RESEARCH PAPER

Preformulation Studies of Insulin: A Foundation for Next-Generation Therapeutics

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ABSTRACT

Background: The formulation of effective peptide delivery systems demands knowledge about the physicochemical attributes of the drug and its suitability for the structuring agent. This research involves the characterization and compatibility evaluation of the drug insulin with the structuring agents lipophilic GMO (glyceryl monooleate) and hydrophilic Poloxamer 407.

Methods: Physicochemical attributes of the active pharmaceutical ingredient (API) were characterized for organoleptic properties, thermal behavior through melting point determination, and moisture content through LOD test. Solubility profiling was performed in various solvents. Lipophilicity-hydrophilicity was determined by partition coefficient (Pka) through n-octanol/phosphate buffer system. Compatibility testing was done using FTIR Spectroscopy by comparison between pure API and physical mixture of API and structuring agents.

Results: The organoleptic test showed insulin to be a beige-brown, odorless, crystalline powder. Melting point of insulin was observed at 234° ± 0.3 C which indicates the purity of the compound whereas LOD was found to be 8.08 % and well below the 10 % pharmacopeial limit. Excellent aqueous solubility of insulin was evident from the fact that it is freely soluble in phosphate buffer pH 6.8, on the other hand it shows low solubility in organic solvents. This has been justified with the help of partition coefficient of 0.0091 and therefore the hydrophilic log P is -2.04. FTIR spectrum of pure insulin showed characteristic bands of proteins including Amide I vibration (N-H bending) at 1631 cm−1 and Amide II vibration (N-H stretching) at 3267 cm−1. From the overlay of compatibility FTIR study, it can be seen that characteristic peaks of aliphatic group at 2916 cm−1 and 2850 cm−1 and individual peaks of carriers (ester carbonyl at 1730 cm−1.

Conclusion: The preformulation studies have succeeded in proving the purity and hydrophilicity of insulin. The most important result is that from FTIR study there was no sign of incompatibility between insulin and selected excipients.

Keywords: Preformulation, Alzheimer's disease, Gel, Insulin, Intranasal
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1. INTRODUCTION

Insulin

Insulin is an endocrinological hormone that lowers blood glucose. Moreover, insulin acts as a growth factor which influences mitogenesis, growth and differentiation. Experimental studies have demonstrated that insulin protected the brain from injury without lowering blood sugar. Insulin possesses neuroprotective activity independently. Insulin causes hypoglycaemia during ischemic brain injury treatment by venous administration.[1] Hypoglycaemia can worsen brain injuries. How insulin may fully perform neuroprotective function? The key point is to define blood glucose range where insulin may show its neuroprotective activity. The purpose of the current research is to analyse the mechanisms of insulin neuroprotection and optimal blood glucose range during insulin administration.[2] The IN approach allows for the administration of insulin into the central nervous system in the presence of minimal systemic uptake and associated adverse peripheral effects. Insulin applied IN is believed to enter via the olfactory and adjacent routes and has been proven to quickly reach cerebrospinal fluid, implying an effective entry into the brain.[3] Twenty years of investigations in healthy people and patients have provided evidence that IN insulin elicits functional metabolic effects, namely decreased food intake and body weight, as well as glucose homeostasis, and

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cognitive effects, i.e. enhanced memory function in both healthy subjects and patients with mild cognitive impairment or Alzheimer's disease; IN insulin is further evidenced to have good safety properties for both acute and repeated administrations of IN insulin. New data suggest that IN insulin is also able to affect neuroendocrine function, sensory perception and mood.[4]

The Dual Function of Insulin: From Peripheric Homeostasis to Neuroprotection

Conventional Physiology and the Metabolic World Pandemic. Insulin is a 51-amino-acid protein discovered more than a century ago by Banting and Best that is now considered the ultimate metabolic regulation factor.[5] Produced by beta cells of pancreas' islets of Langerhans, insulin is the indispensable part of systemic glucose homeostasis. This peptide hormone is involved in glucose uptake in the body's most important peripheric organs – mainly muscle and fat – through GLUT4 translocation to the cell surface, while it decreases production of glucose via gluconeogenesis and glycogenolysis in liver tissue.[6] Diabetes mellitus has developed into a metabolic world pandemic. IDF Diabetes Atlas estimates that hundreds of millions of adults have diabetes worldwide, and this number is predicted to grow exponentially until 2045. For those patients who suffer from Type 1 Diabetes Mellitus (T1DM) and late stages of T2DM, exogenous administration of insulin is not just an option, but an urgent requirement [7]

