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FORMULATION AND CHARACTERIZATION OF INCLUSION COMPLEX OF ACETYL SALICYLIC ACID (ASPIRIN) AND β- CYCLODEXTRIN BY SOLVENT EVAPORATION & ITS COMPARATIVE STUDY TO ASPIRIN ALONE

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ABSTRACT

The aim of present study was to determine whether pharmaceutical and pharmacological activity of aspirin would enhance in form of inclusion complex with β–cyclodextrin or not. Aspirin (Acetyl salicylic acid) a non steroidal anti-inflammatory drug, was formulated in to inclusion complex with β–cyclodextrin (β–CD) by solvent evaporation. The phase-solubility curve was A_L type and K_{st} was found to be 127.8 M^{-1}. FT-IR and microscopic examinatinos were revealed confirmation of inclusion formation. Solubility and permeability of aspirin was enhanced in presence of β–CD. Similarly the pharmacological activity i.e. analgesic and anti-inflammatory activity were also enhanced in presence of β – CD.

INTRODUCTION: Cyclodextrins (CDs) are cylindrical oligosaccharides with a lipophilic central cavity and hydrophilic outer surface. In aqueous solutions, CDs are able to form inclusion complexes with many drugs by taking up some lipophilic part of the drug molecules into cavity [1]. When used in pharmaceutical formulations, they can improve the aqueous solubility, stability, dissolution rate, bioavailability and/or local tolerance of drugs [2, 3].

Cyclodextrin inclusion is a molecular phenomenon in which usually only one guest molecule interacts with the cavity of a cyclodextrin molecule to become entrapped and form a stable association. The internal surface of cavity is hydrophobic and external is hydrophilic; this is due to the arrangement of hydroxyl group within the molecule. Cyclodextrin is to enhance aqueous solubility of drugs through inclusion complexation [4-6].

Acetylsalicylic acid (Aspirin) used for anti-inflammatory property and it is also an anti-pyretic drug. It acts by inhibition of prostaglandin synthesis and COX-II inhibitor. Aspirin induces a long-lasting functional defect in platelets. Orally administered aspirin requires high and frequent dosing because it undergoes extensive pre-systemic metabolism. Also, long term and chronic oral aspirin is associated with serious gastrointestinal side-effects [7]. So, if the solubility and bioavailability of the aspirin can be increased, it will reduce the gastrointestinal side-effects. Preparation of aspirin β -cyclodextrin inclusion complexes was to increase the solubility and reduce the irritation.

The aim of a present study was to compare solubility and permeability of aspirin alone, in physical mixture with β-Cyclodextrin (β -CD) and in inclusion complex with β –CD.

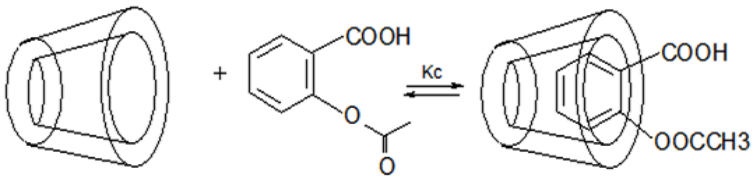


FIGURE 1:- THE PROPOSED MODEL OF ASPIRIN/CYCLODEXTRIN INCLUSION COMPLEX FORMATION

MATERIALS AND METHODS:

Materials: β-cyclodextrin was procured from s d Fine Chemicals (Mumbai). Aspirin IP was gifted from Alta laboratories limited (Raigad).

Equipment: Analytical Balance (Shimandzu Corporation, Japan- AUX 220), Silverson Homogenizer (Remi Industries, RQ-127, Mumbai), FT-IR (Perkin Elmer, Spectrum RX1), UV- Spectrophotometer (Perkin Elmer, Lamda 25), Digital Microscope (Motic, China group B_1 advanced Series) and Franz-Diffusion cell were used for purpose of this study at School of Pharmacy & Technology Management, Mumbai (SPTM).

Methods: Characterization of aspirin and β –CD were carried out. For that Particle size, FT-IR, Solubility and melting point determinations were performed for Aspirin. Similarly for β–CD FT-IR, solubility and LOD were performed. It was compared with certificate of analysis.

