# Design, Development And Characterization of Rutin-Silymarin Loaded Nano-Formulation For Treatment of Asthma

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## I. INTRODUCTION

Asthma is a condition that affects the airways that transport air into and from the lungs. Asthmatics are those who have a chronic or recurring respiratory ailment. Asthmatics suffer from enlarged or irritated airway walls. Swelling or inflammation in the airways makes them more sensitive to irritations, increasing the risk of allergic reactions.Inflammation narrows the airways, allowing less air to travel through to and from the lungs. Symptoms of airway narrowing include wheezing, chest discomfort, breathing issues, and coughing. Asthmatics typically encounter these symptoms at night and in the early morning. Asthma affects around 300 million people worldwide, including over 22 million in the United States. The condition, which affects 6 million children in the US, affects people of all ages but typically begins during childhood. Asthma kills approximately 255,000 individuals worldwide each year[1,2].

## PATHOPHYSIOLOGY AND ETIOLOGY

Asthma triggers Th2 immune responses, similar to other atopic diseases. Asthma triggers can be both allergic (e.g., house dust mites, cockroach remnants, animal dander, mould, and pollens) and non-allergic (e.g., viral infections, exposure to tobacco smoke, cold air, exercise), resulting in chronic airway inflammation. Th2 cells in the airways release cytokines such as IL-4, IL-5, IL-9, and IL-13, which enhance eosinophilic inflammation and IgE synthesis. Asthma symptoms include bronchospasm, oedema, and increased mucous secretion, which are caused by the release of inflammatory mediators such histamine and cysteinyl leukotrienes[3,4].

The release of mediators and cytokines during the early phase of an immune response to an instigating event contributes to the late-phase asthmatic response, which causes airway inflammation and bronchial hyperreactivity[5]. Frequent asthma exacerbations cause airway remodelling, leading to decreased lung function and worsening airway blockage over time.(1/10)Research reveals a genetic link to asthma development.

Asthma susceptibility has been linked to chromosomal regions affecting IgE antibody production, airway hyperresponsiveness, and inflammatory mediators. Additional research is needed to identify the specific genes involved in asthma and the gene-environment interactions that contribute to disease expression[3,5].

## CAUSES OF ASTHMA

### Allergies

Indoor allergies can be caused by animal proteins (mostly from cats and dogs), dust mites, cockroaches, and fungi. The trend towards energy-efficient housing may have led to increased exposure to asthma triggers. Antibodies in the blood can cause allergic reactions, resulting in airway inflammation and asthma symptoms[6].

### Tobacco Smoke

Tobacco smoke has been related to increased risk of asthma, wheezing, and respiratory infections, which can lead to mortality. Children of moms who smoke, as well as those exposed to second-hand smoke, are more likely to develop asthma. Adolescent smoking has been linked to higher asthma risk[7].

### Environmental Factors

Indoor air pollution from mould, household cleansers, and paints can cause allergic reactions and asthma symptoms. Asthma is also linked to indoor environmental factors such as gas stoves emitting nitrogen oxide. Cooking with gas increases the risk of symptoms like wheezing, dyspnoea, asthma episodes and hay fever. Pollution, sulphur

dioxide, nitrogen oxide, ozone, cold temperatures, and excessive humidity can induce asthma in some people. Weather variations have been linked to increased asthma attacks. Cold air can cause airway congestion, bronchoconstriction, secretions, and inefficient mucociliary clearance. Humidity can also cause breathing issues in certain populations[8].

**Obesity**

Overweight adults (BMI 25-30) have a 38% higher risk of asthma compared to non-overweight adults. Obese adults (BMI 30 or more) are twice as likely to get asthma. Some evidence suggests that nonallergic asthma may pose a higher risk than allergic asthma.

**Stress**

Stressed people are more likely to develop asthma. Stress may contribute to an increase in asthma-related behaviours, including smoking. Recent study suggests that stress also affects the immune system[9].

**Genes**

Asthma may be caused by up to 100 genes, which also affect immune system and inflammation. Genetic studies have not yielded consistent results across populations, highlighting the need for additional research to understand the intricate relationships that lead to asthma. Three-fifths of asthma cases are inherited. According to the Centres for Disease Control (USA), having an asthmatic parent raises the risk three to six times[10,11].