The Emerging Concept of "Type 3 Diabetes": Insulin in Central Nervous System

Whereas the physiological actions of insulin on glucose metabolism have been well established in the literature, neurobiological studies conducted during the last two decades have revealed another important front where insulin performs its vital physiological actions, namely, the central nervous system (CNS).[8] Insulin from both exogenous and endogenous sources gains access into CNS through the saturable receptor-mediated process to act on high affinity receptors located mainly in hippocampus, hypothalamus, and cerebral cortex regions. [9]

In CNS, insulin does not function as a key regulator of metabolic fuel regulation but acts as a powerful neuroprotective hormone that coordinates various mechanisms of synaptic plasticity, long-term potentiation (LTP), formation of dendrites and cell survival. [10]

Thus, CNS insulin resistance associated with desensitization of the insulin receptor substrate-1 (IRS-1) signalling pathway has been identified as one of the major pathophysiological causes for the onset of Alzheimer's disease (AD). The striking resemblance of metabolic abnormalities and cognitive impairment has prompted scientists to name Alzheimer's disease as "Type 3 Diabetes." [11]

Insulin administered through intranasal route

It has become impossible for peripheral infusion of insulin to be considered a treatment modality for AD because of the risks of hypoglycemia associated with the peripheral infusion of insulin. [12] Recently, there has been increased interest in direct delivery of drugs to the CNS through intranasal route. The mechanism by which substances are delivered to the brain through intranasal route is believed to be mainly through the olfactory and trigeminal routes. Delivery occurs fast (within 10 min of administration). [13]

2. MATERIALS AND METHODS

For the preformulation studies of Insulin, material which is primarily used is Insulin purchased from Otto Chemie Pvt. Ltd., Mumbai. Glyceryl Monooleate acts as key ingredient in the formulation of cubosomes was procured from Mohini Organics Pvt. Ltd., Malad Mumbai. Poloxamer was procured from Lepid Lifesciences, Pvt. Ltd. Bhiwandi, Rajasthan. Other reagents and chemicals from obtained from Sigma Aldrich chemicals Pvt. Ltd.

Structure of Insulin

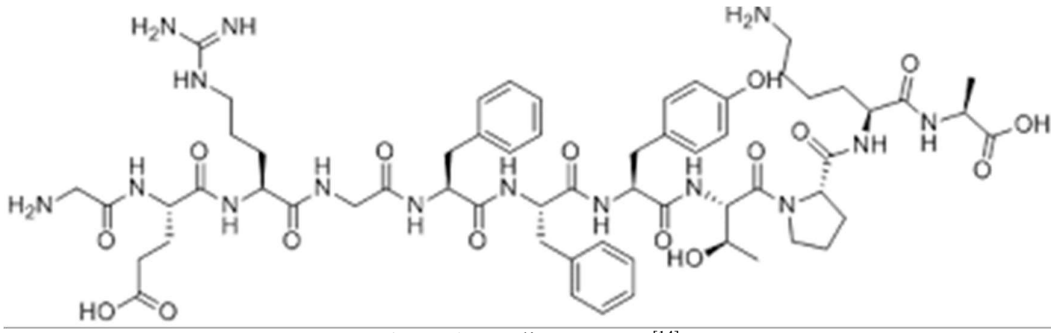


Figure 1: Insulin Structure [14]

3. PREFORMULATION STUDIES

Preformulation are the studies performed before the preparation of formulation. Preformulation plays crucial role and provides a roadmap for the selection of excipients, method and to analyse the purity of the API, also helps to determine the drug excipients interaction. [15] Preformulation study includes:

3.1 Organoleptic Properties-

Morphology and Physical Properties are observed Visually.[16]

3.2 Melting Point

The melting point was determined using capillary method. For the determination of melting point sample taken in fused & sealed capillary tube, filled capillary tube placed in melting point apparatus, observed the melting of the sample. The temperature was recorded from where the melting started up to its liquification.[17]

3.3 Partition coefficient

For the determination of the partition coefficient of insulin, a solvent system consisting of n-octanol and a phosphate buffer solution (typically at physiological pH 7.4) is utilized to simulate the lipid-water distribution. Equal volumes of the pre-saturated non-aqueous (n-octanol) and aqueous (phosphate buffer) phases are transferred into a separating funnel or an airtight vial. A precisely weighed quantity of insulin (e.g., 10 mg) is introduced into the solvent system. The mixture is then subjected to continuous agitation or shaking for a specific duration (often 30 to 60 minutes) to achieve thermodynamic equilibrium, after which it is left undisturbed to allow complete phase separation. The two distinct layers are carefully separated. To remove any suspended particulates or droplets, the phases are filtered using suitable filter paper (such as Whatman filter paper) or centrifuged. The concentration of insulin in each phase is subsequently analysed spectrophotometrically—typically via UV-Vis spectroscopy at 276 nm. [18]