1. **Phase Solubility Study:** The phase-solubility studies were carried out according to the method reported by Higuchi and Connors (1965). Calibration Curve of Aspirin in pH 4.5 acetate buffer was prepared. Aspirin solution in range of 30-250 ug/ml in pH 4.5 acetate buffer was prepared. Absorbance at 273 nm was measured. (Refer **figure 3**). For Phase solubility study 10 mg of Aspirin was added to 100 mL volumetric flask containing pH 4.5 acetate buffer of various amount of β–CD (0.6 to 5 mg). These flasks were sonicated for 4 hrs. These solutions were centrifuged at 5000 rpm for 15 min & filtered through 0.45 µ filter and absorbances were taken. K_{st} was calculated from the straight line portion of the phase solubility diagram according to the following equation

K_{st} = Slope/(1-slpoe)

2. **Formulation of inclusion complex (IC) between Aspirin & β– Cyclodextrin (β–CD):**

a. ***Physical Mixture***: - Aspirin and β –Cyclodextrin were weighed in equal proportion (1:1 molar ration) and crushed using glass mortar pestle.

b. **Solvent Evaporation**: Aspirin was dissolved in ethanol. In to this β–Cyclodextrin was added. (1:1 molar ration). This mixture was stirred continuously. Solvent was evaporated using water bath at 90°c. Dry power was obtained. This inclusion complex was characterized, tested for solubility and permeability.

3. **Characterization of Inclusion complex:**

a. **Microscopy:** Physical mixture of aspirin, β –CD and Inclusion complex were examined under digital microscope. It has represented presence of both as shown in figure 6.

b. **Solubility**: Absorbance of 100 ppm solution of Aspirin, Physical Mixture of Asp + β- CD and its Inclusion complexes were taken at 273 nm and solubility of aspirin in inclusion complex, physical mixture and alone were compared.

c. **FT-IR study:** FT-IR spectra of pure Aspirin, ß - cyclodextrin, with its inclusion complex were obtained by Perkin-Elmer FT-IR spectro-photometer using potassium bromide (KBr) disc method. The sample was scanned from 4,000 to 400 cm^{-1}.

d. **Permeability Study:** Dialysis membrane (Himedia-60 LA 390- 5MT) was kept in water in order to make it hydrate. This membrane was kept in between donor & acceptor receptor. In donor compartment 10 mg of aspirin or Inclusion complex equivalent to 10 mg of aspirin was added. Acceptor compartment was filled with 17 mL of pH 4.5 acetate buffer. This unit was kept on magnetic stirrer at 32 °c. Sample after each time point was withdrawn from acceptor ad in situ condition was maintained. Absorbance of sample was taken.

e. **Animal Study:** The experimental protocol was approved by the institutional animal ethics committee and the care of laboratory animals was taken as per the guidelines of committee for the purpose of control and supervision of experiments on animals, (CPCSEA), Govt. of India.

i. Analgesic activity: The mice (Swiss Albino mice, Female from Haffkine Institute, Lower Parel) were grouped and acclimatized. Different groups of the mice received either water, Std. Aspirin, Aspirin-β cyclodextrin complex each containing a dose of

Aspirin equivalent to 30 mg/kg, 45 minutes before the intraperitoneal injection of 0.1 ml of 0.6% solution of acetic acid I.P. The mice were observed for 5, 10, 15, 20, 25 minutes in individual chambers and the number of writhings were calculated.

ii. **Anti-inflammatory activity:** Female Wistar rats with a body weight of 120-150 gms were starved overnight. To insure uniform hydration, rats receive 3 ml of water by stomach tube (controls) or the Std. Aspirin, Aspirin- β-cyclodextrin inclusion complex containing an Aspirin dose equivalent of 100 mg each. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan in the plantar side of the left hind paw. The paw thickness was measured immediately after injection, again 1 hr 2, 4 and 6 hrs after challenge. Readings were recorded and % inhibition is calculated.

Calculation of Percent Inhibition:

% Inhibition =

$$\frac{\text{Increase in Paw Thickness in Control - Increase in Paw Thickness in Test}}{\text{Increase in Paw Thickness in control}} \times 100$$

RESULT AND DISCUSSION: Aspirin was white crystalline powder having higher solubility in ethanol and methanol, soluble in chloroform and pH 4.5 acetate buffer. Melting point was found to be 132 – 134°C. Mean particle size was found to be 130 µ. Its particle size distribution is represented in **figure 2**. From the IR spectra, structure of Aspirin was confirmed (**Table 1**). It was compared with IR spectra given in IP 2010.

