Preformulation characterization towards design and development of dexibuprofen loaded nanoparticles

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ABSTRACT
The intention of the current study was to investigate the Physico-chemical characteristics of Dexibuprofen loaded nanoparticles. Dexibuprofen is an NSAID - non-steroidal anti-inflammatory drug intended for the treatment of rheumatoid arthritis related symptoms. In current market trends, the tablets and capsule dosage forms captures major market contribution. A systematic evaluation of physico chemical characteristics of the drug powder, it’s characteristics was performed as a basic step at the start of formulation development of the dosage form. The formulation development approach is decided based on the above data. Dexibuprofen nanoparticles was developed by an Ionotropic pre gelation technique comprising Chitosan, Calcium chloride and Sodium alginate as coating material. The preformulation evaluation of Dexibuprofen and it’s compatibility with selected excipients was performed to design an appropriate strategy for the development of Dexibuprofen modified release nanoparticles. The parameters like melting point, pKa, solubility, dissolution and assay method development, solid state stability, solution stability, flow properties, bulk density, microscopical assessment, entrapment efficiency, excipients compatibility, and nanoparticles release profile were evaluated. The results of this study, along with the experimental values, will be discussed in detail.

INTRODUCTION
Dexibuprofen (DEX) is an NSAID intended for the treatment of symptoms associated with rheumatoid arthritis. It acts by cyclooxygenase pathways inhibition and other mediators of inflammation. DEX is a selective Cyclooxygenase-2 inhibitor, which inhibits only cyclooxygenase-2 mediators. It exhibits mild symptoms like abdominal pain, nausea and vomiting, drowsiness, lethargy etc., when compared with other NSAID drugs. Dexibuprofen is S+ isomer of Ibuprofen racemic mixture. The Ibuprofen is a racemic mixture with the combination of both R and S Isomer of Ibuprofen. In which only the S form is active, and the R form is the inactive form. The ibuprofen upon administration, at in vivo, the R(-) form, which is inactive form converts into the active S(+) enantiomer through metabolic conversion (Caldwell et al., 1988; Eller et al., 1998; Lee et al., 1985). Hence, the dexibuprofen dose is equivalent to almost half of the Ibuprofen dose in most of the ethnic groups. In arthritic patients, 1200mg of Dexibuprofen produces analgesia equivalent to 2400mg of Ibuprofen. The drug, upon administra-
tion, achieves limited exposure at the site of activity due to water insoluble in nature of the drug, which results in decreased dissolution rate, which makes inconsistent and incomplete drug absorption. The water insolubility nature of the drug leads to poor patient compliance since the patients need to be administered with high doses of a drug, which shows the difficulty in swallowing the drug due to the large size of doses. The BCS Class II and IV drugs, which has poor water solubility nature, shows challenges in the development of appropriate dosage forms since dissolution is a rate limiting step for these drugs with poor solubility nature in water. The water insolubility nature of most of the drug substances become a rate limiting factor on a fast and continuous absorption rate. In addition to this, BCS Class IV drugs shows challenges in the development of formulations due to limitations in intestinal permeability of drugs (Bittner and Mountfield, 2002; Kaehler et al., 2003; Pierro and Settembre, 2013). As of now, the nanoparticles for Dexibuprofen is not yet reported. Hence, it was decided to make attempts to produce stable, quality Dexibuprofen nanoparticles to improve the release rate of in vitro dissolution and drug delivery to improve the drug efficacy in treatment at a low dose. The scope of the current work was to combine the experimental analysis of formulations with molecular modeling to decide on polymer selection to design optimal nano particle formulations.

The new chemical entities, when synthesized and started showing pharmacological activities in animal studies during the pre-clinical phase and warrants for studies in humans, there is a need for pre-formulation studies before proceeding for further formulation development. As a part of the preformulation evaluation of the drug substance, the critical Physico-chemical properties of drug substances that affects the performance of drug substance and its development towards efficacious dosage form. A complete pysico-chemical properties assessment of drug substances and it’s results are critical inputs to design the strategy for formulation development or support the requirement of modification of the molecule (Venkatesan et al., 2011b; Lachman et al., 1986).