3.4 Loss on Drying

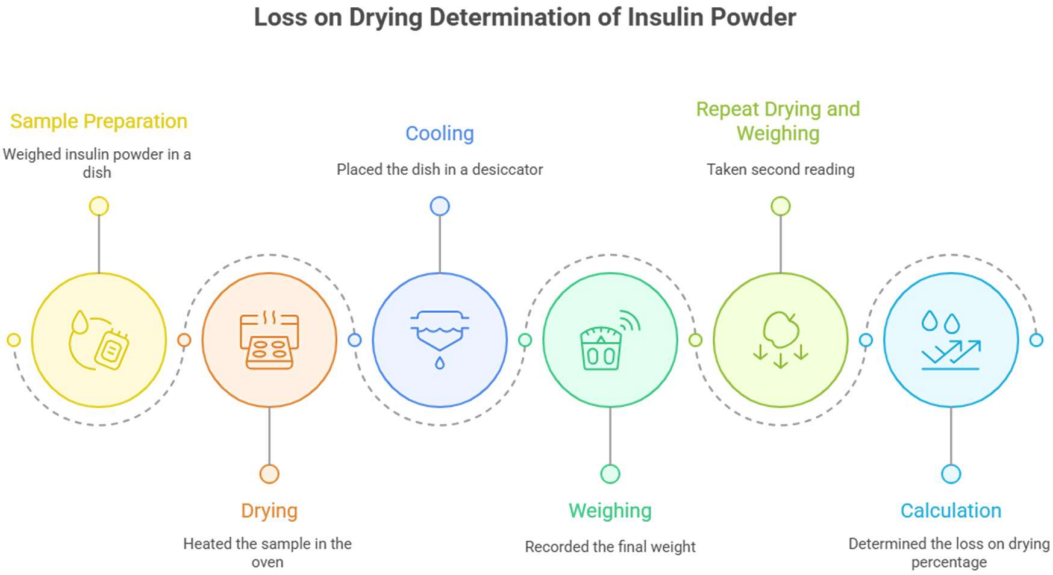


Figure 2: Loss on drying.[19]

3.5 FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy can be used as a very useful, non-destructive method of characterization of insulin based on its structure and conformation. As a peptide hormone composed of 51 amino acid residues in two polypeptide chains, insulin demonstrates specific infrared bands that form its molecular spectrum. The first application of FTIR to insulin is related to determination of integrity of its secondary structure, which includes content of alpha-helices, beta-sheets, and random coils by means of analysis of characteristic Amide I band (1600 – 1700 cm^{-1}, C=O stretching) and Amide II (1500 – 1600 cm^{-1}, C-N stretching with N-H bending) bands. Due to sensitivity of insulin to external factors, FTIR is frequently applied in studies to investigate chemical stability, prove efficient interaction between insulin and drug carrier (polymeric nanoparticles and cubosomes), and identify possible protein aggregation and denaturation. [20]

3.6 Drug Excipients compatibility studies – FTIR

Initial studies are very important to gain the basic understanding of drug and excipients and as well as their interaction. This study includes the drug excipients interaction by the Fourier transform infrared spectroscopy (FTIR), in which the presence of chemical structure was analysed on the basis of peak at a specific wave number. [21]

3.7 Solubility

Solubility screening is a key step that decides if a formulation will be prepared. It helps figure out the best levels for drug loading while stopping early separations or precipitation. By studying how insulin behaves in solubility tests beforehand, it will make sure that formulation will be stable, keeps its structure, and mixes evenly in the liquid crystalline matrix. .[22]

4. RESULTS AND DISCUSSION

4.1 Organoleptic Properties-

Morphology and Physical Properties are observed Visually.