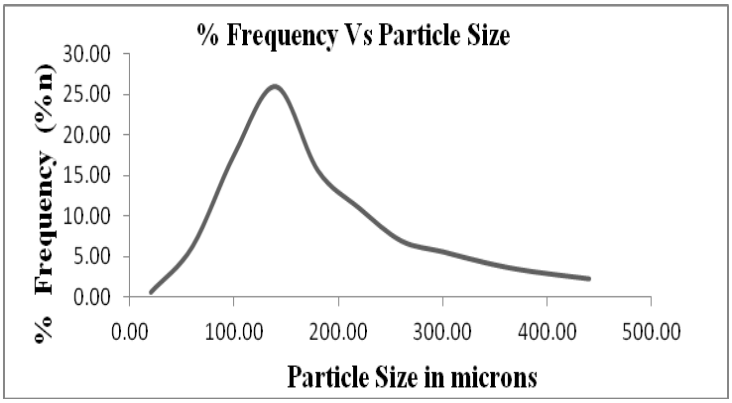


FIGURE 2: %FREQUENCY VS PARTICLE SIZE OF ASPIRIN IN MICRONS

TABLE 1: FUNCTIONAL GROUPS OF ASPIRIN FROM FT-IR STUDY

Frequency (cm $^{-1}$)	Functional group & Vibration
2287.28	: Carboxyl OH
1754.52	: Vinyl ester C=O
1690.70	: Aromatic acid C=O
1605.89, 1575.71, 1484.07	: aromatic C=C stretch
1220.57, 1189.61	: C-O (acid and ester)
755.36	: ortho subst. Phenyl C-H bending

B-CD was found to be soluble in Propylene Glycol (1 part of β-Cyclodextrin was found to be soluble in 175 parts of Propylene Glycol), in water at 30°C – Soluble (1 part in 40 parts of water). It was soluble in 0.1 N HCl (pH 1.23), pH 4.52 acetate buffer, pH 6.80 Phosphate buffer & 0.1 N NaOH. It was found that β-Cyclodextrin was practically insoluble in acetone, ethanol (99%), and methanol. Functional group observed from FT-IR study was represented in Table no.2. % loss was found to be 5.9 %. pH was found to be 5.814 which is in between 5 to 8.

TABLE 2: FUNCTIONAL GROUPS OF B –CD FROM FT-IR STUDY

Frequency (cm $^{-1}$)	Functional group & Vibration
3390.7	: O-H Stretching
2922.2	: C-H Stretching
1651.5	: H-O-H bending
1158.4	: C-O Stretching of COOH
1029.1	: C-O-C Bending

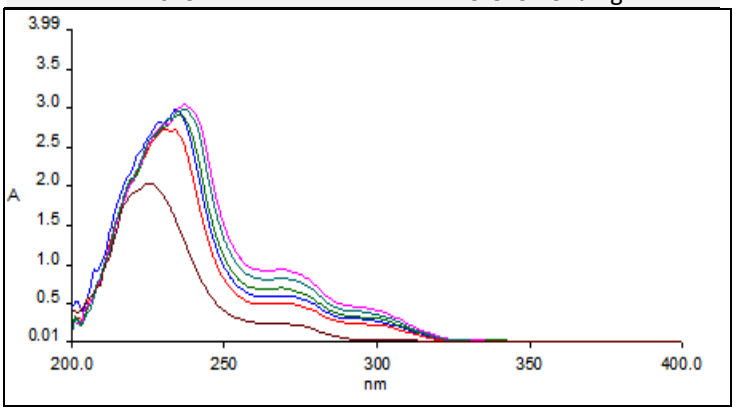


FIGURE 3: ABSORBANCE SPECTRA OF ASPIRIN IN pH 4.5 ACETATE BUFFER (30-250 µg/ml)

From phase solubility curve K_{st} was found to be 127.8 M^{-1}. Since curve was A_L type (figure 5) means as concentration of β–CD increases the solubility will also increased. It suggests that 1:1 ration of aspirin and Betadex is most probable to increase the solubility of aspirin. So, for preparation of aspirin & β– Cyclodextrin Inclusion Complex 1:1 ration was selected.

