RESEARCH PAPER

Design and Optimization of Iguratimod-Based Polymeric Nanocarriers Co-Formulated with Nutraceutical Supplements for Rheumatoid Arthritis Management

Priyanka Keshri [1]*Dr. Avanish Tripathi[2]

[1]*PhD scholar School of pharmacy ITM University Gwalior, M.P*
[2]*Associate Professor School of pharmacy ITM University Gwalior, M.P*
***Corresponding author**:*
Dr. Avanish Tripathi
*Email:ID :*tavani08@gmail.com
Priyanka Keshri
Email:ID priyankakeshri334@gmail.com

ABSTRACT
The disease rheumatoid arthritis (RA) is a persistent, autoimmune, inflammatory system ailment that is marked by inflammation of the synovia, progressive erosion of the articular cartilage, and progressive degradation of the joints. Iguratimod, as a classic synthetic disease-modifying antirheumatic drug (csDMARD), has strong immunomodulatory and anti-inflammatory effects; however, insufficient aqueous solubility, intermittent oral bioavailability, and high first-pass metabolism are its adverse effects on clinical use. The overall goal of the current study was to design and optimize Iguratimod-based polymeric nanocarrier pairs co-formulated with a nutraceutical adjugate, and achieve an enhancement in drug solubility, chemical stability, and therapeutic performance of the constructs in the case of RA management. The creation of Iguratimod-laden nanocapsules, nanoparticle emulsions, and nanocrystals using biocompatible polymers, the inclusion of spray-dried milk powder (SMP) as a nutraceutical co-component, and performance of thorough physicochemical and analytical evaluation of the resulting systems were some of the specific objectives. Polymers that were used to make the nanocarriers were polyvinylpyrrolidone K 90, 8 -cyclodextrin, polyvinyl alcohol, sodium alginate, and chitosan. Compatibility studies using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) have proven the factor of absence of deleterious interactions between chemicals and maintenance of structural integrity of Iguratimod. Assays of ultraviolet visible (UV-Vis) and high-performance liquid chromatography (HPLC) were proved to be validated, with high linearity, precision, and specificity. These findings showed the establishment of consistent nanoscale preparations with a slender particle distribution, suitable zeta -potential readings, elevated drug acquisition capacity, and improved dispersion qualities. To conclude, Iguratimod nutraceutical-polymeric nanocarriers are a potential solution to overcome biopharmaceutical limitations of the first drug and provide a multi-purpose solution with synergistic capacity to enhance the therapeutic effect of the first drug in rheumatoid arthritis..

Keywords: Iguratimod, Rheumatoid Arthritis Management, Nutraceutical Supplements
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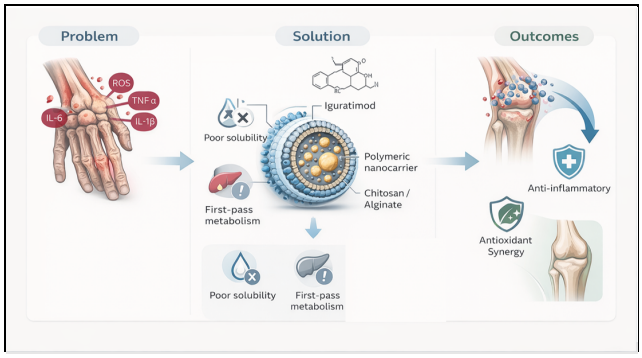
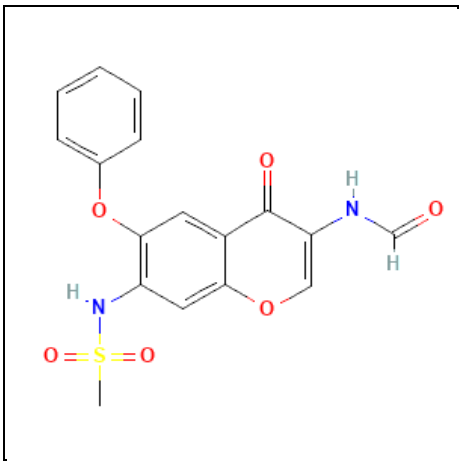


Graphical abstract

INTRODUCTION

Iguratimod produces therapeutic effects through the inhibition of nuclear factor kappa -B (NF -kB) activation, suppression of the production of pro-inflammatory cytokines (including interleukin -1 ¸ (IL -1) interleukin -6 (IL -6) and tumor necrosis factor -Alpha, and regulation of T - and B -lymphocyte functional responses. These steps result in a weakened synovial inflammation and provide immunity to cartilage and bone erosion(Grassi et al., 1998)(Long et al., 2024),

Author for Correspondence:

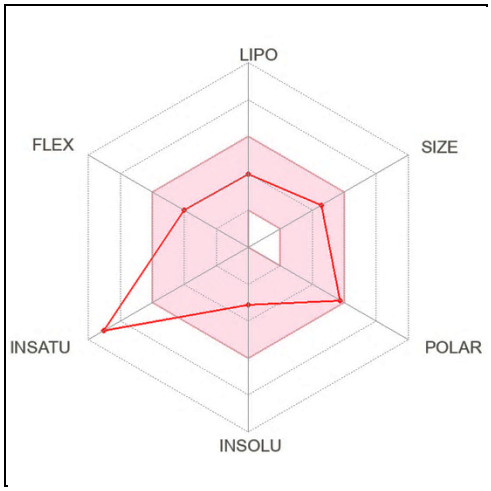


Chemical str of Iguratimod, Molecular Formula C17H14N2O6S

The pathogenesis of rheumatoid arthritis has its base in an unbalanced activity of T -lymphocytes, increased the production of pro-inflammatory cytokines, such as TNF- al, IL-1b, and IL-6, as well as in oxidative stress and enzymes of catabolism that break down connective tissue, all largely driven by an increased level of joint damage(Aletaha & Smolen, 2018). As a result, proper disease management requires a combination of immunomodulatory, anti-inflammatory and antioxidants therapeutic strategies, which should be given in consideration of the complexity of the disorder(S. Li et al., 2021).

The main forms of treatment used in conventional rheumatoid arthritis (RA) are the following: glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDS), biologic agents, targeted synthetic disease-modifying anti-rheumatic (csDMARDs), and conventional synthetic disease-modifying anti-rheumatic (csDMARDs). Though such agents have ability to incur disease activity, they are mostly characterized by side effects, high treatment expenses and poor response(Xie et al., 2020). Several Asian nations have also shown interest in the iguratimod (IGU), which is a small-molecule csDMARD, because it has a good safety profile and strong clinical efficacy in the treatment of active RA. (Jiang et al., 2020). mod inhibits the activation of nuclear factor, reduces the production of interleukin-1B (IL-1B)/interleukin-6 (IL-6), tumor necrosis factor-8 (TNF-A)-, and other pro-inflammatory cytokines and regulates both T- and B-cell responses to alleviate synovial inflammation and protect bone and cartilage integrity(C. Li et al., 2021),(Wang et al., 2021).