Table 1: Organoleptic Properties

S. No.	Organoleptic Characteristics	Inference
1.	Description	Insulin
2.	Color	Beige Brown
3.	Texture	Crystalline
4.	Odour	Odorless
5.	Form	Powder

4.2 Melting Point

Table 2: Melting Point

S. No.	Materials	Melting Point
1.	Insulin	234° ± 0.3 C
2.	Glyceryl Monooleate	36° ± 2 C
3.	Poloxamer 407	58°± 0.6 C

4.3 Partition Coefficient

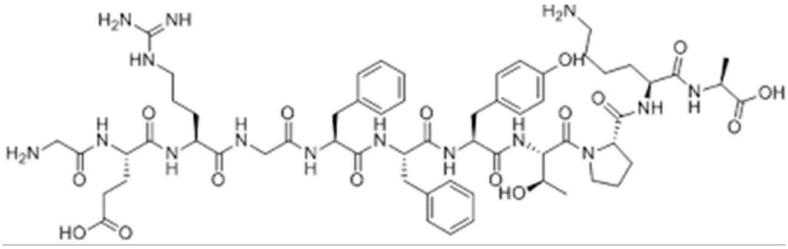
The determined partition coefficient (Pka) of insulin in the n-octanol/phosphate buffer system is approximately 0.0091, yielding a log P value of -2.04. This negative log P value quantitatively confirms the highly hydrophilic nature of insulin, demonstrating its strong preference for the aqueous environment over the lipid phase.

4.4 Loss on Drying

The determined LOD % was 8.08, which depicts that it is in the range i.e. less than 10%.

4.5 FTIR Spectroscopy

Insulin - API



Structure of Insulin

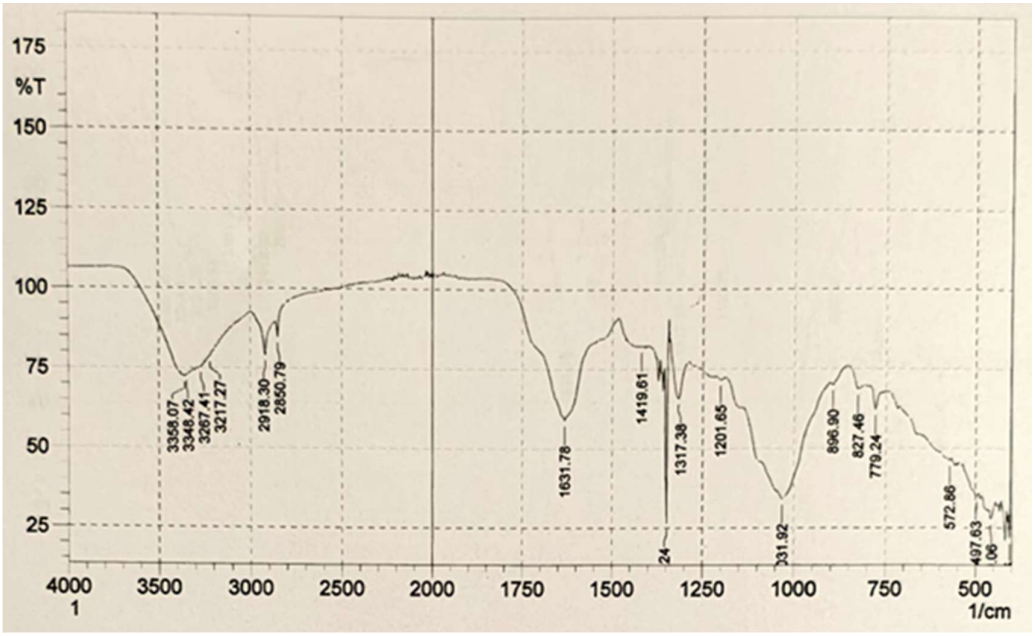


Figure 3: FTIR Graph of Insulin

Table 3: Graph Interpretations

S.No.	Functional Group	Range	Peak
1.	N-H Amide I Bending	1600 – 1700 cm^{-1}	1631 cm^{-1}
2.	N-H Amide II Stretching	3100 – 3300 cm^{-1}	3267 cm^{-1}
3.	C – O Bending	610 – 70 cm^{-1}	572 cm^{-1}

4.6 Drug Excipients Compatibility – FTIR

Insulin + Poloxamer 407 + Glyceryl Mono oleate (Physical Mixture)