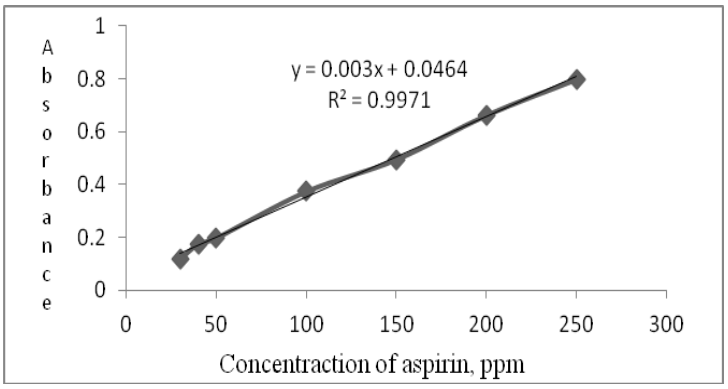


FIGURE 4: STANDARD CALIBRATION CURVE OF ASPIRIN IN pH 4.5 ACETATE BUFFER

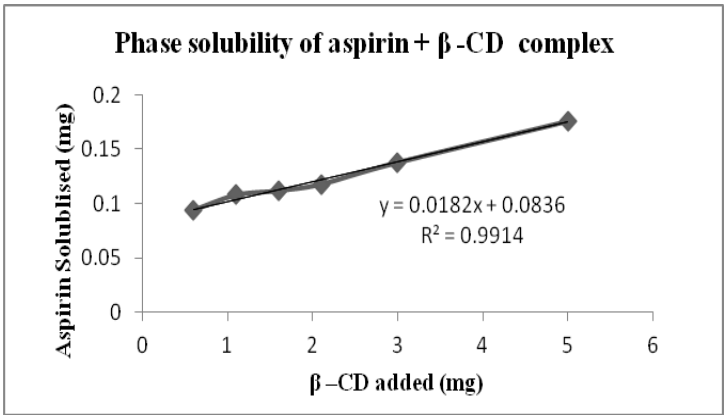
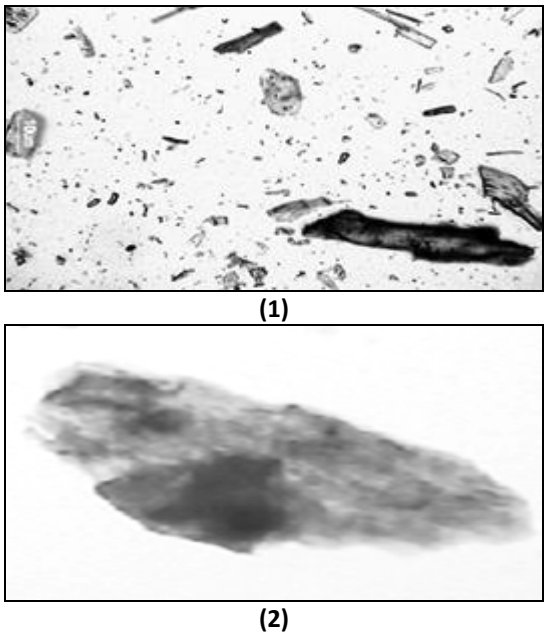


FIGURE 5: PHASE SOLUBILITY CURVE OF ASPIRIN & β–CD IC. (A_L TYPE)

Microscopic examination represented in figure 6. From which inclusion can be observed.



(1)

(2)

FIGURE 6: MICROSCOPIC EXAMINATION OF; 1) PHYSICAL MIXTURE OF ASPIRIN AND β–CD; 2) INCLUSION COMPLEX OF IT

Inclusion complex of ASP + β –CD has shown higher solubility. FTIR studies showed that Peak of Aspirin at 2287, 1575 cm^{-1} were disappeared and the peak at 1448 cm^{-1} shifted to 1458 cm^{-1} in case of Inclusion Complex. Release of aspirin from inclusion complex is higher as compare to aspirin alone. So we can say that permeability of aspirin can be enhanced in presence of β–CD