Although it has promise as a therapeutic agent, the clinical efficacy of Iguratimod is limited by its low solubility in water, high first-pass metabolism, and unpredictable oral bioavailability thus, requiring increased dosage and increasing adverse effects. Polymeric nanoparticles are nanotechnology-based delivery systems that have become strategies of improving these shortcomings by increasing drug stability, extending systemic circulation, and controlled, site-specific release. Polymers that are biocompatible against antirheumatic drugs have been studied widely by using biocompatible polymers like poly (lactic-co-glycolic acid) (PLGA), chitosan, and hyaluronic-acid-based formulations, they assist in accumulating drugs in inflamed joints by increasing vascular permeability and inflammatory cell uptake.(Nozaki, 2021). (J. Li et al., 2013). (Dixit et al., 2025b).



drug-likeness, solubility, and pharmacokinetic prediction

Multi targeted delivery of Iguratimod with carefully chose nutraceutical supplements in polymeric nanocarriers is a rational multi-target approach coupled with embedding strong immunomodulatory action with redox modulating, as well as cartilage protective effects. These hybrid nanotherapeutics could potentially augment drug-nutrient synergism, could improve pharmacokinetics and could target therapeutic payloads in inflamed joints thus limiting systemic exposure and toxicity(Dixit et al., 2025a). It is within this context that the present research aims to design and optimize Iguratimod loaded polymeric nanocarriers co-formulated with nutraceutical agents, and determine their physicochemical properties and in vitro operation as a new platform of handling rheumatoid arthritis.(Fatima et al., 2025).

2.Materials and Methods

The active pharmaceutical ingredient was a poorly water-soluble disease-modifying anti-rheumatic agent called Iguratimod (IGU). During the pre-formulation study, its physicochemical properties including organoleptic properties, solubility profile, melting point (238 0 C), and aqueous pH (7.2) were confirmed. Spray-dried milk powder (SMP) was selected as the nutraceutical supplement due to its good organoleptic properties such as white color, sweet smell, soft and creamy texture, and solubility in aqueous medium, which is partial and makes it a suitable bioactive carrier matrix and synergetic anti-oxidant.

Polymeric stabilizers that were used in nanocapsules, nanoemulsions, and nanocrystals formulation included. polyvinylpyrrolidone K -90 (PVP K -90), 2. 3. polyvinyl alcohol (PVA), sodium alginate (SA), and chitosan (CH). The use of Fourier-transform infrared spectroscopy (FTIR) suggested characteristic functional groups of the individual polymers; thus, confirming the chemical purity and compatibility with Iguratimod (e.g., PVP K -90: N-H stretching at 3450 -1, C=O stretching at 1656 -1; 9- - CD:

O-H stretching at 3422 -1; chitosan: O-H stretching at 3422 -1; sodium alg These polymers were selected due to their ability to stabilize nanosystems, increase solubility and entrapment efficacy.

Solvents and reagents used analytical-grade and included dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) as the only solvents in which Iguratimod had quantifiable solubility (1614 -mg mL -1), phosphate-buffered saline (PBS), acetone, methanol, ethanol and chloroform as reported in the solubility investigation. Surfactants like sodium lauryl sulfate (SLS) were added to assist in the development of nanoemulsions and better the dispersion of drugs. All aqueous preparation was done in distilled water.

Instrumentation for characterization:

The FTIR spectrophotometer can be utilized to prove the existence of functional groups and determine whether pharmaceutical agents are compatible with polymer matrices. As an example, the 3356 -1 -1 O -H stretching, 1637 -1 -1 C = C stretching, 1515 -1 -1 N -O stretching as well as the 1300 -1036 -1 S =O stretching bands are characteristic absorption bands of iguratimod. The thermal profiling and purity were determined with the use of a Differential Scanning Calorimeter (Shimadzu DSC-60). The device was run in a nitrogen ambience with a 20mLmin flow rate in a heating program between 25 C deg to 300 C deg at a specified heating rate of 10 C min -1. A Rigaku MiniFlex 600 X -ray diffractometer was used to test crystallinity and polymorphic transitions. Both raw medications and synthesized nanocarriers were scanned in a 2 per cent of 2 contended to 90 degrees. Brookfield rheometer was used in measuring the viscosity of the nanoemulsion. Iguratimod was quantified using a UV Visible spectrophotometer with a maximum wavelength of 278 2 nm, which allowed the validation of the calibration and the estimation of drugs.All chemicals used were of analytical grade, and all experiments were performed using freshly prepared solutions. Formulations were carried out according to optimized nanocarrier design matrices, using defined concentrations of Iguratimod (50 mg), SMP (50 mg), stabilizers (0.5–2%), and solvents/surfactants as detailed in the formulation code tables provided in the supporting documentation.

4.2 Selection and Justification of Polymers & Nutraceuticals

The success of the creation of Iguratimod-loaded nanopharmaceutical assays will depend on the physicochemical compatibility, functional operation and stability enhancing characteristics of the polymers and nutraceutical supplements used which will be determined by experimentation. The excipients used to prepare nanocapsules, nanoemulsions, and nanocrystals were rationally chosen based on preformulation data that included organoleptic, features, solubility, determination of melting point, pH profiles, Fourier-transform infrared spectroscopy (FTIR), determination of differential scanning calorimetry (DSC) and X-ray diffraction (XRD)(Gore et al., 2026).

4.2.1 The Nutraceutical Supplement's Selection and Rationale (SMP)

SMP was selected as the nutraceutical ingredient because it has functional, therapeutic and formulation benefits. SMP was found to be a stable, white, pleasant-smelling, soft creamy, and creamy material, which can be used in oral nanopharmaceutical systems with the help of organoleptic assessment. Even though its solubility in both water and organic solvents is low, it has an amphiphilic protein-lipid matrix, which does provide a natural colloidal stabilizing milieu, thus easing nanocarrier assembly and enhancing bioactive compounds retention.

Nutraceuticals made of milk have anti-inflammatory, antioxidant and immunomodulatory components e.g. lactoferrin and bioactive peptides, which match the mechanistic requirements of rheumatoid arthritis treatment. The combination of these ingredients is aimed to be used with synergetic effect to enhance Iguratimod anti-inflammatory effect and thus therapeutic efficacy and further offer nutritional value to the patients.(Wedekind et al., 2017)

4.2.2 Detection of wavelength

The spectra were acquired by scanning the solution across the 200-400 nm range after additional dilutions were performed with acetonitrile from the standard stock solution (1000 µg/ml), resulting in a concentration of 10 µg/ml. A wavelength of 256 nm, utilized for detection and quantification, was shown to be the drug's maximum absorption. In figure 2, we can see the drug's spectrum.