The intention of the current study was to evaluate important physico-chemical properties of drug substances like pKa, solubility, melting point, assay and dissolution development, Solid-state stability, solution stability, microscopical properties, bulk density, rheological properties and compatibility study with excipients.

MATERIALS AND METHODS

Dexibuprofen (99.81%) gifted by M/s Shasun pharmaceuticals, Pondicherry and Chitosan purchased from BMR Chemicals, Sodium alginate was procured from Sisco Research Laboratories Pvt. Ltd., Calcium chloride was procured from Avra Synthesis Pvt. Ltd. The acetonitrile (ACN) and Acetone of HPLC grade and analytical reagent grade of dipotassium hydrogen phosphate, tween 80 and phosphoric acid were sourced from M/S SD Fine chemicals, India. The HPLC grade water was prepared using a Millipore system. The Waters HPLC (US) model contains a solvent delivery module, an injector with a 20 µl loop valve and a UV detector, system controller were used. A chromatographic software installed in the computer was utilized to perform an assay of Dexibuprofen. Analytical grade chemicals were used in the study.

Experimental Studies

Solubility Estimation

The Dexibuprofen solubility was estimated in solvents like methanol, ethanol, 0.1N HCl, pH6.8 phosphate buffer and pH7.4 phosphate buffer in-line with the specifications of respective monographs of British pharmacopoeia specifications (Chen et al., 2007; The british pharmacopoeia, 2004).

pH Determination

The pH estimation was performed by shaking for 5 min the drug samples dispersion of 1% w/v in water, and the pH was determined with a digital pH meter (Systronics, model 335, India) (Ohwoavworhua and Adelakun, 2007). The triplicate determination data is presented.

True density

The true density of Dexibuprofen is estimated by the method of liquid displacement. The true density calculation was performed in a pycnometer, based on a mass of powder which displaces the intrusion fluid (toluene) volume (Lachman et al., 1986).

\[
\text{True density } D = \left( \frac{M}{V_p - V_i} \right) \left( \frac{V_p}{V_p - V_i} \right)
\]

Where M is the mass of powder taken for evaluation, Vi is the intrusion fluid volume of the pycnometer, Vp is the total volume of the pycnometer. Triplicate readings were performed for all the estimations, and the average value of triplicates and the data are presented in Table 1.

Determination of compressibility index, bulk density and bulkiness

The Dexibuprofen bulk density is estimated by the three-tap method (Martin et al., 1994). 10g of
Table 1: Physicochemical properties of Dexibuprofen

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Dexibuprofen is a white colour, odourless powder slightly hygroscopic in nature.</td>
</tr>
<tr>
<td>Solubility</td>
<td>Readily soluble in Acetone, Ethanol, Slightly soluble in water.</td>
</tr>
<tr>
<td>True density (g / cc)</td>
<td>1.41 + 0.13</td>
</tr>
<tr>
<td>Bulk density (g / cc)</td>
<td>0.318 + 0.08</td>
</tr>
<tr>
<td>Compressibility Index (%)</td>
<td>14.12 + 0.42</td>
</tr>
<tr>
<td>Angle of repose (°)</td>
<td>28.6 + 0.61</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 + 0.42</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>7.31 + 0.65</td>
</tr>
<tr>
<td>Partition Coefficient</td>
<td>3.41</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>49 – 53°C</td>
</tr>
<tr>
<td>Dissolution of Dexibuprofen tablet after 30min</td>
<td>95%</td>
</tr>
<tr>
<td>LogP</td>
<td>3.5</td>
</tr>
<tr>
<td>pKa</td>
<td>4.85</td>
</tr>
<tr>
<td>Biological half-life</td>
<td>2.2 to 4.7 hours</td>
</tr>
</tbody>
</table>

