Formulation and Evaluation of Duo Flavono Loaded Anionic Polymeric Nanoparticulate System

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Keywords:
Quercetin, rutin, silibinin, three flavonoids are the major flavonols taken in diet. Among these, Quercetin has many benefit but it is poor soluble in water, unstable in gastric fluids which leads to lower therapeutic value and bio-availability. Hence to increase the therapeutic efficacy and bio-availability, quercetin loaded anionic polymeric nanoparticulate system have been developed using nanoprecipitation methods. Quercetin loaded nanoparticulate systems were employed Characrization studies like size of particles, morphology, in–vitro drug releases and storage stability. This research result was revealed that a mean size of particle was 84.4 nanometer (nm) with Polydispersity Index was 0.268 and potential of 10.2 milli volt (mV), morphological studies shown formulated quercetin polymeric nanoparticles were sphere-shaped. The in vitro drug releases studies shown >95% release of drugs within 45 mins. This novel approach of nanocomposite may promise a original system for the safeguard and release of a range of water hating chemicals to target specific tissues using specialized drug delivery.

INTRODUCTION

During the past two decades, nanoparticulate systems have acknowledged substantial interest, because their potent therapeutic efficacy used in drug release systems. Polymeric nanoparticulate systems are colloidal in nature with size range from 10 to 1000 nanometer (nm). Nanoformulation used to prepare using biocompatible polymers which is anionic in nature (Eudragit 100) in which the active moiety can be gallows, adsorbed or chemically combine on the polymer matrix (Sahoo et al., 2002). Quercetin is a cluster of flavonoidal compound, abundantly present in plant derived functional food like berries, red wine, green tea, onions, apples etc (Larson et al., 2012). It has plentiful biological properties have been reported for QU, such as anti- inflammatory (Molina et al., 2003), anti-oxidant, anti-allergic (Kumar and Mohan, 2015), anti - viral, anti-proliferative (Shaji and Iyer, 2012), immunomodulatory (Nday et al., 2015) and anti-carcinogenic effects (Gibellini et al., 2011). Certainly Quercetin is a hydrophobic functional food and activities natural bio-enhancers such as rutin and silibinin were also hydrophobic functional foods and has been reported to inhibits the drug enterohepatic isoenzyme like cyp3A, detoxification reaction such as glucuronidation, sulation of the quercetin present in hepatic and intestinal segment. Hence, these naturally available bio-enhancers were
expected to synergistically enhance the therapeutic and pharmacological activities and also expected to prevent or decrease the metabolism of quercetin thereby to enhance its pharmacological activities. Therefore, the intention of research work was to synthesis and estimate Eudragit (Anionic) nanoparticles (EPO-NPs) encapsulated with quercetin and to determine the effect of quercetin (QU) released from EPO-Nanoparticles encompassed with QU for improved their wide spectrum of pharmacological activity (Wu et al., 2008). EPO is a copolymer based on dimethylaminoethyl methacrylate, butyl, methyl methacrylate. It is severed as a nanocarrier owing to its unique properties such as biodegradability, biocompatibility, hydrophilicity and non-toxic and is also economical. Poloxamer 407 can be used as a surfactant. EPO-Nanoparticles fashioned by ionic cross coupled with poloxamer 407 indicates decreased surface tension between two particles hence it promise to improve stability, drug brimming efficiency and also releasing of drug (Umamahesvari, 2020; Loganathan and Sellappan, 2017).

**METHODOLOGY**

**Materials**

A chemicals utilized in this study were procured from various industrial sources and used as received without any modifications. Quercetin (QU), Rutin (RU) and Silibinin (Sigma Laboratories) Analytical grade ethanol and Poloxamer was obtained from different Chemicals sources.

**Methods**

**Preparation of plain polymeric nanoparticulate system**

An anionic polymer (Eudragit E 100) was diffused in suitable solvent of organic, then which was shifted to aqueous solution containing surfactant under constant stirred using mechanical stirrer. Nanoparticles were composed suddenly and turning the polar phase to milky solution with opalescence. However, the continues stirring was support to cutback the particle size & also to disappear residual solvent in existing nanopreparation.

**Optimization of flavono loaded Eudragit E 100 based nanoparticulate delivery system**

An anionic polymer Eudragit E 100 was suspended with suitable solvent, then it has been transferred to aqueous solution containing surfactant (Poloxamer) under mechanical α stirrer. Nanoparticles were composted simultaneously and turning the polar phase to bluish milky solution with opalescence (Chidambaram and Krishnasamy, 2013). However, the stirring was continued to support reduction in size of particle and also to disperse residual α solvent offered in the nanoformula- tion (Chinnamaruthu and Sellappan, 2014). Though, the physicochemical properties like size of the particle, index of Polydispersibility and zeta potential of the nanoparticles depend upon the process specification bound such as concentration of polymer, Aqueous soluble solvent, organic solvent percentage, organic phase volume, concentration of poloxamer, volume of aqueous solution and duration of stirring (Moorthi et al., 2013). Hence, the factorial design methods was used to optimize the process parameters from lower to higher levels were showed Table 1.

