RESEARCH PAPER

Quality by Design-Based Preformulation Studies of Dual Drug-Loaded Electrospun Nanofibers for Rheumatoid Arthritis

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Abstract
Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder requiring long-term pharmacotherapy, often involving combination drug therapy to enhance efficacy and reduce adverse effects. Nanofiber-based drug delivery systems have gained significant attention for their high surface area, tunable drug release, and potential for localized or controlled delivery. The present study aims to conduct a Quality by Design (QbD)–guided preformulation investigation for the development of a dual drug-loaded nanofiber system intended for rheumatoid arthritis. A systematic QbD framework was applied to define the Quality Target Product Profile (QTPP), identify Critical Quality Attributes (CQAs), and perform risk assessment to determine Critical Material Attributes (CMAs) & Critical Process Parameters (CPPs). Preformulation studies included physicochemical characterization, solubility analysis, drug–drug compatibility, drug–polymer compatibility, thermal behavior and evaluation of solution properties relevant to nanofiber formulation. The findings demonstrate that a QbD-based preformulation approach enables systematic risk reduction for development of dual drug-loaded nanofibers for rheumatoid arthritis management.

Keywords: Quality by Design, Preformulation, Dual drug delivery, Nanofibers, Rheumatoid arthritis
How to cite this article: Gaikwad VM, Bhusnure OG. Quality by Design-Based Preformulation Studies of Dual Drug-Loaded Electrospun Nanofibers for Rheumatoid Arthritis. Int J Drug Deliv Technol. 2026;16(6s): 768-779; DOI: 10.25258/ijddt.16.6s.104

Introduction

Rheumatoid arthritis (RA) is a progressive autoimmune disease characterized by chronic synovial inflammation and joint destruction. RA involves inflammation of linings of joints, accumulation of synovial fliud, which contains enzyme metalloprotinease that causes erosion of cartilage. (Joshi et al., 2022)

Treatment of RA with traditional therapy leads to adverse side effects on gastrointestinal tract, hepatic, cardiac and renal function(Nasra et al., 2022). The low solubility, poor pharmacokinetics behavior and non-targeted distribution of small molecule drugs (DMARDs and GCs), not only hamper their efficacy, but also give rise to multiple adverse effects (Wang et al., 2021). Mono drug therapy approach to treat RA is now being gradually be changing with combination therapy of drugs (Janakiraman et al., 2018). Owing to the limitations of these traditional drugs researchers exploring herbal resources for phytoconstituents for the tratment of RA; Ex Zheng Qing Feng Tong Ning (ZQFTN) is an example of TCM (Traditional Chinese medicine) patented drug that consists of phytoconstituent sinomenine (SIN) and was approved in 2013 by China Food and Drug Administration (CFDA) for RA treatment (Joshi et al., 2022). Combination drug therapy can have synergistic therapeutic effect, reduced side effects and increased safety and efficacy.

Nanofiber-based drug delivery systems have gained significant attention for their high surface area, tunable drug release, and potential for localized or controlled delivery. Nanofiber-based drug delivery systems have high drug loading capacity, large surface area-to-volume ratio, and ability to modulate drug release profiles (Kenry & Lim, 2017; Sun et al., 2019)(Parham et al., 2020). Nanofibers containing combination of drugs can also be prepared (Karthikeyan et al., 2012); Ex. Illangakoon et al. produced fibres of PVP loaded with paracetamol and caffeine (often used together to treat colds and influenza). Also modified release dosage form / extended release system (also known as controlled-

release, prolonged- release, or sustained or slow-release) for releasing drug over prolonged period electrospun fibers can be formulated with appropriate choice of polymers. Due to the side effects of synthetic or chemical drugs sometime results in discontinuation of treatment, so use of the biologically active compounds that are obtained from plants i.e phytoconstituents for the treatment of RA is emerging. Also combination of these synthetic drugs with the phytoconstituents have been suggested; Ex. DMARDS combination with phytoconstituents gives synergestic therapeutic effect and reduced side effects. (Kour et al., 2021)

QbD approach built quality into the formulation from the early stages. According to ICH Q8(R2) guideline, Quality by Design (QbD) is "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and Process control, based on sound science and Quality Risk Management (Gandhi & Roy, n.d.). Preformulation studies aims to understand the fundamental physicochemical properties of the drug substances and excipients, which directly influence formulation and process. In the development of dual drug-loaded electrospun nanofibers, preformulation studies are particularly important due to the complexity of incorporating two active pharmaceutical ingredients into a single delivery system. Furthermore, preformulation studies support risk assessment and rational selection of polymers, solvents, and drug ratios, which are essential for achieving consistent product quality, thereby improving overall product performance in line with QbD principles.