Table 2: Physical properties of Dexibuprofen drug and excipients

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of sample</th>
<th>Description - Initial</th>
<th>Description - Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dexibuprofen</td>
<td>White colour powder</td>
<td>White colour powder. No change</td>
</tr>
<tr>
<td>2</td>
<td>Chitosan</td>
<td>Yellow colour powder</td>
<td>Yellow colour powder. No change</td>
</tr>
<tr>
<td>3</td>
<td>Sodium alginate</td>
<td>Cream colour powder</td>
<td>Cream colour powder. No change</td>
</tr>
<tr>
<td>4</td>
<td>Calcium chloride</td>
<td>White crystalline powder</td>
<td>White crystalline powder. No change</td>
</tr>
</tbody>
</table>

Table 3: Physical properties of mixtures of drug and excipient

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Sample</th>
<th>Initial description</th>
<th>Final description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dexibuprofen</td>
<td>White colour powder</td>
<td>White colour powder. No change</td>
</tr>
<tr>
<td>2</td>
<td>Dexibuprofen + Chitosan</td>
<td>Yellow colour powder</td>
<td>Yellow colour powder. No change</td>
</tr>
<tr>
<td>3</td>
<td>Dexibuprofen + Sodium alginate</td>
<td>Cream colour powder</td>
<td>Cream colour powder. No change</td>
</tr>
<tr>
<td>4</td>
<td>Dexibuprofen + Calcium chloride</td>
<td>White powder</td>
<td>White powder. No change</td>
</tr>
</tbody>
</table>

Table 4: Chemical characterization data of drug and excipient mixture

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Sample</th>
<th>Assay Initial (%)</th>
<th>Assay - Final (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dexibuprofen</td>
<td>99.81</td>
<td>99.80</td>
</tr>
<tr>
<td>2</td>
<td>Dexibuprofen + Chitosan</td>
<td>99.72</td>
<td>99.74</td>
</tr>
<tr>
<td>3</td>
<td>Dexibuprofen + Sodium alginate</td>
<td>99.74</td>
<td>99.74</td>
</tr>
<tr>
<td>4</td>
<td>Dexibuprofen + Calcium chloride</td>
<td>99.76</td>
<td>99.75</td>
</tr>
</tbody>
</table>
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Dexibuprofen was carefully placed into a graduated measuring cylinder of 100ml capacity. Onto a hardwood surface, the measuring cylinder was dropped from a height of 1inch at an interval of 2 seconds for 3 times. Then determination of bulk density was performed using the sample mass divided by sample volume in a graduated measuring cylinder. The sample bulkiness is determined by using the reciprocal of bulk density or the specific bulk volume. The Dexibuprofen compressibility index (I) was determined as per the below formula (Marshall et al., 1991) and the data presented in Table 1.

\[
Compressibility\ Index\ (I) = \left(1 - \frac{V}{V_o}\right) \times \left(1 - \frac{V}{V_o}\right) \times 100
\]

**Angle of repose**

The angle of repose was determined using a fixed funnel and free-standing cone method (Carstensen, 1998). A graph paper was placed on a flat horizontal surface. At the height of 2 cm from the graph to the tip of the funnel, the funnel was clamped. The powder was poured through a funnel in such a way that the apex of the cone reached till the tip of the funnel. The base of the powder cone was measured for it’s average diameter, and the tangent of an angle of repose was determined using the following formula:

\[
Tan\ \alpha = \frac{2h}{D}
\]

Triplicate estimations were performed and the data presented.

**Determination of Partition Coefficient**

The drug substance of 10 mg was transferred to 50ml of pre-saturated n-Octanol with water, and the same was shaken well. To this solution, 50ml of pre-saturated distilled water with n-Octanol was mixed, and the mixture was shaken by a mechanical shaker for the time period of 24 hours. Both the phases were separated after 24 hours. The concentration in each phase was determined by measuring...
the absorbance value in each phase and by using the below formula (Shobha et al., 1994).