**HERBS**

The most common reasons for using traditional medicine are that it is less expensive, more closely aligns with the patient's ideology, alleviates concerns about the side effects of chemical (synthetic) medicines, meets a desire for more personalised health care, and allows greater public access to health information. Herbal medicines are primarily used to promote health and treat chronic diseases rather than life-threatening ones. However, when contemporary medicine fails to treat an illness, such as advanced cancer or novel infectious diseases, the use of traditional medicines grows. Furthermore, traditional remedies are often regarded as natural and safe, that is, non-toxic[12].

**NANOTECHNOLOGY**

A drug's pharmacokinetics and pharmacodynamics are strongly impacted by its physical and chemical properties, as well as the type of formulation employed to administer it. Nano-DDS can regulate and increase the performance of many medications in ways that traditional formulations cannot. For example, nano-DDS can be capitalised to encapsulate drugs and thus (i) increase their solubility, (ii) protect them from degradation, (iii) enhance their epithelial absorption and increase their blood circulation time, (iv) target the drugs to specific cells/tissues/organs, releasing them in a controlled manner in response to a specific stimulus, and (v) enhance their uptake by cells[13,14,15].

**RELEVANCE AND MOTIVATION**

Asthma is a chronic respiratory disorder that affects more than 260 million people worldwide. According to the World Health Organisation (WHO), it is one of the most common noncommunicable illnesses, resulting in a significant public health burden.Creating effective formulations is critical to improve the quality of life for millions of patients who use inhalers, pills, and other antiasthmatic drugs. Inhaled therapy are the most popular way to treat asthma. However, providing continuous and precise drug delivery to the lungs remains a difficulty, especially in chronic care.Advanced formulations can improve drug absorption and targeting, resulting in fewer adverse effects and better therapeutic outcomes.

The project provides an opportunity to use Quality by Design (QbD) concepts, with a focus on ensuring that formulations are robust, reproducible, and meet safety and efficacy standards. Modern technologies such as nanoparticles, liposomal delivery systems, and dry powder inhalers (DPIs) can improve treatment efficacy while reducing systemic exposure to the drug. Current formulations may fail to appropriately address issues such as patient compliance (due to the complexities of inhaler use), environmental concerns (CFC-free formulations), and drug resistance.

Innovative formulations that target severe asthma or address steroid resistance can close major therapeutic gaps.The development of new antiasthmatic formulations is aided by regulatory incentives for novel medicines, particularly orphan drug designation for severe asthma variations.The expanding market for respiratory medications, driven by rising diagnosis rates, promises a lucrative potential. Formulations that improve on existing medications can win significant market share and receive regulatory approval for a faster launch.

An antiasthmatic formulation project is extremely important due to the global health impact of asthma, the

clinical need for more effective and personalised treatments, and the prospect for scientific innovation in drug delivery methods. Combining scientific research with industry and regulatory developments makes this initiative both motivating and significant.

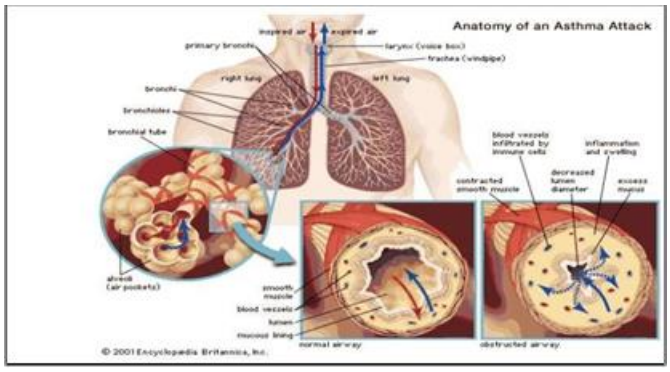


**Figure No. 1:**
Anatomyofasthmaattack.Asthmaischaracterizedbyacomplex interaction of airflow obstruction, airway hyper responsiveness and inflammation.