The objective of the preformulation study was to evaluate the physicochemical properties, solubility, stability, and compatibility of the selected drugs and polymers to support the Quality by Design–based development of dual drug-loaded electrospun nanofibers for rheumatoid arthritis. The study aimed to identify critical material attributes and select suitable formulation components to ensure stable, compatible, and reproducible nanofiber formulation. The present work focuses exclusively on a QbD-guided preformulation study for a dual drug-loaded nanofiber system intended for rheumatoid arthritis.

Quality by Design (QbD) Framework for Preformulation

2.1 Quality Target Product Profile (QTPP) – Preformulation Perspective

From a preformulation perspective, the Quality Target Product Profile (QTPP) defines the desired characteristics of the dual drug-loaded electrospun nanofiber system to ensure safety, efficacy and quality (Singh, 2014). The target product is intended to deliver two therapeutic agents effectively for the management of rheumatoid arthritis, with suitable drug loading, uniform distribution and controlled drug release. Preformulation studies focus on selecting drugs, polymers, and solvents with appropriate physicochemical properties, stability, and compatibility to support smooth electrospinning and consistent nanofiber formation (Nazari et al., 2020).

- Quality Attributes of electrospun nanofibers were as follows:

QTPP Attribute	Target Value	Justification
Dosage form	Electrospun nanofiber	Enables flexible, high-surface-area drug delivery
Route of administration	Local or systemic (as intended)	Non-invasive, localized or systemic delivery
Drugs loading	Dual APIs	For synergistic, combination therapy
Drug release profile	Immediate release of one API followed by Sustained release of other	Matches therapeutic need (e.g., fast pain relief + prolonged anti-inflammatory)
Drug–polymer compatibility	No chemical or physical interaction between drugs and polymer	Ensures drug excipient compatibility
Content uniformity	Uniform drug distribution	Ensures consistency and efficacy
Fiber morphology	Smooth, bead-free fibers with uniform diameter	Directly affects drug release, mechanical and aesthetic quality
Stability	6–12 months minimum under ICH conditions	Shelf life requirement
Appearance	Uniform, no discoloration or cracking	Patient acceptability

2.2 Identification of Critical Quality Attributes (CQAs)

CQAs are defined as the physical, chemical, biological, or microbiological properties or characteristics that must be controlled within predefined limits to ensure the desired quality, safety and performance of the final product (Singh, 2014).

For dual drug-loaded nanofiber-based drug delivery systems, the following CQAs were identified based on the Quality Target Product Profile (QTPP)

- Fiber diameter
- Fiber Morphology (uniformity, presence or absence of beads)
- Drug encapsulation efficiency
- Drug content uniformity
- In vitro drug release profile
- Wettability (contact angle)
- Mechanical integrity

QbD-Based Rationale for Selected CQAs

Critical Quality Attribute (CQA)	Impact on Product Quality & Performance	QbD Rationale for Inclusion
Fiber diameter	Influences surface area, drug distribution, release kinetics, and reproducibility of performance	Identified as a critical physical attribute affecting drug release behavior and consistency of the nanofiber system
Fiber morphology (uniformity, bead formation)	Non-uniform or beaded fibers may cause inconsistent drug loading and uncontrolled release	Considered critical as morphology reflects material compatibility and directly affects functional performance
Drug encapsulation efficiency	Determines actual drug dose delivered and therapeutic effectiveness	Included to ensure adequate drug incorporation and minimize dose variability in dual drug systems
Drug content uniformity	Ensures dose accuracy and prevents localized over- or under-dosing	Critical for quality assurance, particularly for combination therapy requiring precise dosing
In vitro drug release profile	Governs therapeutic effectiveness and duration of action in chronic diseases such as rheumatoid arthritis	Identified as a key performance attribute linked to clinical outcome and patient compliance
Wettability (contact angle)	Affects hydration, drug dissolution, and initial drug release	Included due to its influence on nanofiber–biological fluid interaction and release behavior
Mechanical integrity	Ensures ease of handling, storage stability, and application performance	Considered critical to maintain physical integrity throughout product lifecycle

2.3 Preliminary Risk Assessment for Selection of CPP (Critical Process Parameters) & CMA (Critical Material Attributes):

Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) were identified through literature review, prior knowledge of electrospinning-based nanofiber systems and their potential impact on predefined Critical Quality Attributes (CQAs). Attributes and parameters that were expected to have a direct or indirect influence on fiber morphology, drug loading, release behavior, and mechanical properties were considered critical in accordance with the Quality by Design framework (Yu et al., 2014).

