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PREFORMULATION STUDIES OF INDOMETHACIN EMPLOYED FOR DEVELOPMENT OF PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Preformulation studies are described as the process of optimizing the delivery of drug through determination of physico-chemical properties of the compound that could affect drug performance and development of an efficacious, stable and safe dosage form. A thorough understanding of these properties may ultimately provide a rational formulation design or it supports the need for molecular modification. In this present study we aimed to develop the analytical methods of indomethacin as well as we also determined some physiochemical parameters of that. It has been seen that the drug indomethacin having highest solubility in methanol and all the standard plots were shown linear. Indomethacin is a crystalline and hydrophobic drug and the poor flowability of it proves a need of granules to develop a solid dosage forms.

INTRODUCTION

Since the regulatory expectations for approval of a generic drug product have become increasingly challenging and also to avoid setbacks at a later stages during the development , it is very important that sufficient efforts are made on generating the preformulation data at the initial stages during the development work for a generic formulation. This study aims to provide preformulation information, which is an essential part in the development of a robust Sustained Release Dosage Forms. Various preformulation studies were carried out including drug physio chemical characterization, Drug-Excipients compatibility studies. The physical properties of the drug like Solubility profile, Bulk / tapped densities, flow properties and particle size were also studied. The indomethacin drug was found to be having good compatibility with majority of excipients studied. The drug is found to be of fluffy nature due to low density and the drug was also observed to be having very poor flow characteristic, hence it was concluded that direct filling of powder mixture to fabricate a solid dosage was not possible. Therefore, granulation method was considered to prepare a solid dosage in-order to improve bulk density and flow-ability of blend [1-5].

These Preformulation investigations may merely confirm that there are no significant barriers to the formulation development. Certain Preformulation tests which are an important part of formulation were performed and are enlisted with procedure and result as under [5-6].

The purpose [5, 7-10] of preformulation study is to establish,

- physicochemical parameters of drug
- physical characteristics
- compatibility with common excipients55

There are many techniques available to characterize compounds. In 1985, the U.S. Food and Drug Administration (FDA) indicated (The Gold Sheet) that the principal physicochemical techniques that could be used to characterize compound should include

- Melting point
- Infrared spectroscopy
- Particle size determination
- Flow property
- Thermal analytical techniques (e.g. DSC, DTA, TGA, etc.)
- Phase solubility analysis
- Solution-pH profile determination

Drug properties:

The following properties [8] of the active ingredient are most commonly evaluated during preformulation study.

Organoleptic properties:

Appearance - White to off white crystalline powder
Odor – Odorless or slight odor
Taste – Bitter taste
Texture - Fluffy

Particle size distribution:

The particle size Distribution of the drug (active ingredient) was measured using Master sizer, whose principle is Laser diffraction, in wet method. The sample is analyzed as dispersion by dispersing in light liquid paraffin as a dispersant [6-9].

Measure of drug's compressibility:

The compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of interparticulate interactions. In a free flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poor flowing materials there are frequently greater interparticle interactions and a greater difference between the bulks and tapped densities will be observed

Drug – Excipient Compatibility Studies

Drug was mixed with certain proportions [6, 11-12] with all excipients to be used in our formulation and charged at 40^{o}C / 75% relative humidity (RH) conditions for one month. The physical properties were monitored regularly. We observed any color change in the mixture and the flow of blends were not changed during the period. The powder X ray Diffraction pattern of selected samples was recorded using PANalytical X'pert PRO X-ray diffractometer having X'celerator detector. There was no significant change in XRD patterns of drug with starch, HPMC, Crospovidone, Magnesium stearate indicating absence of interaction between drug and these excipients. The XRD pattern of the drug and the excipients are as follows and were found to be compatible.

Analytical Data Management Applied to Preformulation/Formulation

Preformulation is increasingly moving toward front-loading as many number and types of studies as possible in order to reduce the risks of late stage attrition and to minimize costly problems. Despite the increase in volume, these studies cannot be allowed to delay time to market. This means that extensive characterization of greater numbers of candidate forms and evaluations based on a myriad of criteria create logistical problems in organizing, sharing, communicating, and evaluating data in a coordinated way.