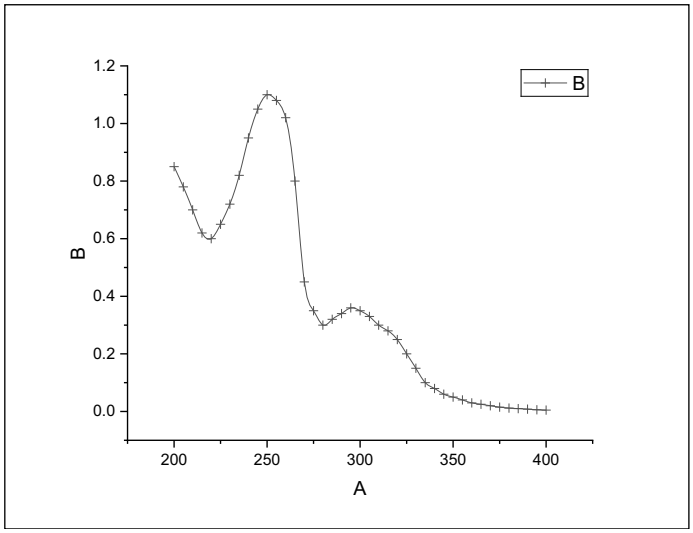


Figure 2 Detection of wavelength

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a. Polyvinylpyrrolidone K-90 (PVP K-90)

Polyvinylpyrrolidone K90 was selected as the major stabilizer due to its high molecular weight, high absorbent properties, and film forming properties. The spectroscopy 3450 cm -1 (NH stretching), 2955 cm -1 (C -H stretching) and 1656 cm -1 (C=O stretching) observed by Fourier -transform infrared spectroscopy demonstrated the integrity and non-reactivity of polymers with Iguratimo.

Its steric stabilization and hydrogen bonds also form a vital part in avoiding agglomeration in a nanosuspension and a

nanocapsule. PVP based formulations (F1-F 5) exhibited good compatibility and dispersibility characteristics in the preformulation matrix.

b. β-Cyclodextrin (β-CD)

The incorporation of β -cyclodextrin was specifically aimed seeking to increase the aqueous solubility as well as elevate the inclusion-complex stability of drugs which exhibit limited levels of solubility. The infrared spectroscopy in Fourier transform showed characteristic OH IS lines at 3422- 1157 -1029 cm -1 and C-O-C vibrational bands in the spectrum of 1157-1029 cm -1, which supported its canonical cyclic oligosaccharide structure. IBG Iguratimod has a very limited solubility in water, most organic solvents, and except dimethyl sulfoxide and N,N-dimethylformamide (solubility 1416 mglmL-1); solubilization of 258g of iguratimod was required by 2 -cyclodextrin in the nanocrystal and nanocapsule nanotechnology formulations (F6F10) to obtain some level of solubilization, crystallinity control, and mit

c. Polyvinyl Alcohol (PVA)

PVA is an amphiphilic polymer in which the amphiphilic polymer chains have contributed to high levels of steric stability and to the increase of uniformity of nanoparticles hence its selection as a stabilizing agent. The Fourier-transform infrared spectroscopy (FTIR) representation that displays an OH stretching in the 3379 forgotten and CH stretching at 2939 forgotten are a support of the maintenance of functional integrity and compatibility of Iguratimod. In addition, PVA ability to reduce interfacial tension makes it the best in stabilization of surfaces of drug nanocrystals in a top-down milling process or in the precipitation type of fabrication(Xiao et al., 2018).

d. Sodium Alginate (SA)

This was because sodium alginate was added due to its biocompatibility, gel-forming and controlled-release properties. The FTIR analysis showed 1619 cm -OH at 1619 cm - and 3441 cm -OH at 3441 cm -, which corresponded to the structural suitability of the composite nanocarriers. Alginate is a hydrophilic polymer with ionic potential of interaction, and is capable of forming a matrix in nanoemulsions and nanocrystals, with mucoadhesive properties that have the potential to increase gastrointestinal residence and subsequent Iguratimod bioavailability.

e. Chitosan (CH)

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4.2.3 Rationale for Polymer Selection Across Nanocarrier Types

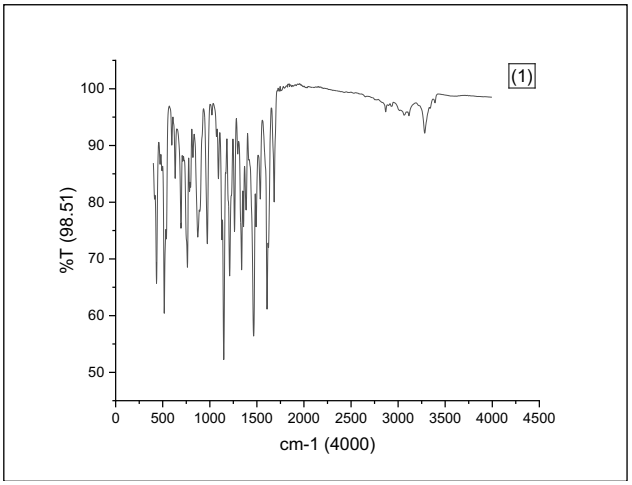
Nanocarrier	Key Polymers Used	Scientific Justification
Nano capsules	PVP K-90, β-CD	Solubility enhancement, steric stabilization, improved encapsulation efficiency
Nanoemulsions	SA, CH	Stabilization of droplets, controlled-release potential, mucoadhesion
Nanocrystals	PVA, SA, CH	Prevention of aggregation, improved wetting, controlled crystallinity

4.3 Drug–Polymer and Drug–Nutraceutical Compatibility Studies

To determine the existence of physicochemical interaction of Iguratimod with various polymers, namely, polyvinylpyrrolidone K -90, 0 -cyclodextrin, polyvinyl alcohol, sodium alginate, and chitosan, and the nutraceutical supplement SMP, compatibility studies were conducted before development of the formulations. The tests used X-ray diffraction (XRD), the differential scanning calorimetry (DSC), and the Fourier-transform infrared spectroscopy (FTIR), all of which give information about the integrity of functional groups, thermal transition, and crystallinity. The findings showed that the stability, identity and performance of Iguratimod was not negatively influenced by excipients chosen as nanocapsule, nanoemulsion and nanocrystal delivery method. These results support the compatibility of the selected excipients and Iguratimod.

4.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

To examine the potential physicochemical interactions between Iguratimod, the identified polymers, and the nutraceutical supplement embedded in the nanocarrier formulation, the current study used Fourier Transform Infrared (FTIR) spectroscopy. The main aim of the experiment was to determine the drug compatibility with the excipient and also to determine the integrity of the Iguratimod with the arrangement of the formulation constituents after blending. Avoiding the impact of an applied sample size FTIR analysis was done in an appropriate spectral range with a calibrated FTIR spectrophotometer to identify functional groups(Pu et al., 2021).