Identification of CMAs and CPPs

i. CMA: CMA identified were as follows

1. Drug – Solubility, Crystallinity
2. Polymers- Concentration, Molecular weight, hydrophilicity.
3. Solvent - Ratio of solvent, Volatility, compatibility with both drugs.
4. Excipients – Type and concentration

ii. CPP identified were as follows

1. Applied voltage
2. Flow rate
3. Needle–collector distance
4. Collector design (flat/rotating)
5. Ambient conditions (humidity, temp)
6. Solution prep sequence (drug order, mixing, filtration)

Critical Material Attributes (CMAs) for Dual Drug-Loaded Nanofibers

A. API-Related CMAs

CMA	Why It Is Critical (QbD Rationale)
Drug solubility	Determines miscibility in polymer solution and uniform drug distribution within nanofibers
Drug–drug compatibility	Incompatibility may cause precipitation, degradation, or phase separation in dual-drug systems

Melting point / thermal stability	Drug may degrade under thermal stress during electrospinning and solvent evaporation
Crystallinity / polymorphism	Influences drug release behavior, stability, and encapsulation efficiency
LogP / pKa	Affects drug–polymer interaction, diffusion through polymer matrix, and release kinetics
Maximum feasible drug loading	Excessive loading may compromise fiber integrity, morphology, and content uniformity

B. Polymer-Related CMAs

CMA	Why It Is Critical (QbD Rationale)
Polymer type (e.g., PVP, PCL, PLA)	Determines hydrophilicity, mechanical strength, and drug release characteristics
Molecular weight	Influences solution viscosity, fiber formation, and mechanical integrity
Polymer concentration	Controls solution viscosity and chain entanglement, essential for uniform fiber formation
Polymer–drug compatibility	Affects encapsulation efficiency, stability, and release behavior
Polymer blending ratio	Impacts mechanical properties, wettability, and drug release modulation

3. Solvent -Related CMAs

CMA	Why It's Critical (QbD Rationale)
Solvent type and ratio	Influences drug solubility, fiber drying rate, morphology
Volatility / evaporation rate	Affects bead formation and fiber surface properties
Toxicity / residue potential	Especially critical for oromucosal or skin-contact applications

Critical Process Parameters (CPPs) for Dual Drug-Loaded Nanofibers

CPP	Why It Is Critical (QbD Rationale)
Applied voltage	Controls jet stretching, whipping instability, and fiber diameter
Flow rate (solution feed rate)	Influences fiber thickness, morphology, drug loading, and bead formation
Needle–collector distance	Determines solvent evaporation time and fiber solidification
Collector design (static/rotating)	Affects fiber alignment, deposition uniformity, and mechanical integrity
Ambient temperature	Influences solvent evaporation rate and polymer solidification behavior
Relative humidity	High humidity may cause bead defects, fiber fusion, or phase separation
Stirring/mixing time	Ensures homogeneity of dual drug–polymer solution

A QTPP–CQA–CMA–CPP linkage matrix was constructed to establish a systematic relationship between target product characteristics, critical quality attributes, material attributes and process parameters. This matrix served as the foundation for the preliminary risk assessment and facilitated identification of high-risk variables requiring further investigation.

QTPP–CQA–CMA–CPP Linkage Matrix for Dual Drug-Loaded Electrospun Nanofibers

QTPP Attribute	Linked CQA(s)	Key CMAs (Material Attributes)	Key CPPs (Process Parameters)
Dosage form: Electrospun nanofiber	Fiber diameter, fiber morphology	Polymer type, polymer molecular weight, polymer concentration, solution viscosity	Applied voltage, flow rate, needle–collector distance
Route of administration (local/systemic)	Wettability, mechanical integrity	Polymer hydrophilicity, polymer blending ratio	Ambient humidity, collector design
Dual drug loading	Drug content uniformity, encapsulation efficiency	Drug solubility, drug–drug compatibility, polymer–drug	Mixing time, drug addition sequence

		compatibility	
Drug release profile	In vitro drug release	Drug crystallinity, LogP/pKa, polymer hydrophilicity, polymer concentration	Voltage, flow rate, ambient temperature
Drug–polymer compatibility	Encapsulation efficiency, stability	Drug solubility, crystallinity, polymer type	Solution preparation sequence
Content uniformity	Drug content uniformity	Drug solubility, polymer–drug miscibility	Flow rate, mixing time
Fiber morphology	Fiber diameter, morphology	Polymer concentration, solvent type and ratio, surface tension	Voltage, humidity, needle–collector distance
Stability (6–12 months)	Mechanical integrity, morphology	Polymer molecular weight, crystallinity, residual solvent content	Collector type, spinning duration, ambient conditions