Efficiency gains of 20% to 80% have been demonstrated for various steps in the workflow:

- Reduces the time spent collecting and processing analytical data versus interpreting
- Speeds up decision-making and reporting by providing a single point of access
- Facilitates interdepartmental and worldwide collaboration via Web-based access

MATERIALS & METHODS

Procurement of drugs and chemicals

Drug indomethacin was procured from Dr. Reddy's Lab., Ahmedabad,India and other chemicals were purchased form CDH, Mumbai, India.

Physical appearance:

Indomethacin was inspected visually for physical appearance. It was physically characterized on the basis of organoleptic properties like color, odor and taste. All the physical parameters were compared with reported parameters.

Identification of drug:

Melting point:

The most important reason to determine the melting point [6] is to know about drug purity. The sample was previously dried, to determine the melting range. This determination was obtained using a digital capillary melting point apparatus (Cam bell Electronics, Bombay) by capillary fusion method. A capillary was taken and bringing it near the burner flame then sealed its one end. The open end of the capillary tube was pushed in to a small heap of drug, so that a small plug of the powder was collected in the open end and the tube was tapped gently, so that collected drug was settled down .This process was repeated several times. Then the capillary tube was placed in the melting point determination apparatus and observed the temperature at which sample changes its state from solid to liquid. The experiment was performed in triplicate. The temperature at which starts to melt was noted with the help of thermometer compared with earlier reported value.

Ultraviolet spectrum:

10 mg drug was dissolved in a mixture of 1 ml of 1M hydrochloric acid and 9 ml of methanol R and diluted to 100 ml with the same mixture of solvents. Then from this solution 10 ml was taken and volume was made up to 100 ml with a mixture of 1 ml of 1M hydrochloric acid and 9 ml of methanol R, to make solution concentration of 10 µg/ml & the resulting solution was scanned between 300-350 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The UV Spectra of the drug was recorded and

compared with reported absorption maximum (318 nm).

IR Spectrum:

The IR spectrum of pure drug was obtained in potassium bromide pellet by FTIR Spectrophotometer (Prestige-21, Shimadzu, and Tokyo, Japan) to monitor the identifications of drug between the ranges of 400 to 4000 cm^{-1}. The IR Spectra of the drug were recorded and compared with reported spectrum.

Thermal analysis:

The thermal analysis of pure indomethacin was carried out by differential scanning calorimetry (DSC) equipped with a thermal analysis data system (Perkin Elmer, California, USA). Samples weighing 3-5 mg were heated in flat-bottomed sealed aluminum pans over a temperature range of 40-250ºC at a constant rate of 10ºC/min under nitrogen purge (50 ml/min) .

Assay:

0.300 g indomethacin was dissolved in 75 ml of acetone, through which nitrogen (free from carbon dioxide) has been passed for 15 min. A constant stream of nitrogen was maintained through the solution. 0.1 ml of phenolphthalein solution was added in it and resulting solution was titrated with 0.1M sodium hydroxide and end point was noted. At the same time a blank titration was also carried out.1 ml of 0.1M sodium hydroxide is equivalent to 35.78 mg of $C_{19}H_{16}ClNO_4$.

Development of analytical methods:

Scanning & preparation of standard curve of indomethacin was carried out by using following solvent systems:

[1] Purified Water,
[2] Methanol,
[3] 0.1 N Hydrochloric acid (pH 1.2),

Scanning of indomethacin solution in various solvent systems:

Purified Water:

1 mg of indomethacin was dissolved in 100ml of Purified Water, so as a solution of 10µg/ml was prepared as a stock solution. From this 3ml was taken and the volume was made up to 10 ml to make solution concentration 3µg/ml. The resulting solution was scanned by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The absorbance was taken in triplicate manner. The absorbance maxima (λ_{max}) were noted.

Methanol:

10 mg of indomethacin was dissolved in 100ml of various respective solvent systems separately, so as a solution of 100µg/ml was prepared as a stock solution. From this 0.8ml was taken and the volume was made up to 10 ml to make solution concentration 8µg/ml. The resulting solution was scanned by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The absorbance was taken in triplicate manner. The absorbance maxima (λ_{max}) were noted.

