Optimizing Azathioprine Preformulation and Exploring its Interactions with Excipients

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Abstract
Preformulation studies investigate the physical and chemical properties of a drug substance, alone and in combination with other excipients. The goal of these studies is to develop a safe, effective, and stable dosage form. Preformulation studies are initiated once a new molecule is seeded. They focus on the concepts of physicochemical properties, which are vital for any new drug molecule and/or proteins/peptides. These properties affect the therapeutic efficacy and the development process of their specific dosage form. The stage of development known as preformulation is when the physicochemical qualities are defined by a set of parameters. Among these are characteristics of solid states such crystal form, water sorption behaviour, surface property, particle size and shape, other mechanical properties, solubility, dissolution behaviour, stability, partition coefficient, and ionisation constant. Drugs are almost always given a compounded property rather than being delivered as a pure chemical ingredient. These can range from quite easy fixes to intricate medication delivery schemes. through the incorporation of suitable excipients or additives into the formulations to offer legitimate and specific medicinal actions. Achieving a consistent, therapeutic response to a drug incorporated in a formulation that can be manufactured on a wide scale with repeatable results is the main goal of dosage form design. The main goal of designing a dosage form is to provide a medicine incorporated in a formulation with a predictable, therapeutic response that can be manufactured on a wide scale with consistent product quality. Preformulation studies are typically used to characterize and determine the stability of the medicinal ingredient. Since stability testing is the main method used to determine when pharmaceutical products should be put into clinical trials and under what storage conditions, it is imperative that the stability profile of any novel component be determined before moving further with product development. Throughout the course of the drug product's lifecycle, stability studies are essential to establishing and guaranteeing its safety, efficacy, and quality. stability information for the drug substance are used to determine optimal storage and packaging conditions for bulk lots of the material. Studies are designed to degrade the solid drug substance and appropriate solutions, allowing the determination of the degradation profile.

Keywords: Preformulation Studies, Physicochemical Properties, Stability Studies, Solubility, Dissolution Behaviour, Stability, Partition Coefficient, Ionisation Constant.

Introduction

A drug's in-vivo destiny is determined by the drug delivery system it is integrated into [Mehnert, W. et al., 2001]. The 1950s saw the first commercialization of formulations based on lipids. The market was then opened to the introduction of Diazemuls, Diazepam-Lipuro, and Etomidate-Lipuro. Intralipid was first marketed as a safe fat emulsion for parenteral nutrition. The decreased pain and inflammation at the injection site may be the cause of lipid delivery systems' growing popularity [1]. One benefit that lipid colloidal carriers specifically provide is an increase in the bioavailability of medications that are not very water soluble. Compared to polymeric nanoparticles, mixed micelles, liposomes, nano-emulsions, and nano-suspensions, melt-emulsified nanoparticles based on room-temperature solid lipids have a number of benefits. Despite being good drug transporters, polymeric nanoparticles have a few associated drawbacks, including the toxicity of the polymer, inclusion of residues from organic solvents

used during formulation, and scaling up of production. On the other hand, the solid lipid nanoparticles provide the subsequent benefits [2].

Solid lipid nanoparticles are composed of solid lipids and have a mean diameter that ranges from 50 to 1000 nm as determined by photon correlation spectroscopy (PCS). Emulsions that are utilised for parenteral delivery can yield SLNs by substituting solid-state lipids for liquid-state lipids. Surfactants are typically used to physically stabilise SLNs. When compared to polymeric nanoparticles, SLNs have a significant advantage in that high-pressure homogenization procedures can be used to create and manufacture them. Despite being good drug transporters, polymeric nanoparticles have a few associated drawbacks, including the toxicity of the polymer, inclusion of residues from organic solvents used during formulation, and scaling up of production. On the other hand, the solid lipid nanoparticles provide the subsequent benefits [2,3].

