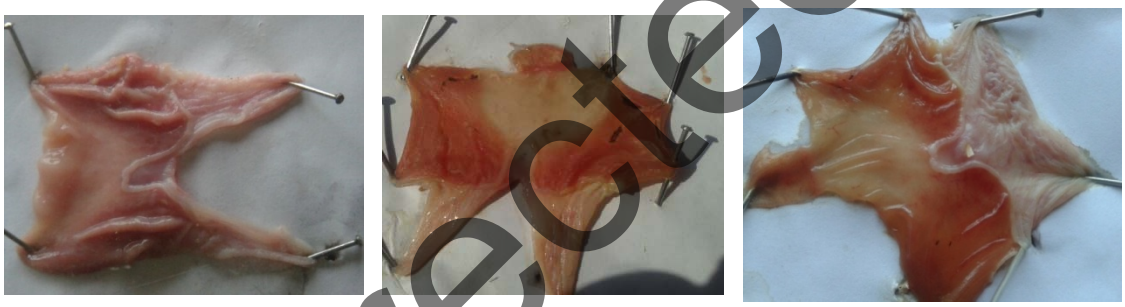
Evaluation of anti-inflammatory activity

Anti-inflammatory activity was carried out for pure ibuprofen and agglomerates. Table 5 shows the results of paw edema and percentage inhibition of carrageenan-induced paw edema in rats treated with pure ibuprofen and agglomerates. An extremely significant ($p<0.001$) inhibition of carrageenan induced paw edema was observed in animals treated with ibuprofen agglomerates in comparison with control during the entire 5 h duration of the study, significant ($p<0.05$) inhibition of carrageenan induced paw edema was observed in animals treated with ibuprofen agglomerates in comparison with ibuprofen pure drug during the entire 5 h duration of the study (Table 5). This may be due to increased dissolution of agglomerates over pure drug, leading to better absorption and onset of action of drug. Also ibuprofen is a propionic acid derivative its dissociation constant is 5.3, so it get easily absorbed in the acidic pH of stomach as it is in ionised form in acidic pH, reported by Damineni Saritha et al (22). Moreover due to high wettability of agglomerates solubility of agglomerates was increased. Hence, agglomerates showed better anti-inflammatory activity over the pure drug. Therefore, the results of the *in vivo* studies clearly demonstrate that the ibuprofen agglomerates showed better anti-inflammatory activity over the pure drug, thus confirming the better therapeutic efficacy and same phenomenon was reported by Saritha D et al and Liles JH et al (16, 23).

Table 5: Anti-inflammatory activity of pure and ibuprofen agglomerates

Group	Treatment	Time (hr)	Paw volume mean	% inhibition
Control	1% CMC suspension	5	0.51	--
Standard	20 mg (100 mg/Kg) pure Ibuprofen	5	0.29	43.13
Test	20 mg(100 mg/Kg) Ibuprofen agglomerates	5	0.14	72.54

Ulcerogenic potential study was carried out for pure ibuprofen and agglomerates. Pure ibuprofen produced haemorrhagic steaks with high intensity than the agglomerates. Pure ibuprofen treated rat's stomach shows haemorrhagic steaks with high intensity (severity score-1.5); whereas agglomerates treated rat's stomach shows red coloration (severity score-0.5) (Figure 6). No ulcers were observed on stomach of rats which are kept control. From ulcerogenic potential study it can be concluded that ibuprofen agglomerates showed better ulcerogenic potential activity which may be due to better absorption and bioavailability of ibuprofen. Similar results were reported by Nagaraju et al (27).



Control | **Treated with pure ibuprofen** | **Treated with ibuprofen agglomerates**

Figure 6: Photographs of isolated stomach of rats for ulcerogenic potential studies.

Conclusion

Ibuprofen agglomerates that were prepared by simple spherical crystallization technique exhibited improved micromeritic properties and dissolution rate. FTIR, DSC and XRD study showed that there is no change in the crystal structure and polymorphism was not occurred. Due to the significant improvement of micromeritic properties, this technique may be used for formulation of ibuprofen tablets by direct compression method. The agglomerates were found to be having better anti-inflammatory activity in the rats when compared to pure drug due to improved solubility.

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