0.1 N HCl (pH 1.2):

1 mg of indomethacin was dissolved in 100ml of 0.1 N HCl (pH 1.2), so as a solution of 10µg/ml was prepared as a stock solution. From this 2ml was taken and the volume was made up to 10 ml to make solution concentration 2µg/ml. The resulting solution was scanned by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The absorbance was taken in triplicate manner. The absorbance maxima (λ_{max}) were noted.

Preparation of Standard Curve of indomethacin in different solvent systems: purified Water & 0.1 N HCl (pH 1.2):

1 mg of **indomethacin** was dissolved in 100ml of respective solvent systems separately, so as a solution of 10µg/ml was prepared as a stock solution. From this 0.2, 0.4, 0.6, 0.8, 1 & 2ml was taken and the volume was made up to 10 ml to make solution concentration 0.2, 0.4, 0.6, 0.8, 1 & 2µg/ml. Absorbance of the resulting solution was measured by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan) at respective absorbance maxima (λ_{max}). The absorbance of each dilution was taken in triplicate manner and a concentration vs absorbance was plotted for preparation of calibration curve.

Methanol:

10 mg of indomethacin was dissolved in 100ml of various respective solvent systems separately, so as a solution of 100µg/ml was prepared as a stock solution. From this stock solution 0.1, 0.2, 0.4, 0.6 & 0.8ml was taken and the volume was made up to 10 ml to make solution concentration1,2,4,6 & 8µg/ml. Absorbance of the resulting solution was measured by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan) at respective absorbance maxima (λ_{max}). The absorbance of each dilution was taken in triplicate manner and a concentration vs absorbance was plotted for preparation of calibration curve.

Solubility Study:

The drug is to be tested for its solubility because solubility is directly related with the release of the drug from the formulation, hence absorption of the drug into blood stream. Solubility study was carried out by "Shake flask method" as given in United States pharmacopoeia [6]. The solubility of indomethacin was

studied in the solvents of different pH range (1.2-8.0), water, methanol, ethanol & polysorbate 80. Standard buffer solutions were prepared as per the procedure given earlier [8].The solubility of indomethacin was determined by adding excess amount of drug in vials with respective solvent system and kept under agitated conditions at 25°C in a water bath shaker for 24 hours. The dispersions were filtered through a 0.45 µm pore filter and analyzed for the quantity of drug dissolved. The drug quantity was calculated by taking the absorbance of a known concentration of drug (standard curve) in respective solutions. The solubility was plotted against the pH of the medium & respective solvent systems.

Determination of Partition Coefficient:

Partition coefficient [6-9] is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. If the substance is added to the immiscible solvents in an amount insufficient to saturate the solutions, it will still become distributed between the two layers in a definite concentration ratio. If C_1 and C_2 are the equilibrium concentration of the substances in a $solvent_1$ and $solvent_2$, the equilibrium expression become $\mathbf{C_1/C_2=K}$ .The K is known as distribution ratio, partition coefficient. No convention has been established with regard to whether the concentration in the water phase or in the organic phase should be placed in the numerator.

The partition coefficient of indomethacin was determined in n-octanol : water. Accurately weighed amount of drug (20 mg) was transferred in to a rubber stopper (wrapped with butter paper) containing 20 ml each of octanol and water the mixture was shaken onrush action shaker for 2 hours. Both the phases were separated using separating funnel and aqueous was analyzed by double beam Shimadzu UV-1700 spectrophotometer, to determine the amount of drug after suitable dilution using separating funnel.

The partition coefficient of the drug in phases was calculated by using the following formula as given below:

$$\text{The PartitionC oefficient , K} = \frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}}$$

Crystal Characteristics:

The Crystal Characteristics [6, 8, 10-13] of the pure drug was studied by using Magnus microscope. Conventional light microscope uses a series of lenses to bend light waves and create a magnified image. Approximately 2 mg of drug was taken in a slide and it was covered with cover slip and it was observed at 60X magnification and snap shot was taking with the help of camera.