By molecular weight, 55% of azathioprine is equivalent to 6-mercaptopurine; 88% of this conversion occurs once the drug is absorbed into the plasma. Assuming 100% oral absorption, these pharmacological variations result in a conversion factor of 2.08 when converting 6-mercaptopurine to an equivalent dose of azathioprine.Despite the fact that these drugs are frequently taken interchangeably and have similar metabolic routes, there are significant variations in the bioavailability of the drugs that may affect their therapeutic efficacy and clinical responsiveness.

Material and Method

Solid lipid nanosuspensions are generally composed of 5 to 10% solid lipid excipient and 2 to 5% surfactants. In the case of NLC, a liquid lipid fraction representing 0.1 to 30% of the lipid content is added to the formulation. Azathioprine was obtained as a gift sample from the Zydus Pharmaceuticals Pvt. Ltd., New Delhi.

Preformulation Studies

Preformulation deals with the authentication of a drug by its various physical and chemical properties, these are considered as an important factor in the formulation of a stable, effective and safe dosage form [4,5].

Organoleptic properties

The drug sample was analyzed for physical appearance and compared with the standard.

Melting Point

It is among the criteria used to assess the purity of the medicine. One technique for figuring out a drug sample's melting point was the capillary method. A tiny quantity of powder was placed within a capillary tube. After the tube was put in a melting point equipment, its temperature was noted. Both the temperature at which the powder started to melt and the temperature at which it melted entirely were recorded. [6]

Partition coefficient of drug:

Because it influences the drug's bioavailability, release rate into the dissolution media, and ultimately the pharmaceutical product's therapeutic efficacy, the solubility of the drug is a crucial physicochemical feature. The greatest volume or mass of the solute that dissolves in a given volume or mass of a solvent is used to represent a molecule's solubility in different solvents. It is defined as the ratio of the unionized drug distributed between the organic and aqueous phases at equilibrium. To determine the partition coefficient of a drug, first of all we took two parts of drug and each part of the drug was 5 mg weight [7,8]. Then one part of the drug was dissolved in a 10 ml organic phase and another part of the drug was dissolved in a 10 ml aqueous phase. On the next step we mix both solutions of Azathioripne and stirred the resultant solution for 1 hour. After this we kept this resultant solution is a packed test tube for 24 hours at room temperature. After 24 hours we centrifuged the resultant solution and both phases were separated. Then samples were taken from both solutions and drug concentration was determined by using UV-visible spectrophotometer.

UV Spectroscopy Spectral analysis:

UV absorption spectroscopy is one of the best techniques for identifying contaminants in organic compounds [9]. Additionally, it can be applied to ascertain the absorbance at a certain wavelength. Additional peaks that can be compared to those of normal raw material can be observed as a result of contaminants in the sample. UV Spectral Analysis is hence an essential feature for qualitative drug detection.A standard solution containing 100µg/ml of the drug sample has been prepared for the spectrum analysis using SGF (pH-1.2), SIF (pH-6.8), and (pH-7.2) as solvents. The preparation process for SGF (pH-1.2), SIF (pH-6.8), and (pH7.2) is illustrated in Appendix 1. At 200–400 nm, UV scanning was carried out with a UV spectrophotometer (UV-1800, Shimadzu, Japan)

FT-IR Spectral Assessment

FT-IR can be used to identify unknown compounds, assess the consistency or quality of a sample, and calculate the quantity of each component in a mixture. Potassium bromide (KBr) powder was used to create an FT-IR spectrum of a powdered medication utilising an FTIR (IR Affinity-1, Shimadzu, Japan) from 400–4000 cm-1. The sample (Drug:KBr :: 5:95) was put into the sample holder and its infrared absorption spectra were measured. Thus, FT-IR spectra have been examined for the qualitative identification of drug samples. After that, the drug's infrared spectra was obtained and contrasted with the reference [10, 11].

Result and Discussion

PREFORMULATION STUDIES

Identification of drug sample

The drug was identified according to Indian Pharmacopeia (I.P.) 2014.

Physical appearance

Visual inspection and physical appearance of Azathioripne revealed odourless orange powder.