Pure Iguratimod, individual polymer (polyvinylpyrrolidone K -90, 2 -cyclodextrin, polyvinyl alcohol, sodium alginate and chitosan), and respective physical mixture of them were prepared to analyze them. Fine trituration of every sample was done using spectroscopic grade potassium bromide (KBr) and the sample crushed to transparent pellets using a hydraulic pressure. The spectra were then recorded and compared so as to detect typical absorption bands and also to detect any form of shift, disappearance or formation of new peaks which could be an indication of chemical interaction or incompatibility.

The FTIR spectrum of pure Iguratimod indicated that the compound had bound spectral peaks in terms of functional groups with O–H stretching vibrations in the range of 33561338 at 14161338 cm -1 under the range of 33561338, C=C stretching at 1637cm -1, N-O stretching at 1515cm -1, O-H bending at 14161338, and S=O stretching at These maxims were used as measures to evaluate drug intactness. The nutraceutical component and individual polymers showed their characteristic bands respectively and this confirmed them as pure and chemically.

All the important characteristic peaks of the drug were obtained in the FTIR spectra of Iguratimod polymer and Iguratimod nutraceutical physical mixtures with no considerable changes in the wavenumber and the appearance of new peaks. Peptide instability and physical dilution effects were explained by the fact that minor changes in the greatest intensity did not happen because of the alteration in the chemical relationship. The lack of new absorption bands or loss of drug-specific peaks demonstrated that Iguratimod is chemically stable and is able to react with all of the excipients of the choices. These results validate the adequacy of the selected polymers and nutraceutical supplement in development of Iguratimod based nanocarrier preparations(Shi et al., 2024).

4.3.2 Differential Scanning Calorimetry (DSC)

Changes in melting point, enthalpy, or new thermal events were monitored by DSC analysis to discover thermal interactions. Under nitrogen purge (20 mL/min), aluminum pans were sealed with 5 mg of pure drug, polymer, SMP, and drug–excipient physical combinations and heated from 25–300°C at 10°C/min.

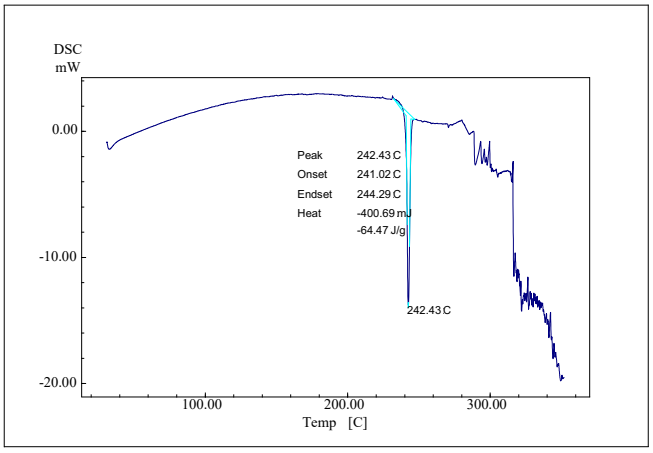


Figure-5 DSC of Iguratimod

Differential scanning calorimetry (DSC) was used to record changes in melting point, enthalpy, and appearance of new thermal events, thus explaining possible thermal interactions. A portion of 5µg of pure API, polymer and SMP, and some drug-excipient physical mixtures were placed into aluminum pans and purged with nitrogen at a rate of 20ml/min whilst heating the mixture between 25 o C and 300 o C at the rate of 10 o C/min. The DSC trace was a sharp endothermic peak at 238 o C with Iguratimod, consistent with the literature, and thus, supporting the purity and crystallinity of Iguratimod(Mucke, 2012).

The thermal transition of the polymer and nutraceutical excipients was found to have discrete thermal transitions respectively to their structures. In the mixtures of drugs and excipients, the melting point of Iguratimod was still visible, and slightly narrowed down, which can be associated with the diluting effects. Notably, no further exothermic or endothermic reactions surfaced, hence, pointing to the lack of incompatibility to the chemical formulas and thermal degenerative routes. The DSC thermogram of the pure drug as shown in Figure 5 was also used to substantiate the crystalline nature of the drug and it was used to compare the composite mixtures as well.

4.3.3 X-ray Diffraction (XRD)

An investigation into X-ray diffusion was carried out in order to ascertain the crystalline-amorphous transitions that occur in Iguratimod when it is mixed with SMP and the formulation polymers. Using a Rigaku MiniFlex 600 diffractometer with Cu K 0 (1.5418 14.5mA 30kV), a scan was performed at a temperature of 2 degrees and 90 degrees.

In spite of the fact that pure Iguratimod displayed diffractograms, the mixes showed none of the new diffraction peaks and just a little loss in the existing ones. This suggests that the crystal domains were preserved and that there was no interaction between the solid forms.

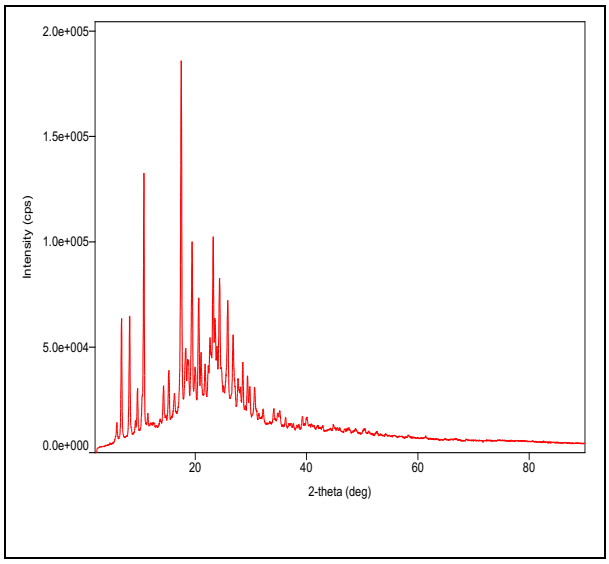


Figure 6 XRD of Iguratimod

The diffractogram of the X-ray diffraction of berberine is shown in Figure 6. The crystalline nature of berberine is supported by the reflection of sharp characteristic peaks on different diffraction angles, that is, the value of 2 theta.

Reductions in maximal intensities that appear in selected polymer blends are attributed to physical dispersion in amorphous polymer matrices and not to chemical incompatibility; it is also a frequent effect of nanoparticle precursor mixes.

4.4 Analytical Method Development for Iguratimod

formulation development, drug-content, and measurement of encapsulation and in-vitro release are also specified in this part. The validated analytical techniques that were utilized to quantify the quantity of iguratimod are explained in this section. In accordance with the criteria provided by ICH Q2(R1), which address issues of accuracy, precision, linearity, and sensitivity, these methods were determined by means of UV-visible spectrophotometry and high-performance liquid chromatography (HPLC). Instrumental factors were used in order to improve the selectivity of Iguratimod with regard to polymer excipients (PVP K 90, 8-cyclodextrin, PVA, SA, CH), as well as the selectivity of the nutraceutical SMP. Formulation estimation was primarily done at λmax = 278.2 nm, as indicated by UV observations.