X-ray diffractometry of drug sample was investigated using Philips XRD Machine set up with generator (PW1830), Goniometry (PW 1820) and diffractometer (PW1710, Eindhoven & Almelo, Netherlands, Europe).Cu Kα radiation was Fused (30 kV,50mA with an α1/α2 ratio of 0.5). The XRD patterns were recorded at diffraction angels (2θ) with 4^o/min scanning speed, and 5^o-45^o 2θ range.

Determination of bulk properties [6, 8-14] of pure drug:

Bulk Density:

Bulk density was determined by measurement in graduated cylinder (USP method I). A quantity of 25 gm of material was weighed accurately and passed through sieve (# 22) to break up any agglomerates and introduced into a 100 ml measuring cylinder without compacting. The powder was leveled carefully and the unsettled apparent volume Vo was noted to the nearest graduated unit. The bulk density was calculated in gm/ml by the formula: **M/Vo**.

Tapped Density:

After determination of the bulk density, the cylinder was tapped mechanically by mounting on a holder in a mechanical tapped density tester that provided a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped for 500 times initially and the tapped volume Vt was measured to the nearest graduated unit. The tapping was repeated for an additional 750 times and the tapped volume was measured. Final tapped volume was measured and tapped density was calculated by the formula: **M/Vt**.

Compressibility Index and Hausner's Ratio:

The Compressibility Index and Hausner's Ratio [3-5] are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of inter-particulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter-particle interactions, and a greater difference between the bulk and tapped densities will be observed.

. These differences are reflected in the compressibility index or Carr's index (CI) and the Hausner ratio (HR) which is calculated using the following formulas –

Carr's Index = 100 * (TD – BD) / TD

Consolidation index is related to the flow of powder as follows.

Carr's consolidation index	Flow
5 – 15	Excellent
12 – 16	Good
18 – 21	Fair to passable
23 – 35	Poor
33 – 38	Very poor
> 40	Very very poor

Hausner ratio = TD / BD

<= 1.25 – good flow

Angle of repose:

The angle of repose is a relatively simple technique for estimating the flowability of a product. A glass funnel was held in place with a clamp on a ring support over a glass plate. Approximately 50 gm of powder was transferred into the funnel keeping the orifice of the funnel blocked by thumb. As the thumb was removed, the powder was emptied from the funnel. The height of the pile (h) and radius of the base (r) measured with the ruler. The angle of repose was determined by the following equation.

$\theta = \tan^{-1}(h/r)$

Relationship between θ and Powder flow:

θ	Powder flow
< 25	Excellent
25 – 30	Good
30 – 40	Passable
> 40	Very poor

RESU LTS & DISCUSSION

The drug indomethacin was characterized for the physical appearance and it was found a Pale-yellow odorless powder with fluffy texture. It was tabulated in table 1. The melting point of indomethacin tabulated in table 1. The maximum wave length of UV spectra of indomethacin in methanolic 0.1 N HCl (90:10) tabulated in table 1 & it was also compared with the earlier reported data.

Table 1: Physico-chemical Properties of Indomethacin.

S.no.	Parameter	Reported / standard	Observed
1	**Physical appearance**	Pale-yellow odorless powder	Fully complied
3	**Melting point**	158°C-162°C	160-162 °C
4	**UV Spectra**	λ_{max} at 318 nm in methanolic 0.1 N HCl (90:10)	Fully complied
5	**Drug Assay (%)**	98-101 (Dried basis)	99.69±0.84
6	**Partition Coefficient**	Log P=3.51(octanol/water)	3.46±0.02
7	**DSC Study**	162°C	163.19°C

The FTIR peaks of indomethacin responsible for characteristic functional group were identified and interpreted & it was also compared with the earlier reported data. FTIR peaks and spectra of indomethacin are exposed in table 2 and figure 1.

Table 2: Interpretation of FTIR Spectra of Indomethacin.