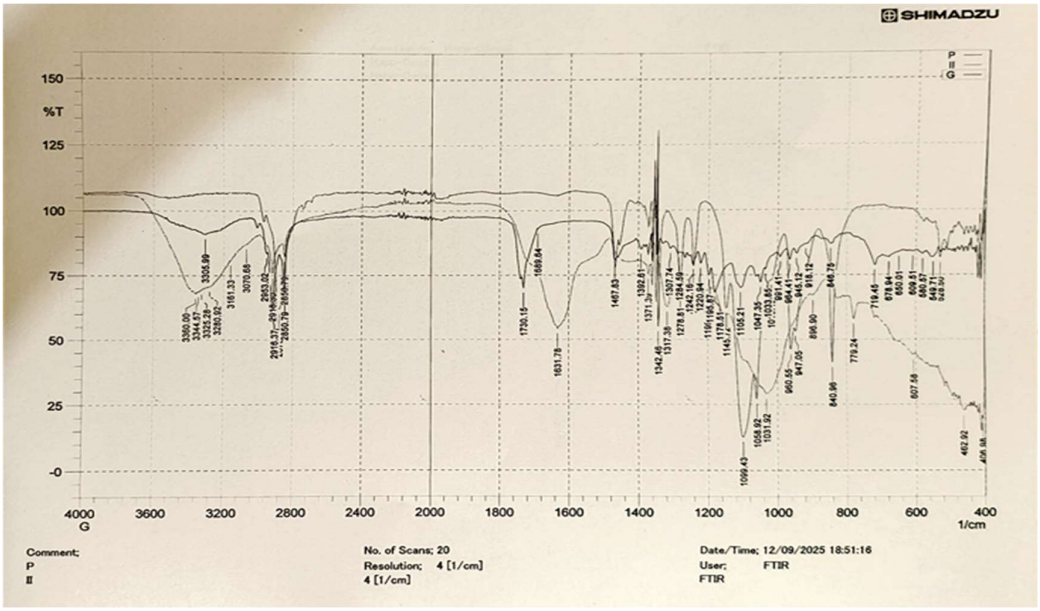


Figure 4: FTIR Graph of Physical mixture

The overlay demonstrates that the signature aliphatic peaks (2916 cm^{-1} and 2850 cm^{-1} are consistently maintained throughout all three samples, suggesting the core matrix structure remains stable. However, the prominent appearance of the 1730 cm^{-1} peak in some curves and the broad 1631 cm^{-1} feature in others highlights the successfully distinct chemical compositions of the individual ingredients versus the final formulation blend.

4.7 Solubility

Table 4: Graph Interpretations

S. No.	Solvent	Solubility
1	Water	Soluble
2	Methanol	Slightly Soluble
3	Ethanol	Practically Insoluble
4	Glycerol	Slightly Soluble
5	Propylene Glycol	Slightly Soluble
6	Phosphate Buffer 6.8	Freely Soluble

4. CONCLUSION

The current research carried out a complete pre-formulation assessment of insulin, whereby the physicochemical properties were evaluated and the compatibility of insulin with the selected excipients (Glyceryl Monooleate and Poloxamer 407) was determined. The results of the organoleptic analysis showed that the insulin drug was a brownish/beige powder with no odour at all. It was established that the melting point of insulin is 234° ± 0.3 C, which is perfectly in line with the melting point as provided in literature, thereby proving the purity of the active pharmaceutical ingredient (API). The melting points of Glyceryl Monooleate (36° ± 2 C and Poloxamer 407 (58°± 0.6 C) confirmed their thermal identity. The Loss on Drying (LOD) was determined to be 8.08% (which is within the acceptable pharmacopeial limit of below 10%), indicating the presence of adequate moisture level that would not impact the stability of the peptide. The hydrophilicity was further confirmed using partition coefficient test in which the drug exhibited the negative value of log P = -2.04 (P approx 0.0091) demonstrating high level of hydrophilicity of the compound. In case of FTIR interpretation of insulin the

intact structure of the protein was shown with clear presence of typical primary protein peaks: Amide I bending vibrations at 1631 cm^{-1}(N-H) and Amide II stretching vibration at 3267 cm^{-1} (N-H). Most importantly, drug-excipient compatibility studies via FTIR overlay of physical mixture proved that the key structural units of insulin and its carriers were retained. It should be emphasized that the consistent retention of characteristic aliphatic absorption bands at 2916 cm^{-1} and 2850 cm^{-1}, along with the presence of typical ester carbonyl (1730 cm^{-1} and Amide I (1631 cm^{-1}), confirm the absence of any adverse chemical interaction between insulin and selected excipients. In summary, the data obtained on physicochemical properties and intrinsic stability of the drug, along with excellent compatibility with excipients, show that the insulin is highly suitable to be used in advanced lipids/polymer-based drug delivery systems such as nanocarriers.

Conflict of Interest

The authors report no conflicts of interest in this research work.

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