A preliminary risk assessment (RA) was conducted in accordance with the Quality by Design (QbD) to evaluate the potential impact of critical process parameters (CPPs) and material-related attributes on the predefined critical quality attributes (CQAs) of the dual drug-loaded nanofiber system. The objective of this assessment was to identify high-risk factors that may significantly influence nanofiber properties

Each CPP was assessed for its potential impact on fiber diameter, fiber morphology, drug release behavior, encapsulation efficiency, and mechanical integrity. The level of risk was categorized as High, Medium, or Low, depending on the extent of influence on the respective CQA. Parameters exhibiting high risk across multiple CQAs were identified as critical and suggest further investigation during subsequent formulation and process optimization studies.

Table: Preliminary Risk Assessment of Critical Material and Process Parameters (Nazari et al., 2020; Singh, 2014)

Parameter	Type	Fiber Diameter	Fiber Morphology	Drug Release	Encapsulation Efficiency	Mechanical Integrity	Overall Risk
Drug solubility	CMM	High	Medium	High	High	Medium	High
Drug crystallinity	CMM	Medium	Medium	High	Medium	Medium	Medium
Polymer concentration	CMM	High	High	Medium	Medium	High	High
Polymer molecular weight	CMM	High	High	Medium	Medium	High	High
Polymer–drug compatibility	CMM	Medium	Medium	High	High	Medium	High
Solvent system (type/ratio)	CMM	High	High	High	Medium	Medium	High
Applied voltage	CPP	High	High	Medium	Medium	Medium	High
Flow rate	CPP	High	High	High	High	Medium	High
Needle–collector distance	CPP	High	Medium	Medium	Medium	Medium	Medium
Ambient	CP	Me	High	Me	Low	Medi	Me

humidity	P	dium		dium		um	dium
Ambient temperature	CPP	Medium	Medium	Medium	Medium	Low	Medium
Collector type/ speed	CPP	Low	Medium	Low	Low	High	Medium

 = High Risk → Must be controlled/tuned precisely
 = Medium Risk → should be monitored.
 = Low Risk → Minimal effect or easy to control

2.4 Physicochemical Characterization (CMAs)

1. Organoleptic Properties of API:
Sample of drug sulfasalazine and quercetine was evaluated for its physical appearance like color, odor, taste crystalline nature etc.

2. Melting point- Melting point of sulfasalazine & quercetine was determined by using capillary method.

3. Solubility: Solubility of both the drugs sulfasalazine & quercetine was tested in organic solvents like water, methanol, ethanol, DMF, DMSO etc
Solubility of individual drugs was determined by adding excess amount of drug in 10ml of solvent, stirring at room temperature followed by filteration though 0.45um filter and then analyzed supernatant at respective; at 359 nm by UV spectrophotometer for sulfasalazine and 370nm for quercetine (Haleagrahara et al., 2018; Mattam & Krishna Sailaja, 2016; Psimadas et al., 2012).

2.5 Polymer Solubility and Spinnability Screening

Different polymers were screened for solubility in various organic solvents, including methanol, ethanol, DMF, DMSO, and acetone. Polymer solutions at different concentrations (10–30% w/v) were prepared in solvents in which complete polymer dissolution was observed. In addition, mixed solvent systems such as ethanol:DMSO, ethanol:DMF, acetone:DMF, and water:ethanol were evaluated to assess their suitability for polymer dissolution.
The spinnability of the prepared polymer solutions was assessed by electrospinning using a 2 mL syringe at a fixed flow rate of 0.05 mL/min. Polymer solutions capable of producing continuous fibers were further evaluated. Initial fiber formation and morphology were examined using an optical microscope. Based on solubility behavior and spinnability screening, a suitable polymer and solvent system were selected for subsequent studies.

2.6 Drug– Excipient Compatibility Studies

FTIR Spectra: To study drug excipient interaction FTIR spectra of individual drug and polymer sample was recorded and also spectra of their physical mixture were taken.

DSC Study: Thermal analysis of drug, polymer & physical mixture was done by DSC and thermograms were obtained.
XRD study: Powder X-ray diffraction patterns of pure drugs, polymer(s), and physical mixtures were recorded using an X-ray diffractometer.