S.NO.	Reported values (cm^{-1})	Observed values (cm^{-1})	Responsible Functional groups
1	750	754.38	C-Cl
2	925	929.53	-COOH out of plane
3	1230	1235.05	C-O Stretch
4	1450	1451.09	O-CH3
5	1600	1607.81	Aromatic C=C Stretch
6	1715,1695	1716,1696.43	C=O Stretch
3	3400-2500	3400-2500	Aromatic C-H Stretch,-COOH (s)

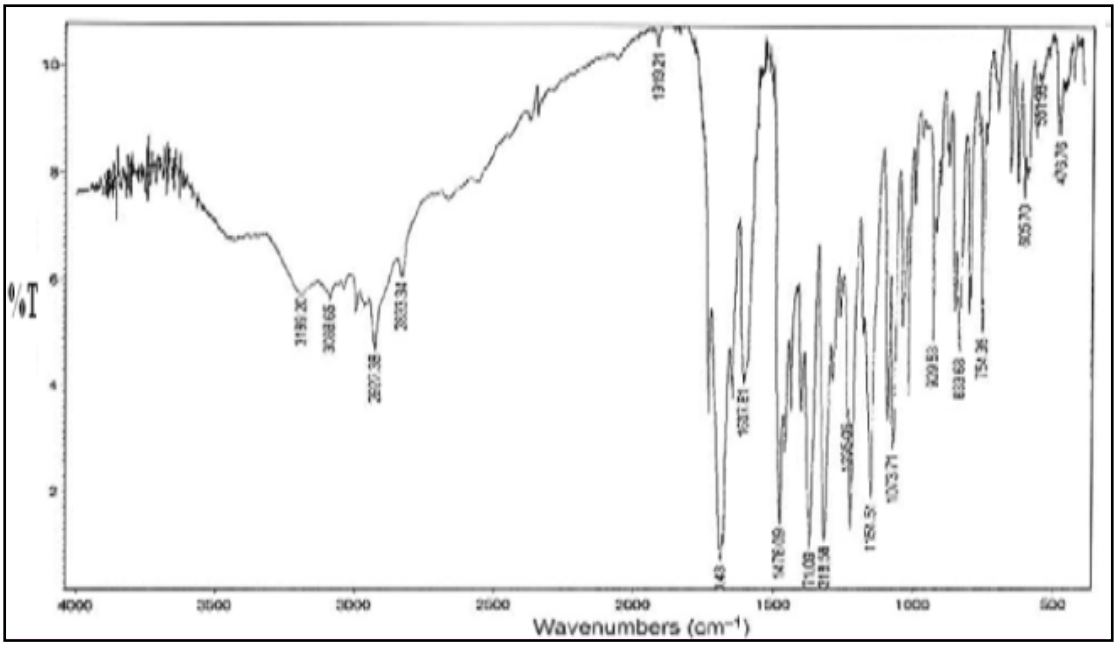

Figure 1: FTIR Spectra of Indomethacin.

The assay result of indomethacin on dried basis was calculated and tabulated in table 1 & it was also compared with the earlier reported data.

Scanning & preparation of standard curve of indomethacin solution in various solvent systems:

Table 3: Data for preparation of Calibration curve of indomethacin in water (λ_{max} 265 nm).

Concentration (µg/ml)	Absorbance at 265 nm
0.2	0.009 ± 0.003
0.4	0.031 ± 0.012
0.6	0.045 ± 0.02
0.8	0.058 ± 0.034
1	0.077 ± 0.036
2	0.163 ± 0.041

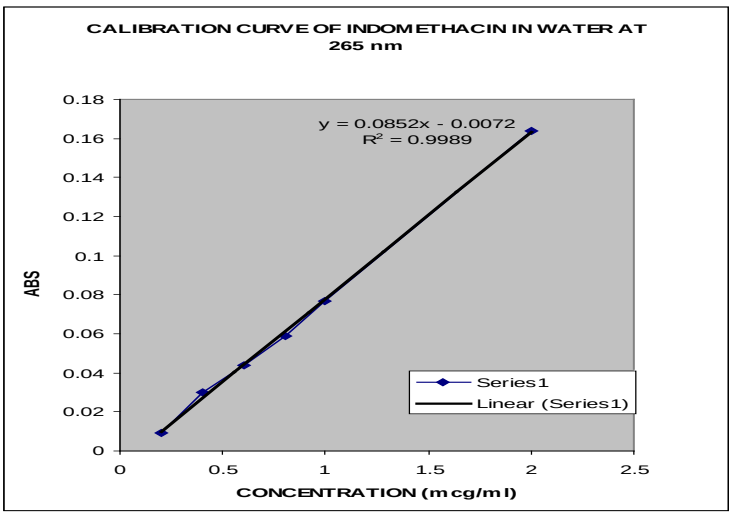

Figure 2: Calibration curve of indomethacin in water (λ_{max} 265 nm).