Partition Coefficient =

\[
\frac{\text{Drug concentration in Octanol}}{\text{Drug concentration in water}} \times 100
\]

**Percentage of moisture loss**

The percentage moisture loss of Dexibuprofen nanoparticles was estimated since the drug is hydrophilic in nature. The Dexibuprofen polymeric nanoparticles were initially weighed and placed in a desiccator at 24 hours at an environment of calcium chloride. Once the sample achieved constant mass without further change in mass, the final mass was recorded and used for calculation of % moisture loss (Ghosh et al., 2007; Karuppusamy and Venkatesan, 2017b).

\[
\% \text{ of moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

**Dissolution**

The USP Type II (Paddle) dissolution apparatus at 50rpm of paddle speed. 900ml of pH7.4 phosphate buffer was used as the dissolution medium, maintained at a temperature of 37 ± 0.5°C. 5 ml of aliquot sample was withdrawn at each interval and replaced with phosphate buffer, pH 7.4 of equal volume. The percentage release of Dexibuprofen was determined by using the HPLC method (Venkatesan et al., 2012; Valliappan et al., 2013).

**FT IR studies of Drug polymer**

The FT IR spectrometric characterisation of drug-polymer was performed to determine the interactions between drug and polymer. The mixture of drug sample of 2% (w/w) to that of potassium bromide and dry KBr used. The mixture was grinded as fine powder in a mortar and pestle, and the same was compressed at 10000 PSI hydraulic pressure to form potassium bromide discs. The scanning of each potassium bromide disc was performed at a resolution of 2 cm⁻¹ using Happ-Genzel apodization for 10 times, and the spectrum was recorded (Valliappan et al., 2013; Velavan and Venkatesan, 2014; Karuppusamy and Venkatesan, 2017a).

**Differential scanning calorimetry**

Dexibuprofen 2mg was transferred into an aluminium pan, covered with a lid and crimped. Dexibuprofen with a mixture of chitosan, Sodium alginate and calcium chloride of 2mg was transferred into an aluminium pan, covered with a lid and crimped. The samples of Dexibuprofen with formulation excipients was prepared. Then, the same was transferred into an aluminium pan covered with a lid. The aluminium pan is crimped by a crimper. The samples were subjected to temperature changes in differential scanning calorimetry instrument (Velavan and Venkatesan, 2016a; Venkatesan et al., 2011a; Velavan and Venkatesan, 2016b; Kowshik et al., 2019).

**Preparation of Dexibuprofen Nanoparticles**

**Chitosan Gel preparation**

The chitosan of 100mg was mixed with a glacial acetic acid solution of 0.2 mg/ml to achieve 0.1% Chitosan gel. This mixture was continuously stirred for 2 hrs. Allowed this solution to stabilize overnight. Clear Chitosan gel was obtained. Similarly, different concentrations of chitosan gels were prepared (Venkatesan et al., 2009; Velavan et al., 2015; Madhusudhan et al., 2010).

**Ionotropic Pre Gelation Method**

Dexibuprofen nanoparticle (DNP) was prepared in a two step process by ionotropic pre gelation technique. Polyanion pre gelation with calcium followed by polycationic pre gelation with calcium chloride, followed by crosslinking with polycation. In a beaker, a Sodium alginate solution of 0.5% w/v was taken. To this, Dexibuprofen was mixed to obtain an alginate pre-gel. To the alginate pre gel, 18 mM calcium chloride solution of 2.5ml was added dropwise at stirring speed of 800rpm for 60min. To the above pre gel, chitosan solution of different concentrations of 5ml each was added as drops over a period of 120 min. The solution was centrifuged at 10000RPM for 45 min (Gupta et al., 2009; Gohel et al., 2005; Saravanan et al., 2004).