## II. LITERATURE REVIEW

1. Aftab Ahmad(2022): has published review article Pharmacological Strategies and Recent Advancement inNano-Drug Delivery for Targeting Asthma and from that he found Numerous benefits have already been demonstrated in the administration of medications and immunisations utilising nanotechnology for asthma treatment. Asthma, a chronic inflammatory disorder, affects a wide range of cells and components. As a result, asthma offers a diverse set of molecular targets that could be combined with nanoparticles to achieve therapeutic efficacy. To address the inadequacies of pharmaceuticals, nanotechnology has emerged as a critical weapon in the fight against drug resistance. This study examined the advantages and applications of nanoparticles as medication delivery vehicles in asthma. The use of nanotechnology combined with inhaled administration has accelerated the development of asthma treatments. Many research are still in their early stages and must be evaluated for clinical relevance, despite promising preclinical results. In the future, researchers should concentrate on therapeutic nanomedicine, molecular mechanisms, performance enhancement, and potential toxicity[16].
2. David Ong Cherk Yong et.al, (2019): has worked on Preparation, characterization and in-vitro efficacy of quercetin loaded liquid crystalline nanoparticles for the treatment of asthma and from that they found effectively encapsulate quercetin into liquid This indicates that quercetin LCN may be used as a novel medication delivery system for the management of asthma. This method will address the problems related to quercetin, including its short half-life, limited solubility, and poor bioavailability. The present study's findings support its translation into other related pulmonary inflammatory disorders, such as lung cancer and chronic obstructive pulmonary disease (COPD), which will assist give pulmonary clinics a new focus[17].
3. Amjad Hussainet.al,(2023): has worked onNanoformulation of curcuma longa root extract and evaluation of its dissolution potentialThe nanosuspension exhibited a satisfactory rate of dissolution, with the highest observed dissolution occurring at pH-7. This is anticipated to be the optimal setting for the absorption of C. longa in any kind of solvent, medium, or bodily fluid. Additionally, the nanosuspension's great effectiveness was demonstrated by the increased antibacterial and antioxidant properties when compared to the coarse extract. Significant antibacterial activity was demonstrated by the C. longa nanosuspension against E. coli and A. niger, with zone of inhibition values of 13.7 ± 0.16 and 11 ± 0.06, respectively. With an IC50 of 123.8 μg/mL, the antioxidant activity of the nanosuspension was found to be the highest, surpassing even that of ascorbic acid[18].
4. Muhammad Qasim et.al (2023): has worked onMolecular mechanism of Ferula asafoetida for the treatment of asthma:network pharmacology and molecular docking approachsubstances (assafoetidin, cycloside, farnesiferol-B, and farnesiferol-C) can be very helpful in understanding how F. asafoetida works to treat asthma. As a result, network pharmacology offers a wide range of uses and presents a viable strategy for reducing the prevalence of asthma in drug discovery in the future[19].
5. Allison Clyne et.al (2020): has worked on Molecular docking and networkconnections of active compounds from the classical herbal formula Ding Chuan Tang the 155 network docking connections that DCT-compounds generated with proteins that are also targeted by asthma genes point to the possibility that DCT-compounds may exert therapeutic effects by inhibiting or promoting proteins linked to biological networks and pathways involved in asthma disease[20].
6. Ondrej Misiket,al (2024): has worked on Nebulization and in vitro upper airway deposition of liposomal carrier SystemsWhen choosing a nebuliser technology, factors other than formulation stability should be taken into consideration. These include the aerosol's aerodynamic characteristics and how it deposits in respiratory traction. This work outlined the potential of a particular liposomal system for pulmonary medication administration to the lung from these two perspectives: aerosol deposition in

human airways and system stability during nebulisation, which together make up a sophisticated strategy utilising extremely uncommon techniques. The study's conclusions indicate that this kind of intricate testing is required for accurate drug delivery efficacy prediction[21].

7. Swati Devendra Raysing et.al (2022): has worked on Formulation and evaluation of Doxofylline-loaded polymeric micelles for pulmonary administration produced micelles are discovered to be resilient, stable over an extended period of time, and secure for inhalation. The experimental results that are reported here achieve every goal that the study had in mind. If more thorough research is done for pre-clinical lung distribution tests, lung deposition and permeability investigations, and cellular uptake analysis, the created micelles formulation may make it to the commercial market[22].