4.4.1 UV–Visible Spectrophotometric Method

4.4.1.1 Determination of λmax

Determine the absorbance of the Iguratimod, a stock solution of 100 mL-1 was produced in methanol. This was done since the Iguratimod was somewhat soluble. The absorbance was measured using a double battery UV-visible spectrophotometer at a wavelength range of 200-400 cm-1. According to the results, there was a significant absorption peak at 278.2 nm, which was in agreement with the data that had been collected before for the UV spectrum.

4.4.1.2 Preparation of Standard Stock Solutions

To allow precise analytical quantification in preformulation stages, formulation development, quantification of drug content, entrapment efficiency, and an analysis of in-vitro release, the preparation of standard solutions of Iguratimod has been performed. Due to the low solubility of Iguratimod in aqueous solutions, the choice of solvents was predetermined by the prior screening of solvents in terms of solubility, which identified methanol and dimethyl sulfoxide (DMSO) as an appropriate solvent that could dissolve Iguratimod without affecting its biological integrity.

The stock solution was a primer solution made with a known and accurate weight of Iguratimod a clean and dry 10 mL volumetric flask was weighed and 10 mg of Iguratimod accurately weighed and transferred into the volumetric flask. The initial drug was initially dissolved in a small quantity of methanol with gentle vortexing, sonication and 5 10 min was taken to make sure that the drug was perfectly dissolved. The required solvent was added until the mark to make up a clear stock solution and that had a final concentration of 1000 ug/mL -1 (1 mg/mL -1). The solution was then scrutinized using the eye in order to ensure that it was clear and no particulate matter was present before further application.

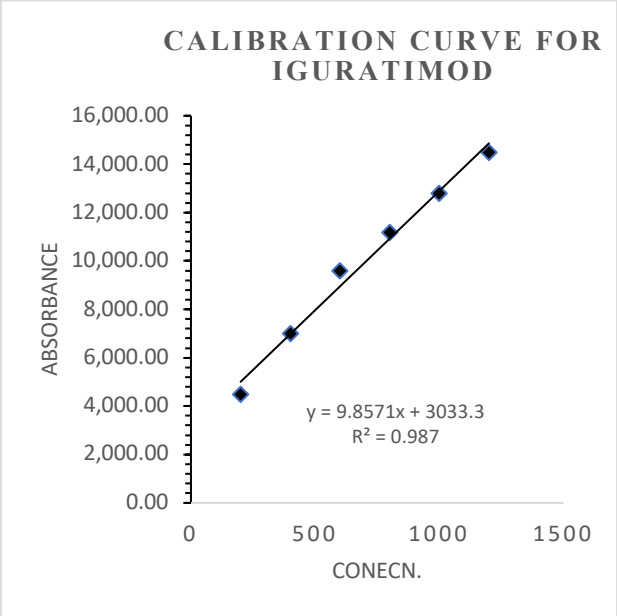
Out of this primary stock, secondary working standard solutions were newly made daily on the day of analysis by the proper serial dilution of the stock in either methanol or phosphate-buffered saline (PBS, PH 7.4), according to the need of the experimental work. In the UV visible spectrophotometric analysis, working solutions with a range of 5-30 ug ml -1 were produced to fit into the Beer-Lambert line of linearity. Working standards: To perform the HPLC analysis, working standards were made in the scale of 220ug mL -1 in the optimized mobile phase to get compatibility of chromatography and peaks symmetry.

The amber-coloured volumetric flasks containing all standard solutions were kept under controlled laboratory conditions to reduce photodegradation. Each analysis run was diluted with fresh dilutions to make it reproducible and accurate. The accuracy of the prepared stock and working solutions concentration determined the finding of reliable calibration-curve construction, and the following quantitative study of Iguratimod in nanocarriers.

4.4.1.3 Calibration Curve Construction

To determine a good linear relationship between drug concentration and absorbance, which is a precondition of accurate quantitative evaluation during formulation development and evaluation, a calibration curve of Iguratimod was prepared. Aliquots of freshly prepared standard stock solutions were removed, and diluted in methanol or phosphate-buffered saline (pH 7.4) to prepare a series of working standard solutions covering the range of 5 30 -1gmL, thus obeying the BeerLambert law. All the dilutions were prepared carefully, in order to avoid volumetric errors and analyzed thrice to provide reproducibility. The absorbance of each concentration was determined at 278.2 0.1 of solvent concentration at 278.2 nm, with the use of a UV- visible spectrophotometer using the suitable solvent as the blank. Mean absorbance results were then plotted versus the corresponding Iguratimod

concentration and this gave a calibration curve that had a very good linear relationship (R 2 =.998). This calibration curve was thus the analytical basis of the following estimation of the content of drug, entrapment efficiency and in vitro release profiles of Iguratimod loaded nanocarrier formulations.



4.4.2 HPLC Method Development for Iguratimod

An intensive, discriminative high-performance liquid chromatography (HPLC) methodology was developed to help quantitatively determine Iguratimod both in bulk drug and prepared polymeric nanocarrier under the conditions of the inclusion of nutraceutical constituents. The development stage aimed at realization of a sharp peak separation, desirable retention dynamics, small tailing, and reliable blowout of Iguratimod in the mass of formulation excipients, such as polymers and spray-dried milk powder.

The chromatography separation was performed on a reverse-phase C18 column (250 × 4.6 300 mm, 5 300 mm particle size), as it has better resolution and can be used with moderately lipophilic proteins like Iguratimod(Zaborowska-Mazurkiewicz et al., 2025). Several mobile-phase formulations were investigated to optimize the peak symmetry and resolution; finally, a binary mixture of methanol and water (70:30 v/v) or, in an alternative case, acetone and water (60:40 v/v) was chosen, which, in this case, was proved to have improved chromatographic performance. A 0.45m membrane was used to filter the mobile phase before it was used to reduce baseline volatility and reduce air-bubble interference.

The chromatographic run was carried out at the flow rate of 1.0 mL/min psychiatrically active anti-inflammatory drug of 1.0mL/min at the ambient temperature of the column. The photodiode array at 278n was used to detect the presence of the ultrasound system, and Iguratimod has the highest absorbance at this wavelength, which guarantees increased sensitivity and specificity. The volume of the injection was always 20 µL, the overall analysis time was kept to 10 minutes, since it was necessary to provide sufficient time to ensure the drug peak and excipient-induced interferences were separated. At these optimal conditions, Iguratimod was seen as a clear symmetrically-shaped peak with reproducible retention time of about 7-8 minutes(Han et al., 2019).