**Encapsulation efficiency (EE)**

The Dexibuprofen nanoparticles, which are freshly prepared, mixed in 10ml of water & centrifuged in an ultracentrifuge at 10000 rpm for 20 min. The supernatant solution was diluted appropriately. This solution was scanned at 220nm in a UV spectrophotometer using blank and control samples of nano particles, and the absorbance values were recorded. The drug encapsulation efficiency was estimated by calculating the difference between the mass of the initial drug and free drug present in supernatant liquid (Martin et al., 1994; Marshall et al., 1991; Ghosh et al., 2007).
Entrapment efficiency = 
\[ \frac{\text{Content of drug}}{\text{Quantity of drug added in each formulation}} \times 100 \]

**Particle Size Distribution of Nanoparticles**

The diameter and particle size distribution of nanoparticles was estimated using Mastersizer 2000 (Malvern Instruments, Malvern, UK) by laser diffractometry. The Nonidet P40 solution of 0.1% in distilled water of around 2 to 3ml was taken. To this, about 10 mg of nanoparticles were dispersed using an ultrasonic bath for several minutes. The aliquot of nanoparticle suspension was taken and placed in the small volume recirculation unit (Saravanan et al., 2004; Zinutti et al., 1994; Sundar et al., 2010; Gohel et al., 2005; Rastogi et al., 2007), which in turn was circulated at 3500rpm. Each sample was estimated for triplicate analysis, and the mean of the volume distribution was reported.

**RESULTS AND DISCUSSION**

The drug substance bulk characteristics like flow properties, compressibility, packing densities etc., were significantly affected based on the Particle size distribution estimation is a critical parameter for pharmaceutical technologists to assess. The Dexibuprofen nanoparticle, Particle size distribution histogram presented in Figure 1.

The pre-formulation characteristics results such as solubility, true density, bulk density, compressibility index are presented in Table 1.

The physical description and assay results of drug excipient samples confirmed that there were no changes in physical description and no significant change in assay results. This confirmed that the Dexibuprofen was found to be compatible with the added excipients. The data were summarized in Tables 2, 3 and 4.

DSC study is used to allow evaluation of possible incompatibilities between different components blended in specific composition of formulation as per the appearance, shift and disappearance of peaks in the corresponding enthalpies. DSC curves (presented in Figures 1 and 2) were evaluated to assess DEX compatibility with excipients of interest. In DSC determination, the heat loss or gain of sample due to change in physical or chemical characteristics was measured as a function of temperature. An endotherm of symmetric and sharp in nature can represent the relative purity, whereas the curve of asymmetric and broad in nature represents, presence of impurities. The loss of moisture from the molecule is represented by endothermic peaks formed below 340°C in DSC measurement. The physical characteristics of Dexibuprofen, as well as drug-polymer interaction, were assessed by DSC analysis. The thermograms of drug substance, polymer and physical mixture were shown presented in Figures 2 and 3. The thermograms showed the characteristic peaks of drug substances at a melting point of 52.6°C. This confirmed that the drug-polymer interaction is not there.

The spectral data from FT IR was shown from Figures 4 and 5. The FT IR spectra of Dexibuprofen and Dexibuprofen with a mixture of excipients had shown that there are no significant differences in the spectrums.

**CONCLUSIONS**

The pre-formulation evaluation is an important aspect in the assessment of the properties of drug substances, which will help to perform an appropriate risk assessment for formulation development.

The pre-formulation assessment starts at the lead optimization phase and extends till the preliminary stages of development. These data are critical in a later phase of the development of these drug compounds. Hence, it is inferred that the per formulation characterization is critical to finalise the development strategy. The data generated at the pre-formulation stage varies based on qualitative and quantitative aspects of drug substance, equipments, knowledge of personnel etc. In the current assessment, we have successfully completed the characterization of physico-chemical properties of Dexibuprofen like morphology, particle size, solubility, pH, partition coefficient etc. The data generated in this study is informative to design development strategies for modified release formulations, especially sustained-release nanoparticle formulation of Dexibuprofen.

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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