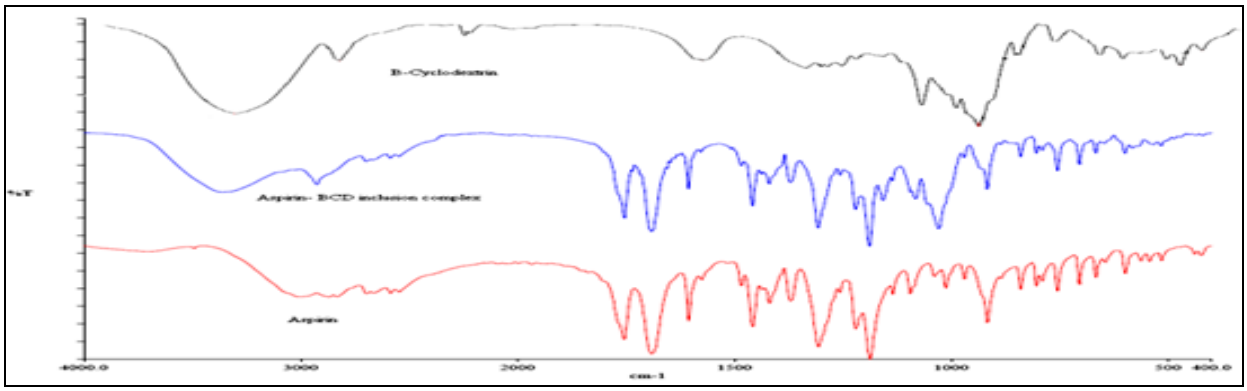


FIGURE 7: FTIR SPECTRA OF β–CD, ASPIRIN & ASPIRIN + β–CD IC

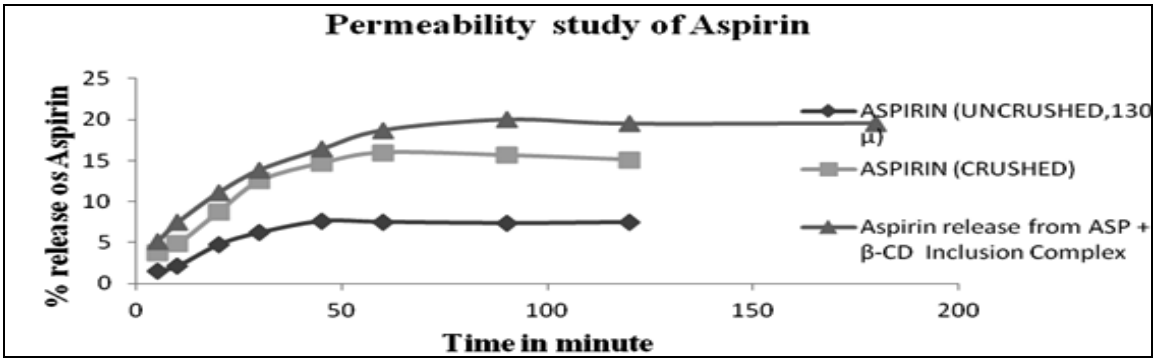


FIGURE 8: PERMEABILITY OF ASPIRIN

Analgesic activity of Aspirin in β-cyclodextrin (β–CD) revealed significant increase in analgesic activity compared to that of the Standard drug alone in equivalent dose. From anti- inflammatory result we can conclude that Inclusion complex of aspirin and β-CD has higher activity as compared to drug alone in equivalent dose.

TABLE 3: NUMBERS OF WRITHES VALUES EXPRESSED AS MEAN ± SEM

Groups	No of writhings (After 25 mins)
Control	34±0.40
Standard (Aspirin) (30 mg/kg)	16±1.47***
Asp+ β-CD IC (60 mg/kg)	10±0.8***,##