Determination of absorption maxima (λ_{max} at 259nm)

The λ_{max} of Azathioripne in water was measured in Shimadzu 1800 UV/visible spectrophotometer and found to be 259 nm. For identification of drug, first of all drug 50 mgdissolved in 100 ml of water and then we took 3 ml of solution in 25 ml volumetric flask andvolume make up to 25 ml water after that rinse the cuvette with methanol subsequently. Then after absorption maxima recorded by the U.V. spectrophotometry and wavelength range were selected between 200-400 nm. Absorption maxima (λ_{max}) of Azathioripne were recorded at 259 nm in water.

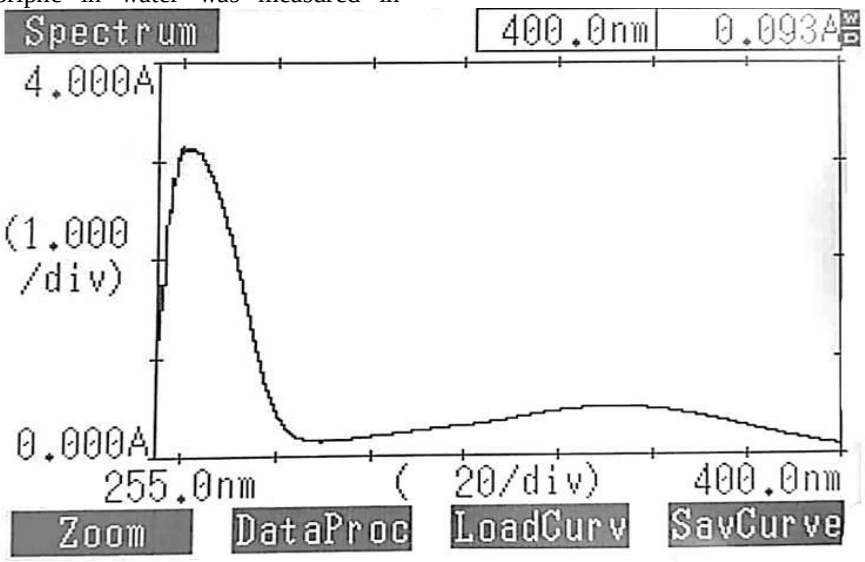

Figure 1: Graph of absorption maxima for Azathioripne

Infrared spectral determination of drug

The infrared spectral analysis was analysed by using FT/IR-4600typeA model. The Nujol method was used for the preparation of the sample. In this method drug was distributed (dispersed) in non-volatile Nujol (liquid paraffin) which having a similar refractive index to drug and then IR spectroscopy conducted. For the preparation of the sample, 10 mg drug was pulverized in a mortar and pestle. Then two drops of Nujol was added and mixed to distribute the drug in Nujol. Apply the paste to a KBr crystal plate (liquid cell) and sandwiched it with another plate. Then this drug sample subjected to scanning between 1999.820 to 400.197 cm-1 (wave number cm-1). According to graph the identification score of drug was 0.978. This score was based on an algorithm of the correlation coefficient.