2.7 Preliminary Electrospinning Trials

Electrospinning Process

Preliminary electrospinning trials were conducted to formulate nanofiber using the selected polymer–solvent system identified during preformulation screening; from spinnability screening
Quercetin solution prepared with ethyl cellulose 10% in ethanol. Sulfasalazine solution with 12%w/v of PVP K-90 was prepared in ethanol: DMF solvent system.
For both solutions individual electrospinning (monoaxial/Uniaxial) was done and nanofibers were formed. Variation in the voltage, spinneret- to- collector distance and flow rate for the single- liquid process was done so as to select the value that gives best fiber formation. Fibers produced were collected on aluminium foil, observed by holding them up to light and further by optical microscope. Initial fiber assessment done by optical microscopy to saw the bead-less, smooth fibers.
Electrospinning was performed with 2ml syringe loaded with drug containing polymer solution, using Super ES-2 E-spin Nanotech m/c (Channabasweshwar Pharmacy College, Latur MH). To assess the effect of applied voltage, electrospinning was carried out at voltages ranging from 15 to 20 kV while maintaining a constant flow rate and needle–collector distance (15 cm). The influence of flow rate was evaluated by varying the flow rate between 0.01 and 1.0 mL/min at fixed voltage and distance. Similarly, the effect of needle–collector distance was studied by adjusting the distance between 10 and 20 cm while keeping voltage and flow rate constant. All experiments were conducted at ambient laboratory conditions (25 ± 2 °C). (Bhusnure et al., 2021; Sa'adon et al., 2021; Sipos et al., 2019)

2.8 Preliminary Nanofiber Characterization

Electrospun nanofiber mats obtained during preliminary electrospinning trials were initially examined using an optical microscope to qualitatively assess fiber formation. The surface morphology of electrospun nanofibers was examined using scanning electron microscopy. These preliminary morphological evaluations provided early confirmation of process

feasibility and supported risk-based selection of critical process parameters.

3. Result & Discussion

3.1 Risk Assessment of Critical Material and Process Parameters

A systematic risk assessment was conducted at the preformulation stage to identify and prioritize CMAs and CPPs that could potentially impact the CQAs of dual drug-loaded electrospun nanofibers. The assessment was performed in accordance with Quality by Design (QbD) principles described in ICH Q8(R2), with the objective of establishing process understanding.

Risk Assessment of Critical Material Attributes (CMMs)

Drug-related attributes such as solubility and crystallinity were identified as high-impact material attributes due to their direct influence on drug dispersion within the polymer matrix, encapsulation efficiency and in vitro drug release behavior. Poor drug solubility or high crystallinity may result in drug precipitation during electrospinning, leading to heterogeneous fibers and inconsistent release profiles. Therefore, drug solubility was classified as a high-risk CMM affecting multiple CQAs. Polymer-related attributes, including polymer type, molecular weight and concentration, were also categorized as high-risk material attributes. Polymer concentration and molecular weight play a critical role in solution viscosity and chain entanglement, which directly govern fiber diameter, morphology, and mechanical integrity. The solvent system (type and ratio) was identified as another high-risk CMM, as it influences drug solubility, polymer dissolution, solution conductivity and solvent evaporation rate during electrospinning. Variations in solvent properties can significantly affect fiber morphology, drug encapsulation efficiency and release characteristics. Drug–polymer compatibility, although confirmed experimentally through FTIR, DSC, and XRD studies, was conservatively assigned a high impact on functional CQAs such as drug release and encapsulation efficiency.

Risk Assessment of Critical Process Parameters (CPPs)

Among process-related parameters, applied voltage and flow rate were identified as high-risk CPPs due to their dominant influence on jet initiation, jet stability, fiber stretching, and solvent evaporation (Nazari et al., 2020). As a result, these CPPs were classified as high risk for multiple CQAs, including fiber diameter, morphology, and drug release. Needle–collector distance was categorized as a medium-risk CPP. While it significantly influences solvent evaporation and fiber drying, its impact on CQAs is generally less severe and more easily controlled compared to voltage and flow rate (Pelipenko et al., 2015). Ambient conditions such as humidity and temperature were also assigned medium risk, as fluctuations can affect fiber morphology and surface characteristics but are typically manageable under controlled laboratory conditions.

Risk Prioritization and QbD Implications

Based on the combined risk assessment, material attributes related to polymer characteristics, solvent system, and drug solubility, along with process parameters such as applied voltage and flow rate, were prioritized for experimental evaluation during preformulation and preliminary electrospinning trials. Medium-risk parameters were monitored to ensure process consistency, while low-risk parameters were considered to have minimal impact on product quality.