## III. AIM & OBJECTIVES

**AIM:**

Design, development and characterization of Rutin- Silymarin loaded nano-formulation for treatment of Asthma.

**OBJECTIVES:**

1. In sillico study of herbal phytoconstituents with protein of disease.
2. Optimization of surfactants and polymer by using trial error batches of nano-formulation by suitable method.
3. Preparation nano-formulation of rutin and silymarin by using suitable method.
4. Characterization of prepared nano-formulation for

a. Determination of Rutin content
b. Determination of silymarin content
c. Average particle size
- Particle size
- Zeta potential

d. Drug-Excipient compatibility study
e. In-vitro drug release study
f. Stability study

**PLAN OF WORK**

1. Exhaustive review of literature
2. Selection of suitable surfactants as well as other additives
3. Procurement of materials.
4. Preformulation Study
5. Screening of surfactants, and polymers
6. Preparation nanosuspension of rutin and silymarin by using antisolvent precipitation method.
7. Characterization:

a. Average Particle Size and Zeta potential

b. Drug Content Determination

c. In-vitro drug release

d. Stability study

10. Results and discussion
11. Conclusion
12. Compilation and submission

## IV. METHODOLOGY

| Sr. No. | Parameters | Method |
|---|---|---|
| 1. | Preformulation Study | Uv-Visible spectrophotometer, visual observation, solubility. |
| 2. | Preparation of polyherbal nano-formulation | Preparation and optimization polyherbal nanoformulation loaded with Rutin and Silymarin by using suitable method like:<br>• Spontaneous nanoprecipitation<br>• Solvent casting<br>• Direct dissolution<br>• Solvent evaporation method<br>• Dialysis method etc. |

| | | |
|---|---|---|
| 3. | Characterization of optimized polyherbal nano-formulation | • Drug content<br>•Particle size and Zeta potential<br>• *In-vitro* drugs release<br>• *In-vitro* stability study, etc. |

**Facilities Available, Instruments Required:**

**• Equipment's / Instruments required:**

UV-Visible Spectrophotometer, Electronic Balance, Magnetic Stirrer, Centrifuge, Micropipette, Rotary Evaporator, Mechanical Shaker, Particle Size Analyzer, etc.

**• Equipment's / Instruments available:**

Electronic Balance (Shimadzu AUW 220D), Magnetic Stirrer (Remi Equipment Pvt. Ltd.), Centrifuge (Remi Equipment Pvt. Ltd.), UV-Visible Spectrophotometer (Agilent Technology Carry 60 UV-vis UV-1800 Shimadzu, Japan), Micropipettes (Swastik Instrument Private Ltd. Mumbai), Mechanical Shaker (Remi Equipment Pvt. Ltd), Rotary Evaporator (Indosati Scientific lab equipment), Ultrasonicator (Quality Equipment and Instrument), etc.

**Equipment's / Instruments to be outsourced:**

Like Particle size analyzer, zeta potential, Differential scanning colorimetry (DSC), etc.

**Chemicals:**

**Lipids:** Phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), polyethylene glycol (PEG) etc.

**Polymers:** Tocopherol polyethylene glycol succinate (TPGS), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) etc.

**Solvents:** Ethanol, water, methanol, alcohol, acetone, ethyl acetate, glycerin, chloroform etc.

**Excipients:** Sucrose, trehalose, β-cyclodextrins etc.

## V. RESULT AND DISCUSSION

**Preformulation Study**

**Identification Test**

The identification tests of Rutin and Silymarin were performed using following techniques. The findings are described in the set of values found in table no 1 and table no 2 from the results we conclude from that rutin and silymarin in pure form.