*** P < 0.001 Statistically significant with respect to Control

P < 0.01 Statistically significant with respect to Std. Aspirin

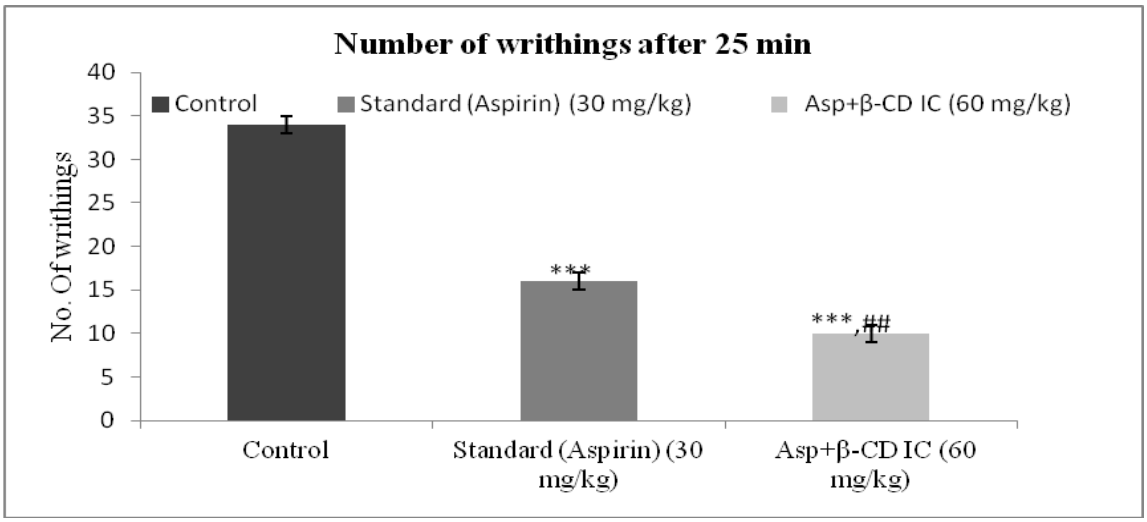


FIGURE 9: ANALGESIC ACTIVITY RESULTS

TABLE 4: %INHIBITION AT DIFFERENT TIME INTERVALS

Group	% Inhibition at time after Carrageenan injection					
	0hr	1hr	2hr	4hr	6hr	8 hr
Control (vehicle)	-	-	-	-	-	-
Aspirin (100mg/kg)	-	55.5%	79.4%	82.9%	78.8%	62.5%
Aspirin- β CD Inclusion Complex (200 mg/kg)	-	61.1%	84.3%	90.24	100%	100%

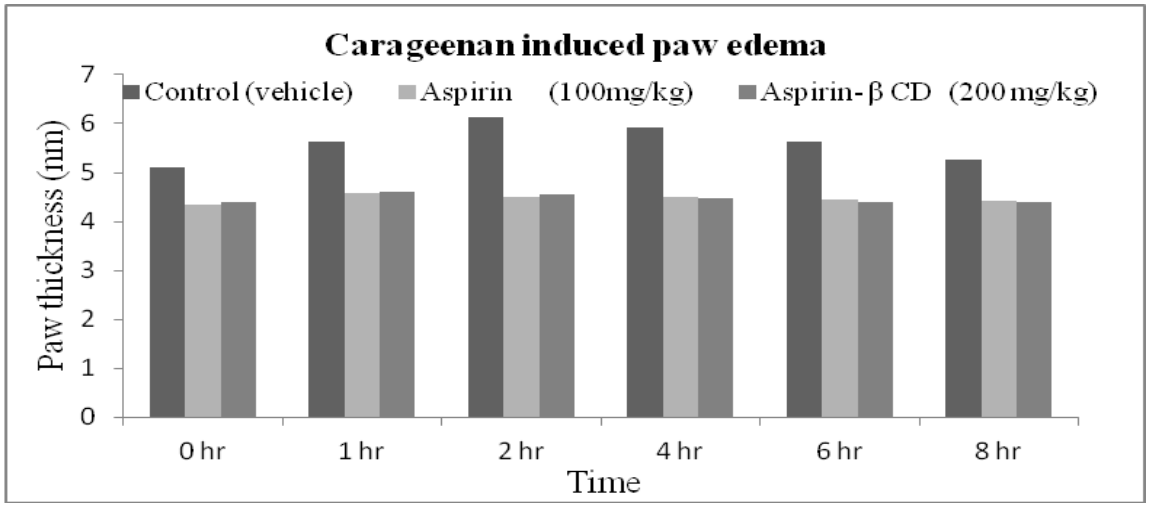


FIGURE 10: GRAPH REPRESENTING RESULTS OF ANTI-INFLAMMATORY ACTIVITY

CONCLUSION: From the characterization formation of inclusion complex was confirmed. Permeability and solubility of aspirin was enhanced in inclusion form. Similar behaviour was observed for pharmacological activity those are analgesic and anti-inflammatory.

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