3.2 Physicochemical Characterization:

The drug was observed for organoleptic characteristics such as color, odor, and taste. Basic physicochemical attributes supports early identification of CMAs related to drug identity and physical state. The absence of abnormal odor or discoloration was studied suggested low risk of degradation or impurity-related concerns at the preformulation stage.

Properties	Sulfasalazine	Quercetine
Color	Bright yellow fine powder	Yellow crystalline
Odor	Odorless	Odorless
Taste	Tasteless	Bitter
Melting Point	248^0C	316^0C

From a Quality by Design perspective, melting point serves as a critical indicator of the solid-state nature and thermal stability of the active pharmaceutical ingredients. The high melting points observed for both sulfasalazine and quercetine suggest a stable crystalline structure with low susceptibility to thermal degradation under electrospinning conditions. Consequently, melting point was identified as a supportive CMA for assessing thermal risk, rather than a limiting factor for process feasibility.

Solubility of sulfasalazine

Solvent	Observation	Interpretation
Distilled water	Practically insoluble	Practically insoluble; suspension formed
Chloroform	Practically insoluble	Practically insoluble
Ethanol (96%)	~0.6 mg/mL	Poorly soluble
Methanol	~0.7 mg/mL	Limited solubility; incomplete dissolution

Acetone	~0.2 mg/mL	Very low solubility
Ethyl acetate	Practically insoluble	Practically insoluble
DMF	~70 mg/mL	Freely soluble
DMSO	~80 mg/mL	Freely soluble; clear solution

The poor aqueous solubility of sulfasalazine was identified as a critical material attribute (CMA) with potential impact on drug distribution and release behavior. The high solubility observed in solvents such as DMF and DMSO supported their suitability for electrospinning solution preparation and reduced the risk of drug precipitation during processing. Based on these findings, drug solubility was categorized as a high-risk CMA and was considered during solvent system selection in the preliminary risk assessment.

Solubility of Quercetine

Solvent	Observation	Inference
Distilled Water	Practically insoluble	Practically insoluble; yellow suspension.
Acetone	~2 mg/mL	Moderate solubility.
DMF (dimethyl -formamide)	~110 mg/mL	Very high solubility.
DMSO (dimethyl sulfoxide)	~120 mg/mL	Very high; gives deep yellow solution.
Ethanol (95%)	~2 mg/mL	Good solubility; improves with mild heating.
Methanol	~5 mg/mL	Freely soluble;
Ethyl acetate	~3 mg/mL	Moderately soluble.
Chloroform	~1 mg/mL	Limited solubility.

Quercetin exhibited extremely low aqueous solubility and formed a yellow suspension in distilled water. Moderate to good solubility was observed in organic solvents such as acetone, ethanol, methanol, ethyl acetate and chloroform. In contrast, quercetin demonstrated very high solubility in polar aprotic solvents, including DMF and DMSO, forming clear deep yellow solutions. Based on these findings, quercetin solubility was categorized as a high-risk CMA and considered during solvent system selection and preliminary risk assessment.

3.3 Combined Dual-Drug Solubility

A comparative evaluation of sulfasalazine and quercetin solubility revealed that both drugs exhibited extremely poor aqueous solubility and limited solubility in non-polar solvents. In contrast, both active pharmaceutical ingredients demonstrated high solubility in solvents, particularly dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) (Bhutkar & Rashmi Tambe, 2020). The similarity in solubility behavior of the two drugs across the tested solvent systems indicates good compatibility in solution and supports the feasibility of preparing a single, homogeneous dual-drug electrospinning solution.

Based on the combined solubility profiles of both drugs, solvents capable of dissolving both sulfasalazine and quercetin were prioritized. DMF and DMSO were selected due to their ability to solubilize both drugs at high concentrations. Solvents exhibiting poor drug solubility were excluded from further consideration due to their potential to compromise solution homogeneity and process robustness.

3.4 Polymer Solubility and Spinnability Screening

Sr.No.	Polymer	Conc. range studied	Solvent Used	Observation
1.	PVP K-30	38-42%	Ethanol	No proper NF formation
2.	PVP K-30	37-42%	Ethanol & DMF	NF formation observed as the concentration of polymer was increased up to 42%
3.	Polycaprolactone	13-15%	DMF	No proper jet formation and only sprinkling was observed
4.	Ethyl Cellulose	10-15%	Ethanol	Very high viscocity, fiber peeling problem
5.	Ethyl Cellulose	9 to 15%	Ethanol & DMF	Not getting desired viscosity, fibers not

				formed
6.	PVP K-90	8-12%	Ethanol:Water	Fibers formed but sticking to Al-foiled not peeled out properly.
			Ethanol: DMF	Fibers formed.
7.	Ethyl Cellulose with PVP K-90	EC 10% PVP K-90 7.5 to 10%	Ethanol	Good fiber was obtained with EC10% and PVP 7.5%

Preliminary spinnability studies were conducted by electrospinning polymer solutions at different concentrations to identify concentration ranges capable of producing continuous and uniform fibers. Based on these observations, solvent type and polymer concentration were identified as high-risk material attributes and were included in the preliminary risk assessment.