**Table No 1: Identification tests of rutin**

| Sr.No | Physical Properties and tests | Methods | Discription |
|---|---|---|---|
| 1 | Physical state | Visualobservation | Solid |
| 2 | Color | Visualobservation | White |
| 3 | U.V. Spectra | U.V. Visible Spectrophotometer | UV spectra were obtainedatstandard condition and it showedλ max of 358 nm in methanol |

**Table No.2:Identificationtestsofsilymarin**

| Sr.No | Physical Properties and tests | Methods | Discription |
|---|---|---|---|
| 1 | Physical state | Visualobservation | Solid |
| 2 | Color | Visualobservation | Yellow |
| 3 | U.V. Spectra | U.V. Visible Spectrophotometer | UV spectra were obtainedatstandard condition and it showedλ max of 368 nm in methanol |

**Standard calibration curve of rutin**

The absorbance against the conc. Plot for the rutin API has been found to be linear at 358 nm in concentration

range of 1-6 ppm. The drug obeys beer-lamberts law in the range1-6 ppm. Standerd calibration curve values of rutin in methanol are shown in table no. 3 and calibration curve shown in figure no. 2. The $R^2$ value was found to be 0.9987.

**Table No. 3: Calibration curve values of rutin in methanol**

| Sr.No | Concentration (µg/ml) | Absorbance (λ max) |
|---|---|---|
| 1 | 1 | 0.174 ±0.03 |
| 2 | 2 | 0.246 ±0.05 |
| 3 | 3 | 0.329 ±0.04 |
| 4 | 4 | 0.42 ±0.06 |
| 5 | 5 | 0.504 ±0.01 |
| 6 | 6 | 0.591 ±0.05 |
| 7 | 7 | 0.689 ±0.04 |

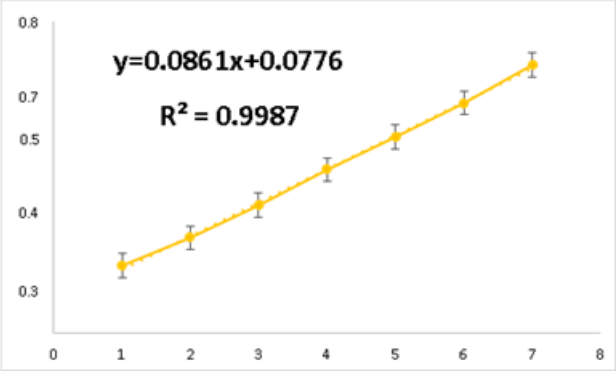


**Figure No. 2: Calibration curve of rutin in methanol**

**Calibration curve of silymarin**

The absorbance against the conc. Plot for the rutin API has been found to be linear at 368 nm in concentration range of 1-5 ppm. The drug obeys beer-lamberts law in the range1-5 ppm. Standard calibration curve values of rutin in methanol are shown in table no. 4 and calibration curve shown in figure no. 3. The $R^2$ value was found to be 0.9981.

**Table No 4: Calibration curve values of silymarin in methanol**

| Sr.No. | Concentration | Absorbance |
|---|---|---|
| 1 | 1 | 0.255 ± 0.03 |
| 2 | 2 | 0.465 ± 0.04 |
| 3 | 3 | 0.678 ± 0.03 |
| 4 | 4 | 0.941 ± 0.05 |
| 5 | 5 | 1.176 ± 0.02 |

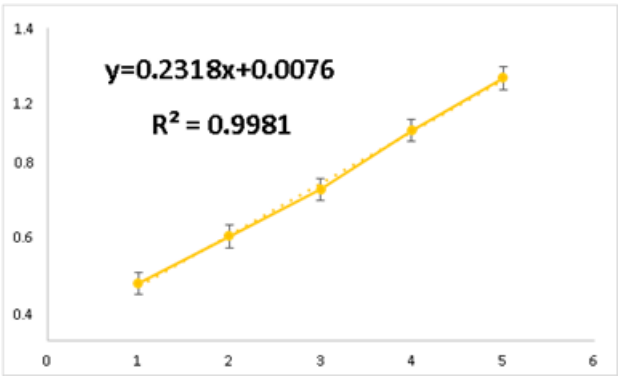


**Figure No. 3: Calibration curve of silymarin in methanol**

**Preparation and characterization of nanosuspension**
**Selection of polymer**

Two type of polymer was used to prepare batches of nanosuspension (SLS and PEG 8000) and from that PEG 8000 batch showed good entrapment efficient is selected as good polymer. Different batches of polymer with different ratio are shown in table no. 5 from that PEG 8000 gives good batches so we analyzed it in different ratio with entrapment efficient which is showed in table no. 6. from that 1:10 ratio gives good entrapment efficiency so that ratio was used in the final batches.