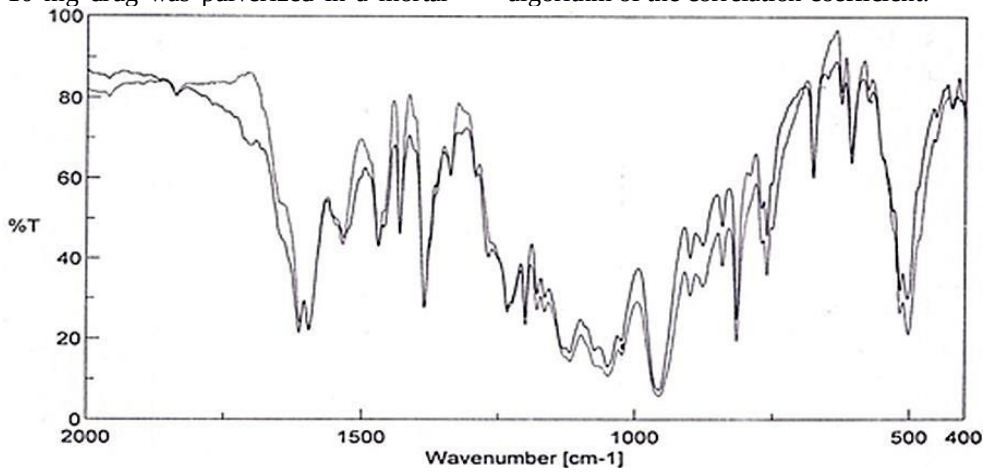

Figure 2: FT-IR spectrum of Azathioripne

Melting point determination by melting point apparatus

First of all switch on melting point apparatus and then measure the temperature of the instrument with the help of an analytical thermometer. A capillary was taken and one end of this capillary was sealed by using a burner. Now we filled the Azathioripne in the capillary tube. Place the capillary in the melting point test apparatus. Then the drug was started to melt after keeping in the melting point apparatus at a significant temperature. We noticed that the drug gets melted at the range of 196-198 °C.

Partition coefficient of drug

N-Octanol: water, n-octanol:PBS (pH 5.5) & n-Octanol:PBS (pH 6.8) partition coefficient

It is defined as the ratio of the unionized drug distributed between the organic and aqueous phases at equilibrium. To determine the partition coefficient of a drug, first of all we took two parts of drug and each part of the drug was 5 mg weight. Then one part of the drug was dissolved in a 10 ml organic phase and another part of the drug was dissolved in a 10 ml aqueous phase. On the next step we mix both solutions of Azathioripne and stirred the resultant solution for 1 hour. After this we kept this resultant solution is a packed test tube for 24 hours at room temperature. After 24 hours we centrifuged the resultant solution and both phases were separated. Then samples were taken from both solutions and drug concentration was determined by using UV-visible spectrophotometer.

Table 1: Partition coefficient of Azathioripne

Solvent system	Partition coefficient
n-Octanol : Water	0.867±0.010
n-Octanol : PBS (pH 5.5)	0.719±0.009
n-Octanol : PBS (pH 6.8)	0.785±0.005
Skin pieces : PBS (pH 5.5)	0.563±0.003

*Values are shown as mean ± SD (n = 3).

Conclusion

The drug sample was identified according to Indian Pharmacopoeia (I.P.)/British Pharmacopoeia (BP) 2018. Organoleptic properties like colour, nature and taste have been performed on drug sample. The melting point of Azathioripnewas found in the range of 288-291°Cfollowing the standard specified in the reference. The solubility of Azathioripnein different solvents at room temperature has been determined with the solvents like Water, ethanol, methanol, 0.1N Hcl, PBS pH 6.8 and 7.4. The drug sample FTIR was interpreted and matched with the reference FTIR spectrum. The obtained FTIR Spectra of Azathioripne showed all the prominent peaks of functional groups present in the drug sample. Band spectrum of 3600-3200 cm-1 denoting –OH stretching, 3000cm-1 Showed C-H stretching, 1619cm-1 is the characteristic peak of C=H Stretch of aromatic group, 1131cm-1 marked as C-Ostretching and 685-808cm-1 denoting bending of C-H which ascertained the purity of the drug. These studies proved that the drug sample was authenticated. The current work showed that the ionic gelation approach was a successful means of manufacturing chitosan nanoparticles loaded with primaquine. Critical process factors, such as the concentration of TPP and chitosan, can be adjusted to modify the zeta potential, particle size, and encapsulation efficiency. Studies using FTIR, DSC, and XRD showed that primaquine was effectively contained in the nanoparticles. With the primaquine-loaded chitosan nanoparticles in phosphate buffer saline (pH 7.4), sustained in vitro drug release was seen. Compared to 25 °C or 40 °C, the nanoparticle formulation PN3 is found to be more stable at 4 ºC. Furthermore, it reveals that following intravenous delivery to mice, the nanoparticles dispersed mostly in the liver because of their smaller particle size (100–200 nm) relative to blood. The study shows that intravenous introduction of chitosan nanoparticles results in effective uptake at the liver. Still, a thorough pharmacokinetic analysis is necessary to investigate efficient hepatic medication delivery.

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