Polymer solubility and spinnability screening demonstrated that polymer type, concentration and solvent system significantly influenced fiber formation (Shahriar et al., 2019). Among the polymers evaluated, PVP K-90 in Ethanol: DMF solvent and a combination of ethyl cellulose with PVP K-90 in an ethanol solvent system produced continuous good fibers. The observed differences in fiber formation can be attributed to variations in polymer viscosity, molecular weight and solvent evaporation behavior. Blending ethyl cellulose with PVP K-90 improved solution spinnability and fiber integrity, highlighting polymer composition and solvent system as critical material attributes.

3.5 Drug– Excipient Compatibility Studies

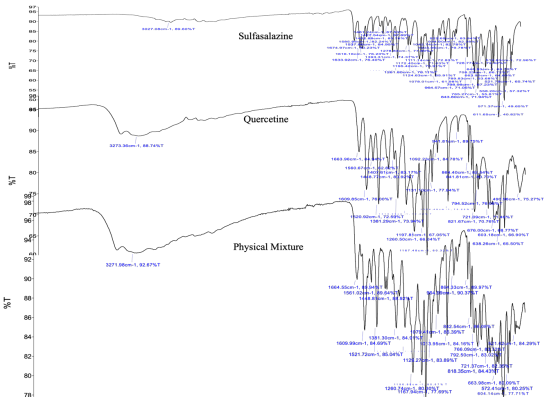


Fig: Overlay FTIR spectra of pure drug sulfasalazine, quercetine and their physical mixture with polymer.

The overlaid FTIR spectra of sulfasalazine, quercetin, selected polymer, and their physical mixture showed the retention of characteristic functional group peaks of both drugs. No additional peaks or significant peak shifts were observed in the physical mixture spectrum, indicating the absence of chemical interaction at the preformulation stage. Based on FTIR analysis, drug–polymer compatibility was identified as a low-risk material attribute.

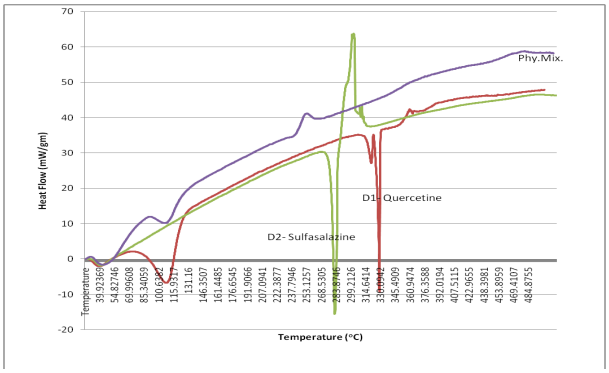


Fig: DSC thermogram of sulfasalazine, quercetin, and their physical mixture with polymer

The DSC thermograms of sulfasalazine and quercetin showed sharp endothermic peaks corresponding to their respective melting points. In the physical mixture, the characteristic melting endotherms of both drugs were retained with slight broadening and reduced intensity.

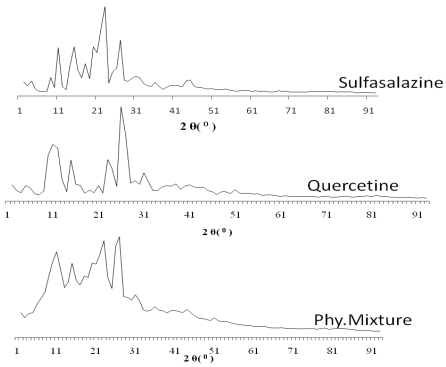


Fig: XRD patterns of sulfasalazine, quercetin, and their physical mixture with polymer

The XRD diffractograms of sulfasalazine and quercetin exhibited characteristic sharp diffraction peaks, indicating their crystalline nature. The reduction in peak intensity observed in the physical mixture is attributed to dilution by the polymer matrix rather than solid-state interaction. The absence of new diffraction peaks confirms that no significant crystalline transformation or incompatibility occurred during physical mixing.