**Table No. 5: Batches for selection of polymer**

| Batch | Drug | SLS | PEG8000 | Water |
|---|---|---|---|---|
| **NSB1** | 5 | 250 | - | 10 |
| **NSR1** | 5 | 250 | - | 10 |
| **NSUA1** | 5 | 250 | - | 10 |
| **NSC1** | 5 | 250 | - | 10 |
| **NSQ1** | 5 | 250 | - | 10 |
| **NSB2** | 5 | - | 250 | 10 |
| **NSR2** | 5 | - | 250 | 10 |
| **NSUA2** | 5 | - | 250 | 10 |
| **NSC2** | 5 | - | 250 | 10 |
| **NSQ2** | 5 | - | 250 | 10 |

**Table No. 6: Batches of PEG 8000 with different ratio**

| Batch | Drug(mg) | Polymer/ Stabilizer (mg) | Water | EE% |
|---|---|---|---|---|
| **NSB1** | 5 | 100 | 10 | - |
| **NSR1** | 5 | 100 | 10 | - |
| **NSUA1** | 5 | 100 | 10 | - |
| **NSC1** | 5 | 100 | 10 | - |
| **NSQ1** | 5 | 100 | 10 | - |
| **NSB2** | 5 | 250 | 10 | 90 |

| | | | | |
|---|---|---|---|---|
| **NSR2** | 5 | 500 | 10 | 86 |
| **NSUA2** | 5 | 500 | 10 | 85 |
| **NSC2** | 5 | 500 | 10 | 98 |
| **NSQ2** | 5 | 500 | 10 | 96 |
| **NSB3** | 5 | 1000 | 10 | 70 |
| **NSR3** | 5 | 1000 | 10 | 89 |
| **NSUA3** | 5 | 1000 | 10 | 91 |
| **NSC3** | 5 | 1000 | 10 | 98 |
| **NSQ3** | 5 | 1000 | 10 | 77 |

## VI. SUMMARY AND CONCLUSION

### SUMMARY:

- The in-sillico study of several herbal compounds with the standard FDA approved drug was done on PyRx software.
- The analytical method UV-Visible spectroscopy was used for construction of calibration curve of rutin and silymarin in methanol.
- Excipients were selected based on trial error batches of NS preparation. The rutin and silymarin loaded NS was prepared by suitable strategy and method, rutin and silymarin loaded NS were prepared using mechanical Stirrer and Probe Sonicator.
- The prepared compounds were analyzed by Particle Size, drug content and entrapment efficiency of compounds in which the results confirmed that successful preparation of ritun and silymarin loaded NS.
- The prepared rutin and silymarin loaded NS were analyzed for particle size and zeta potential by Malvern Zeta sizer, UV-Visible spectroscopy and in-vitro drug release
- The particle size of NS is in nanometer size range. The polydispersity index of NS is less than one which suggested that all the NS formed is mono dispersed or of uniform size.
- The size of rutin and silymarin loaded NS was found to 593.7±5.4 nm with PDI of 0.657±0.009 and Zeta Potential -32.9 mV, 587±6 .8 nm with PDI of 0.331±0.008 and zeta was found to be -39.1 mV respecticely.
- The percent cumulative drug release from the rutin NS and silymarin NS solution was found to be 98.231± 1.54 and 99.289± 1.15 respectively after 50 min. for rutin and silymarin NS loaded tablet 99.879± 1.19 and 97.547 ± 2.24 respectively.
- TheOptimizedbatchofrutinandsilymarinloadedNSwerefo undstableattheendof the more than 150 days with no change in batch.

### CONCLUSION:

Rutin and silymarin loaded NS were successfully prepared. From Rutin and silymarin loaded NS loaded tablets were prepared successfully. Nano particle size may show EPR effect, even formulation is stable refrigerator condition 20 to 80. Formulation shows good in vitro study which shows increase in absorption.

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