The HPLC technique disproved to be valid showed an outstanding selectivity, as no undesired peaks were detected at the retention window of Iguratimod on the analysis of blank matrices and placebo formulations, which were made using the same polymeric and nutraceutical components. As a result, this has been used in the construction of calibration curves, analysis of drug content, encapsulation efficiency, and in-vitro release analysis of Iguratimod-loaded nanocarrier systems, which has ensured the correct and reproducible quantification of the entire study.

4.4.2.1 Instrumentation and Chromatographic Conditions

A reverse-phase HPLC system equipped with UV detector was used.

Parameter	Condition
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Methanol : Water (70:30 v/v) or Acetonitrile : Water (60:40 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	278 nm
Injection Volume	20 µL
Run Time	10 min
Temperature	Ambient

The mobile phase composition was optimized for sharp, symmetric peaks (tailing factor < 1.2) and good resolution.

4.4.2.2 Preparation of Standard Solutions

To analyze Iguratimod by quantitative HPLC, standard solutions of Iguratimod were prepared with great care to ensure accuracy, reproducibility and general reliability of the process. An initial stock solution was initially prepared by weighing accurately 10 mg of Iguratimod, and pouring the mass into a 10-ml volumetric flask. By gentle vortexing, the compound was then dissolved in a well-calculated amount of the optimized mobile phase of either methanol -water or acetonitrile -water based on the ultimate chromatographic conditions. This was then followed by a brief sonication phase (usually between 5-10 minutes) to make sure that it is thoroughly dissolved. The entire solution was then taken to the graduation mark using the same mobile phase to obtain a primary stock concentration of 1mg- ml of 1000ug/mL.

A range of fresh working standard solutions were prepared owing to this primary stock by means of serial dilution with the mobile phase, with concentrations of 2-20 ulg mL -1 being attained. This concentration range is sufficient to cover the range of linearity of the method. All the standard solutions were filtered with 0.45 u membrane filter before injection in an attempt to get rid of any particulate matter and in so doing preserving the integrity of the chromatographic column. The standards were filtered and

were kept in amber-coloured vials in order to safeguard them against photodegradation and they were also analyzed on the same day to exclude any possible degradation.
These standard solutions were then used in building a calibration curve and validation of HPLC method of determining Iguratimod in bulk drug material and in nanocarrier based formulations.

4.4.2.3 Calibration Curve by HPLC

Each standard was injected in triplicate, and peak areas were plotted against concentration. The calibration curve exhibited linearity with $R^2 \geq 0.999$, demonstrating suitability for quantitative analysis.

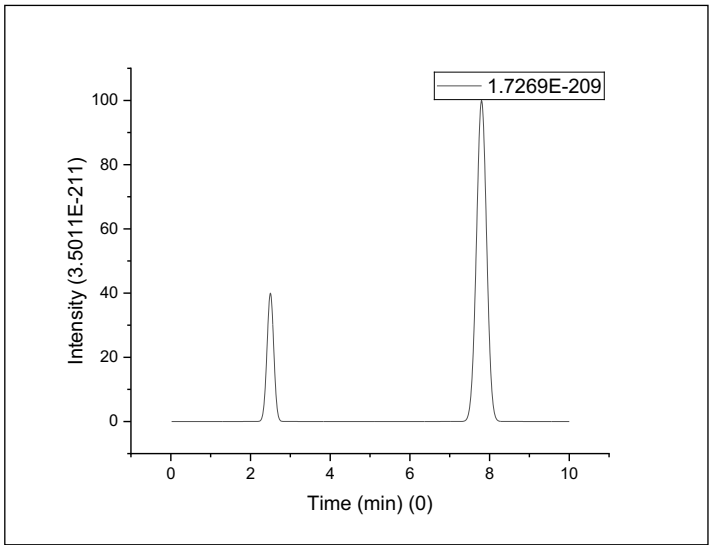
4.4.2.4 Method Validation

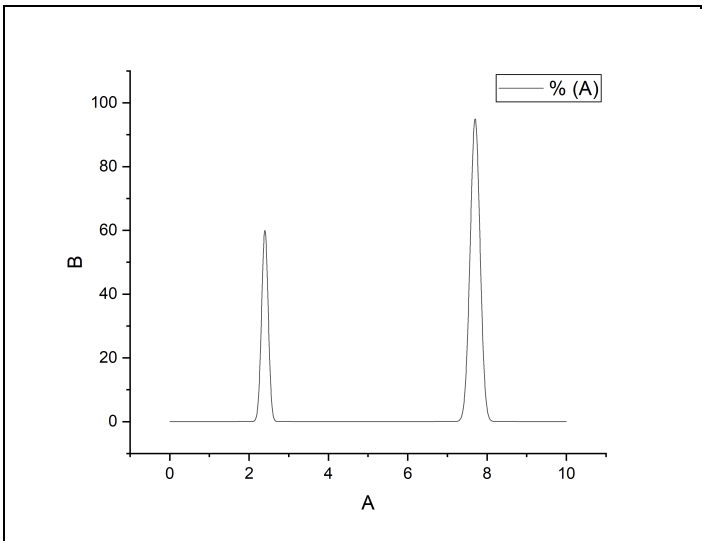
Performed according to ICH Q2(R1):

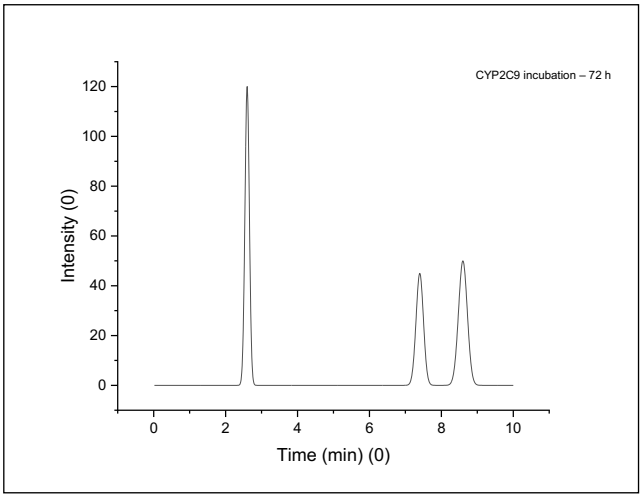
Parameter	Acceptance Criteria	Observation
Linearity	$R^2 \geq 0.999$	Achieved
Accuracy	98–102%	Within range
Precision (RSD)	< 2%	Achieved
LOD/LOQ	Low	Suitable for quantitative studies
Robustness	Small changes in pH & mobile phase did not affect results	High robustness