Table 4: Data for preparation of calibration curve of indomethacin in methanol (λ_{max} 266nm).

Concentration (µg/ml)	Absorbance at 266 nm
1.0	0.141 ± 0.002
2.0	0.285 ± 0.004
4.0	0.491 ± 0.03
6.0	0.720 ± 0.024
8.0	0.995 ± 0.055

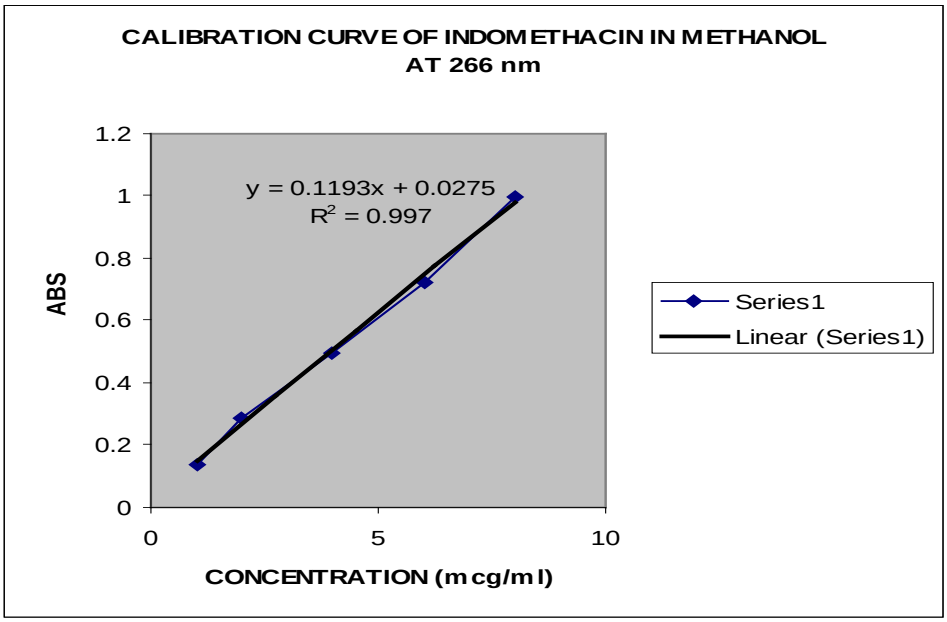

Figure 3: Calibration curve of indomethacin in methanol (λ_{max} 266nm).

Table 5: Data for preparation of calibration curve of indomethacin in 0.1 N HCl (λ_{max} 262nm).

Concentration (µg/ml)	Absorbance at 262 nm
0.2	0.065 ± 0.004
0.4	0.086 ± 0.006
0.6	0.107 ± 0.024
0.8	0.130 ± 0.043
1	0.157 ± 0.05
2	0.276 ± 0.042