Compatibility studies using FTIR, DSC, and XRD demonstrated that sulfasalazine and quercetine remained

chemically, thermally and structurally stable in the presence of the selected polymer system. No significant changes in characteristic functional groups, melting behavior were observed in the physical mixtures.

3.6 Preliminary Electrospinning Trials

CPP	Range Studied	Feasible Range	Observations
Applied Voltage (kV)	15–20	17–19	Stable jet and continuous fibers observed within this range
Flow Rate (mL/min)	0.01–1.0	0.01–0.05	Good fibers obtained in feasible range
Needle–Collector Distance (cm)	10–20	10-20	Good fibers obtained in feasible range

Preliminary electrospinning trials enabled the identification of operable parameter ranges supporting continuous fiber formation. Stable jet formation and uniform fibers were observed only within specific ranges of applied voltage, flow rate, and needle–collector distance, whereas conditions outside these ranges resulted in jet instability or bead formation. These operable ranges were therefore considered critical in the QbD-based risk assessment.

3.7 Preliminary Nanofiber Characterization

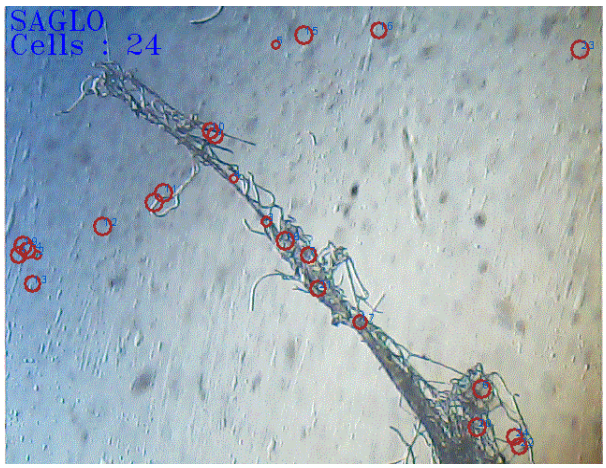


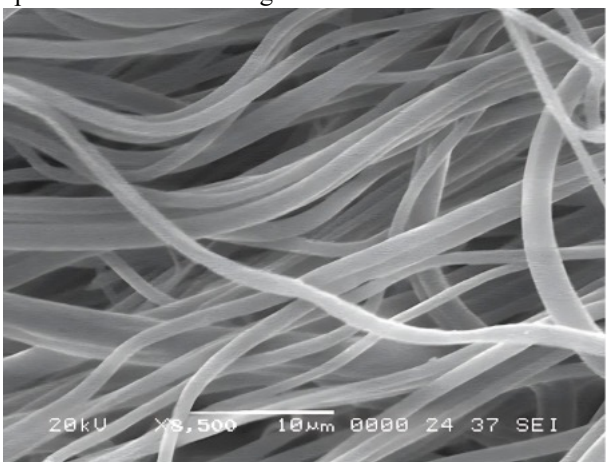
Fig : Optical microscopy image & SEM image of nanofibers electrospun within the feasible process parameter range identified during preliminary electrospinning trials.

Optical microscopy confirmed the formation of fibrous structures, indicating successful electrospinning of the polymer solution. The fibers appeared continuous. SEM analysis confirmed the formation of continuous and uniform nanofibers under the feasible electrospinning parameter range. The observed morphology supports the suitability of the selected process parameters for further formulation development.

Conclusion

Quality by Design (QbD) approach, as described in ICH Q8(R2), were applied to the preformulation development of dual drug-loaded electrospun nanofibers intended for rheumatoid arthritis management. The Quality Target Product Profile (QTPP) was defined based on the intended route of administration and therapeutic requirements. Identified relevant critical quality attributes (CQAs) such as fiber morphology, drug encapsulation efficiency and drug release behavior. A risk assessment was performed to identify critical material attributes (CMMs) and critical process parameters (CPPs) with the highest potential impact on product quality. Material attributes related to drug solubility, polymer characteristics, solvent system, and drug–polymer compatibility were identified as high-risk and were experimentally evaluated through solubility studies, polymer screening, and compatibility assessments using FTIR, DSC, and XRD. Process-related risks associated with applied voltage, flow rate, and needle–collector distance was addressed through preliminary electrospinning trials to establish process feasibility and robustness.

The integration of risk assessment with targeted experimental studies enabled the rational selection of materials and electrospinning conditions, improved process understanding and identified CQAs, CMMs, and CPPs. Overall, this preformulation QbD approach provides a robust foundation for subsequent formulation optimization of dual drug-loaded nanofibers.



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