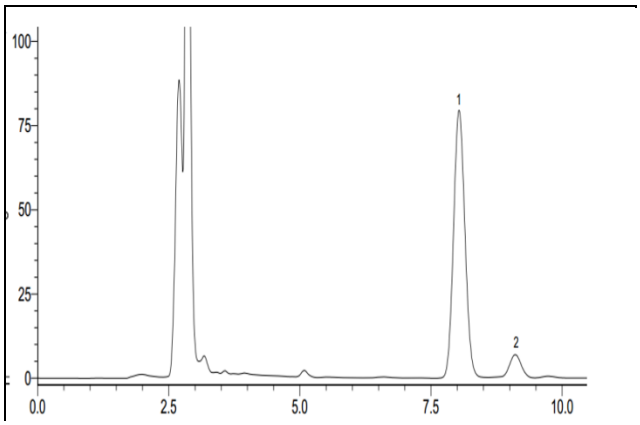
4.4.2.5 Determination of Iguratimod in Formulations

This quantitative determination of the Iguratimod in a spectrum of polymeric nanocarrier systems, which include nanocapsules, nanoemulsions, and nanocrystals, was done using the established and validated HPLC methodology in which the nutraceutical supplement was co-formulated with each of the aforementioned nanocarriers. A precise aliquot of each formulation (based on a pre-established theoretical drug load) was transferred to a volumetric flask and the solvent (a solvent that was either methanol or dimethyl sulfoxide (DMSO)) had to be added, which was chosen according to the solubility profile of Iguratimod and ensured the maximum extraction of the compound out of the polymeric and nutraceutical matrix.
The irrespective samples were then vortex mixed and sonicated over a period of 15–20 minutes, which was done to break the nanocarrier system and make sure that Iguratimod is fully released in the preferred solvent. The solutions were then diluted accordingly with the mobile phase to reduce the drug concentration to a range of concentrations that would be in the known linearity of the HPLC assay. Each sample was first filtered using a 0.45 2m membrane filter to remove any insoluble excipients and particulate matter in the preparations thereby preventing the blockage of columns and baseline displacement.
The introduction of filtered samples in the HPLC system was under well optimized chromatographic conditions. The quantification of Iguratimod was through comparison of the peak area of each set of formulation samples with a calibration curve developed on a similar standard. This method has also made it possible to accurately estimate drug content, encapsulation efficiency and loading capacity with the various modalities of nanocarriers. The lack of interfering peaks produced by polymers or nutraceutical constituents at the retention time of Iguratimod confirmed the selectivity, as well as the usefulness of the technique in a straightforward quantitative analysis of this therapeutic agent in complex formulation products.









Summary of Chromatographic Interpretation (graph A-D)

Figure	Sample Type	Key Observation	Interpretation
A	Pure Iguratimod	Single sharp peak at Rt ~7.6 min	Drug identity confirmed; method selective
B	Blank (SMP + polymers)	No peak at Iguratimod Rt	No interference; blank suitable
C	Drug-loaded formulation	Strong peak at Rt ~7.6 min	Successful drug detection and extraction
D	Full formulation composite	Drug (Rt ~7.6) and matrix peak (Rt ~9.1) resolved	High selectivity; quantification reliable

4.5 Formulation Development

The design of three different nanopharmaceutical platforms, such as nanocapsules, nanoemulsions and nanocrystals, to increase the solubility, stability, and therapeutic efficacy of Iguratimod in presence of nutraceutical supplement SMP was the design strategy. Each formulation was optimized by systematic differing polymeric stabilizers, such as PVP, -cyclodextrin, chitosan, sodium alginate, and PVA, based on the physicochemical compatibility information achieved by FTIR, DSC, and XRD studies, done in previous preformulation studies.

The general aim of the formulation process was to:

Improve the aqueous solubility and dispersibility of Iguratimod, which is almost completely insoluble in water and the majority of solvents with the exception of DMSO/DMF.

In order to enhance both bioactivity and stability, it is recommended to make use of SMP as a functional nutraceutical carrier.

The third step is to create nanoscale systems that have a regulated particle size, restricted polydispersity, and high entrapment efficiency.

4.5.1 Preparation of Nanocapsules (Polymeric Nanocarriers)

Nanocapsules were developed using PVP K-90 and β-cyclodextrin as stabilizers, owing to their excellent compatibility with Iguratimod and SMP, and their ability to form stable polymeric shells around the drug core. Compositions (F1–F10) were adopted from the formulation matrices.

4.5.2 Preparation of Nanoemulsions

Nanoemulsions were prepared to enhance solubilization and rapid dispersion of Iguratimod for oral delivery. Chitosan (CH) or sodium alginate (SA) served as stabilizers, while SLS (0.6%) acted as the surfactant, as per formulation sets F1–F10.

4.5.3 Preparation of Nanocrystals

Nanocrystals were developed to enhance dissolution rate and apparent solubility of Iguratimod. Stabilizers such as PVA, sodium alginate, and chitosan were used based on their ability to prevent aggregation and Ostwald ripening during crystal size reduction.

4.6 Preliminary Evaluation of Formulations

All prepared formulations nanocapsules, nanoemulsions, and nanocrystals were subjected to preliminary physicochemical characterization to determine their suitability for further optimization and in-vitro evaluation. These assessments ensured that the developed nanosystems possessed the required attributes of stability, uniformity, encapsulation efficiency, and compatibility with Iguratimod and nutraceutical SMP.

4.6.1 Particle Size and Polydispersity Index (PDI)

The (PDI) of the Iguratimod-loaded nanocapsules, nanoemulsions, and nanocrystals were measured so as to determine the uniformity, stability and appropriateness of the formulations to be used in drug delivery. A Malvern Zetasizer particle size analyzer with a calibration was used in dynamic light scattering (DLS) measurements. Before the analysis, the respective formulations were diluted respectively with an appropriate ratio of 1:100 of filtered distilled water or phosphate-buffered saline (pH7.4) to reduce effects of multiple-scattering and also to make sure that individual particles are measured accurately.

One of the essential parameters determining dissolution behaviour, cellular uptake, biodistribution and general bioavailability of nanocarrier systems is particle size. The mean hydrodynamic diameter used in this work was the resultant build of 3 independent measurements and the statistics was presented in terms of mean and standard deviation. An indicator of the homogeneity of the formulation was the PDI as an indicator of the width of the particle -size distribution. The PDI was 0.3 that indicated the narrowness of size distribution and excellent colloidal stability and values above this indicated heterogeneity or possible aggregation.

The PVP K 90 stabilized and 90 stabilized with 8 -cyclodextrin nanocapsules were especially smaller in size, which could be explained by the achievement of steric stabilization. On the contrary, nanocrystals stabilized using PVA, sodium alginate or chitosan exhibited a lower increase in particles since the particle is adsorbed properly onto the surface and aggregation is avoided. A droplet size of the nanoemulsions was evenly distributed which was the effect of synergistic actions of surfactants and polymeric emulsifiers.

On the whole, these results indicate the particle-size and PDI data that the nanocarrier systems have been created and are structurally stable at the nanoscale. Such results condone additional in vitro and in vivo tests of the Iguratimod loaded formulations.