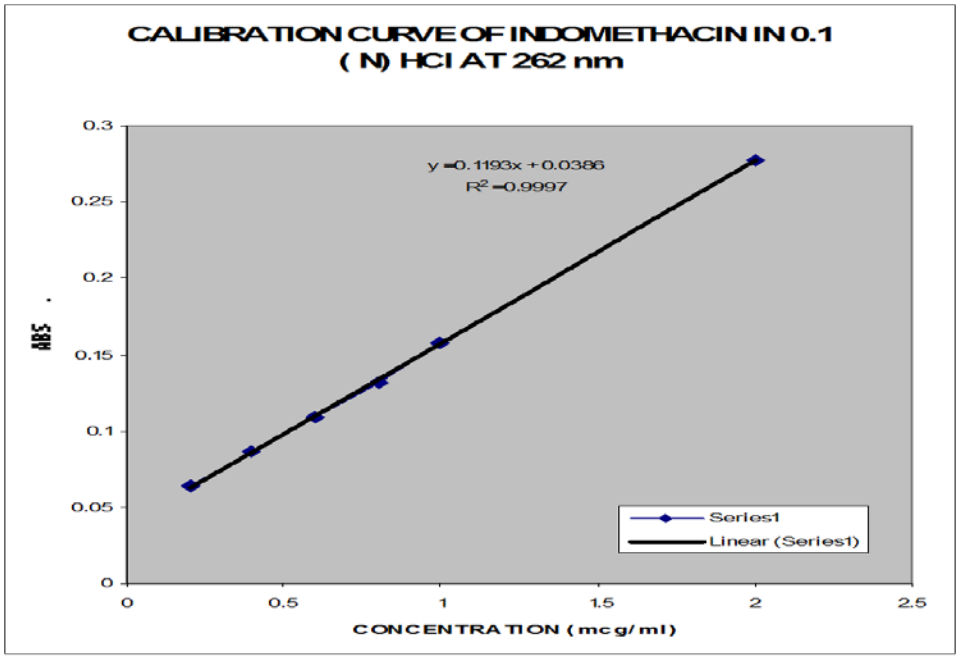

Figure 4: Calibration curve of indomethacin in 0.1 N HCl (λ_{max} 262nm).

Table 6: Summary of all parameters of standard curves in presence of various solvent systems of Indomethacin.

Sr. No.	Solvent System	Equation	(R^2)	Conc. (mcg/ml)	Abs. at ….nm
1.	**Purified Water**	)852X+ 0.0072	0.999	3	0.266 at 265
2.	**Methanol**	l193X-0.0275	0.997	8	0.999 at 266
3.	**0.1 (N) HCl**	l193X+0.035	0.999	2	0.262 at 262

Table 7: Solubility studies of Indomethacin in various solvent systems.

S.NO.	Solvent system	Solubility of indomethacin (mg/mL) at 35 ºC
1	**Water**	0.800±0.060
2	**0.1 (N) HCl**	0.031±0.004
8	**Methanol**	30.010±1.640

The solubility was calculated and reported in Table 7. The Partition Coefficient was calculated and reported in Table 1 and it was also compared with reported value. Crystal characteristics of indomethacin are shown in figures 5 & 6.

Figure 5: Microscopic characters of pure Indomethacin at 60 X.

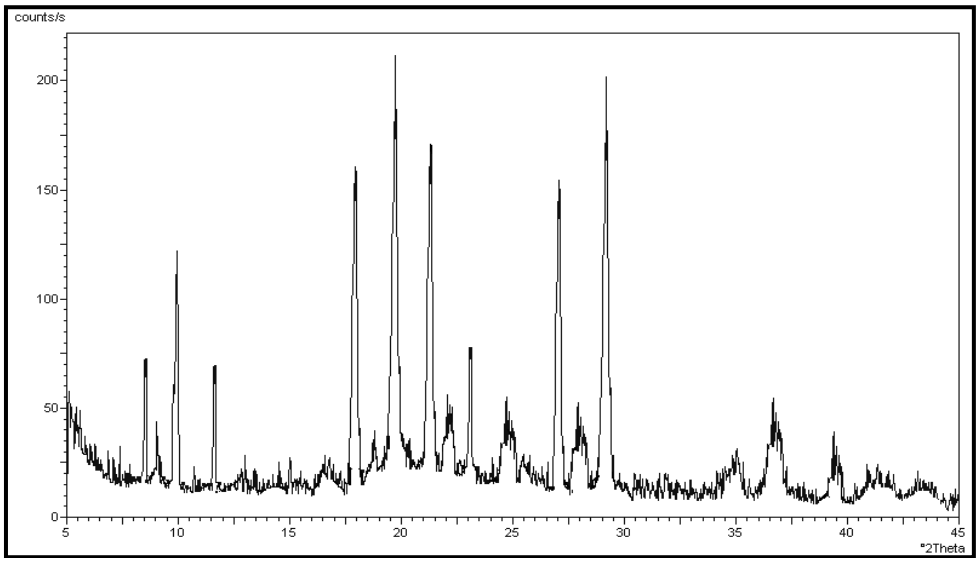

Figure 6: XRD graph of pure drug indomethacin.