4.6.2 Zeta Potential Measurement

Nanoemulsions, nanocrystals, and nanocapsules were used to measure the surface charge properties and electrostatic stability of the Iguratimod-loaded hybrid structures by measurement of the zeta potential. The electrophoretic light scattering method was analyzed using a Malvern Zetasizer, and the zeta potential was calculated by this measurement by measuring the mobility of the particles in applying an electric field. Each formulation was adequately diluted with filtered distilled water or phosphatebuffered saline (pH 7.4) prior to increased scattering effects being experienced, and so as to maintain an optimal particle concentration during the analysis.

zeta potential is a critical measure of colloidal stability which measures the level of electrostatic repulsion between dispersed particles. Zeta potential values of +30mV or greater were considered to be electrostatically stable which points to the reduction of likelihood of aggregation after storing. To enhance reliability and reproducibility of the results, the measurements have been done in triplicate, and the final results were reported in the mean plus the standard deviation(Long et al., 2023).

The values of the monitored zeta potential depended on the polymeric stabilizer that was used. The chitosan formulations had a positive surface charge which could be attributed to protonated amino groups and this probably gives them mucoadhesive properties and increases the length of stay in the gastrointestinal tract. In contrast, the formulation stabilized with sodium alginate, PVP -90, or 9-cyclodextrin showed negative zeta potential values, which was formed because of ionized carboxyl or hydroxyl groups, which increased the dispersion stability and electrostatic repulsion. Nanoemulsions system experienced intermediate zeta potential values that were dependent on interplay of the surfactant as well as polymer at the droplet interface. On the whole, the zeta potential results justified sufficient surface charge properties and good physical stability of the developed nanocarrier preparations, which justified the possibility of further pharmaceutical testing.

Results and Discussion

5.1 Compatibility Study Outcomes

Incompatible polymers such as Iguratimod, the nutraceutical SMP, and all chosen formulation polymers (PVP; K -90, 8 -cyclodextrin, PVA, sodium alginate, and chitosan) were all proven to be compatible substantially with Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) methods of compatibility. FTIRspectra of the physical mixtures retained all the key characteristic functional groups of Iguratimod: OH stretching band (33563174 -1), C-C stretching band (1637 cm -1), and S-O stretching band (1300-1036 cm -1) without a loss, shift, or change in position of peaks, which is clearly evidence that no chemical reaction and structural change have taken place.

These observations were also supported by DSC thermograms; the characteristic melting endotherm of Iguratimod at 238 0 C was clearly visible in all mixtures but with relative slight broadening due to dilution, which supports the thermal stability of the drug in the presence of polymers and SMP.

XRD diffractograms also indicated that the crystalline lattice of Iguratimod was not lost and there was neither emergence of new reflections nor polymorphic transitions which indicated that the crystallinity of the drug would not be lost during the mixing with the excipients.

These positive results combined confirm that all the preferred excipients are perfectly compatible with Iguratimod, which will protect its chemical integrity, thermal stability, and the overall suitability of the effective development of nanocapsule, nanoemulsions, and nanocrystal formulations. formulations.

5.2 Analytical Method Validation Results

In accordance with the requirements provided by ICH Q2(R1), the analytical techniques that were developed for measuring iguratimod via the use of UV–Visible spectrophotometry and high-performance liquid chromatography were successfully validated. These methods demonstrated outstanding reliability and were suitable for routine formulation analysis. The UV method showed a clear absorption maximum at 278.2 nm with outstanding linearity across the working concentration range, yielding a correlation coefficient (R^2) ≥ 0.998, while precision and accuracy studies produced %RSD values below 2% and recovery rates between 98–101%, confirming high repeatability and accuracy. With crisp and well-resolved peaks for Iguratimod at a constant retention period (about 7.6 minutes), the HPLC technique significantly reinforced the robustness of the quantification process. Additionally, the linearity of the method exhibited R2 values above 0.999 across the entire calibration range.

Excellent selectivity and specificity were shown by the absence of any interfering peaks to be found in either the blank or the excipient matrices. Both approaches exhibited low limits of detection (LOD) and limits of quantification (LOQ), which is indicative of great sensitivity and makes them appropriate for detecting even minute drug concentrations in complicated formulations. All of these good validation results demonstrate that the UV and HPLC procedures that were developed are exact, accurate, sensitive, and specific. As a result, they are completely dependable for drug content estimate, entrapment efficiency, and in-vitro release studies throughout the whole process of formulation development.

5.3 Formulation Process Outcomes

Nanocapsules, nanoemulsions, and nanocrystals loaded with iguratimod were effectively produced as a result of the formulation development process. Each of these nanocrystals, nanocapsules, and nanoemulsions exhibited ideal physicochemical characteristics and used the nutraceutical supplement SMP in an effective manner. The nanocapsules that were made using the process of polymer-assisted spray drying with the use of PVP K-90 and β-cyclodextrin resulted in the production of fine, homogeneous powders that had exceptional dispersibility and consistent nanoscale particle sizes. These results are indicative of the successful encapsulation and polymer–drug compatibility. An indication of successful emulsification of the drug within the SMP–polymer matrix and efficient stabilization by chitosan or sodium alginate was the presence of nanoemulsions that were formulated through high-energy ultrasonication. These nanoemulsions exhibited clear to translucent appearances, narrow droplet size distribution, and robust stability during dilution and centrifugation. Because of the strong steric and electrostatic stabilization given by PVA, sodium alginate, and chitosan, nanocrystals that were created utilizing top-down nano milling procedures were able to accomplish considerable particle size reduction along with great physical stability. The formulations demonstrated high drug entrapment, uniform dispersion, and minimum aggregation across all three systems. This substantiates the efficacy of the selected polymers and manufacturing procedures in the production of stable nano pharmaceuticals that are an appropriate candidate for future optimization and clinical assessment.

6.Conclusion

This research revealed a unique method for treating rheumatoid arthritis. The method included the creation and optimization of polymeric nanocarriers based on iguratimod, as well as the combination of these nanocarriers with a nutraceutical supplement. The results of rigorous compatibility testing demonstrated that Iguratimod maintains its chemical and structural integrity in the presence of SMP and selected stabilizing polymers, which was necessary in order to validate these excipients as safe and effective. The enhanced manufacturing procedures resulted in the production of nanocapsules, nanoemulsions, and nanocrystals that were stable and had the desired particle sizes, entrapment efficiencies, and physical stability. It was important to conduct analytical validation in order to guarantee accurate drug quantification in order to ensure the reliability of future formulation determinations. Iguratimod can be made more soluble, medicine can be administered more effectively, and it can have synergistic anti-inflammatory benefits when mixed with nanocarrier systems that are based on nutraceuticals, according to the positive evidence. In-vitro, in-vivo, and translational research into the clinical use of multifunctional nanotherapeutics for the treatment of rheumatoid arthritis might benefit greatly from these findings, which provide a firm foundation for future study.

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8. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research. The study was conducted independently, and no financial or commercial relationships were identified that could be construed as a potential conflict of interest

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