The of bulk properties of pure drug was calculated and reported in Table 8.

Table 8: Bulk properties of pure drug indomethacin.

Bulk properties	Indomethacin
Bulk Density	0.54 gm/ml
Tapped Density	0.83 gm/ml
% Carr's Index	33.72
Husnar's Ratio	1.51
Angle of Repose	30.85° (EF)

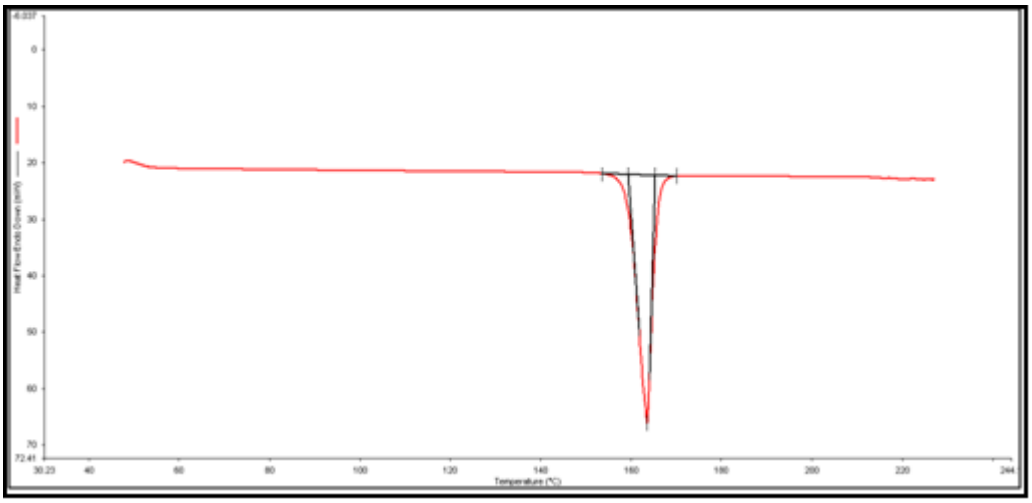

Figure 7: DSC curve of Indomethacin.

Identification: The drug, indomethacin was identified on the basis of M. Pt., UV Spectra & FTIR studies & it was fully complied with the reported & standard values. The melting point (table 1) of indomethacin was 160-162°C, matched with reported melting point which was 158°C-162°C.

Infrared spectroscopy of the model drug was studied for identification purpose. The IR spectra (Figure 1) of drug was compared with that of the Standard peaks (table 2) available in official monograph and identified as indomethacin. Peaks were found according to functional groups present in the model drug as reported in the literature. So the model drug obtained was authentic. The UV spectra of the drug were recorded and the obtained λ_{max} value (table 1) was compared with the peaks those are given in reference books. From the UV spectra the drug was identified as indomethacin. Drug was assayed (table 1) for purity test and it was within the specified limit mentioned in IP. The regression co-efficient obtained from the standard plots (table 6) were nearing about 1.0 and which proved the linearity of the analytical methods. All the calibration curves followed the linear regression. All the models followed Beer-Lambert's law and therefore can be analyzed by UV spectrophotometer.

The solubility values (table 7) were observed at different solvents and the solubility of drug was shown highest solubility in methanol and among all the selected solvent systems least solubility was observed in purified water. The model drug has low solubility and high permeability and hence falls in BCS class II.

The model drug was found to be rod shaped through photomicroscope (figure 5) and crystalline in nature. Sharp and intensed peaks were obtained in XRD analysis (figure 6) and 2θ values were found matching to that present in the literature.

All the flow parameters like angle of repose, compressibility index and Hausner's Ratio were evaluated and shown in table 8. The Carr's index, angle of repose values revealed that the API was having poor flow ability which was confirmed by referring to standard literature. Bulk density and Tapped density values confirming to API specification given in standard official books. All the flow parameters like angle of repose, compressibility and Hausner's ratio are suggestive of poor flow of the model drug.

CONCLUSION

It has been seen that the drug indomethacin having highest solubility in methanol and all the standard plots were shown linear. Indomethacin is a hydrophobic drug and the poor flowability of it proves a need of granules to develop a solid dosage forms.

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