12 experimental runs (Table 2) with 10 process parameters at both lower / higher levels were created using Expert -Design . Prepared quercetin scaffold with flavono polymeric nanoparticles were analyzed their characterization such as zeta potential, polydispersity index (PI) and particle size.

**Formulation of quercetin loaded polymeric nanoparticles using stirring technique**

Single flavono encapsulated with polymeric nanoparticulate systems were fabricated using final optimized formula from factorial design method (Kumari et al., 2010). Approximately 250 milligram of EPO 100 with 50 mg of flavono(s) were suspended with of 80% acetone (20 ml). The prepared organic media was shifted in to beaker contains 100ml of distilled water, weight about 250 mg Poloxamer 188, 25 mg of Poloxamer 407 continuous stirring at 500 ±20rpm flavono encapsulated with polymeric nanoparticulates were produced unexpectedly but the stirring to be continued until 60 minutes to aid the size reduction and vaporized solvent residual all the values shown in Table 3.

**Polydispersity Index (PDI)Particle size, and zeta potential Analysis**

Designed plain (P), Qu, Ru, Si encapsulated flavono polymeric nanoparticulates system were kept 30 days at room temperature. Past Completion of 30 days the nanoparticulate system engaged with characterization studies like zeta potential, particle size analysis, and polydispersity index. (Chinnamaruthu and Sellappan, 2014) concerning 1 ml of prepared plain and flavono loaded polymeric nanoparticulate systems were depilated suitable distilled H2O, then this content was then taken separately into a zeta size cell and calculation was made using Zetasizer (Malvern).

**Surface morphology**

Synthesised plain and flavono Co-capssulated poly-
### Table 1: Parameters used Optimization process at higher/lower levels

| S.No. | Process parameter                          | Lower  | Levels       |
|-------|--------------------------------------------|--------|--------------|
| 1     | Concentration of Polymer                   | 25 (mg) | 250 (mg)     |
| 2     | Organic solvent                            | Acetone | Ethanol      |
| 3     | Organic solvent Percentage                 | 80 %    | 100 %        |
| 4     | Volume of Organic medium                   | 10 (mL) | 20 (mL)      |
| 5     | Concentration of Poloxamer 188             | 25 (mg) | 250 (mg)     |
| 6     | Concentration of Poloxamer 407             | 25 (mg) | 250 (mg)     |
| 7     | Volume of Aqueous phase                    | 50 (mL) | 100 (mL)     |
| 8     | Size reduction influence                   | Sonication | Stirring  |
| 9     | Influence duration                         | 30 mins | 60 mins      |
| 10    | Addition mode                              | Aqueous to organic | Organic to Aqueous |

### Table 2: Factorial design (Plackett-Burman)

| Run   | Acetone | Organic solvent | Percentage of Solvent (%) | Vol of Solvent (ml) | Mode of Addition mg | Con of Pol 188 (mg) | Con of Pol 407 (mg) | Dis H2O (ml) | SO/ST (MT) | Duration (Hr) |
|-------|---------|-----------------|---------------------------|--------------------|---------------------|---------------------|---------------------|--------------|-------------|----------------|---------------|
| 1     | 25      | E               | 80%                       | 10                 | O→A                 | 250                 | 25                  | 50           | ST          | 60             |
| 2     | 250     | A               | 80%                       | 20                 | A→O                 | 250                 | 25                  | 100          | ST          | 60             |
| 3     | 25      | A               | 100%                      | 20                 | O→A                 | 25                  | 25                  | 100          | SO          | 60             |
| 4     | 25      | E               | 80%                       | 20                 | A→O                 | 25                  | 25                  | 100          | ST          | 30             |
| 5     | 25      | A               | 80%                       | 10                 | A→O                 | 25                  | 25                  | 50           | SO          | 30             |
| 6     | 25      | E               | 100%                      | 10                 | A→O                 | 250                 | 25                  | 100          | SO          | 30             |
| 7     | 25      | A               | 100%                      | 10                 | O→A                 | 250                 | 25                  | 100          | ST          | 30             |
| 8     | 250     | E               | 100%                      | 20                 | O→A                 | 25                  | 25                  | 50           | ST          | 30             |
| 9     | 250     | E               | 80%                       | 10                 | O→A                 | 25                  | 25                  | 100          | SO          | 60             |
| 10    | 250     | A               | 100%                      | 10                 | A→O                 | 25                  | 25                  | 50           | SO          | 60             |
| 11    | 25      | E               | 100%                      | 20                 | A→O                 | 250                 | 25                  | 50           | SO          | 60             |
| 12    | 250     | A               | 80%                       | 20                 | O→A                 | 250                 | 25                  | 50           | SO          | 30             |

### Table 3: Fabrication of plain, quercetin loaded polymeric nanoparticles using stirring approach

| Drug (mg) | Polymer (mg) | Organic solvent (%) | OrgPhase Vol (ml) | AquPhase Vol mg | Pol 188 (mg) | Pol 407 (mg) | Stirring Speed (rpm) | Duration of Stirring (min) |
|-----------|--------------|---------------------|-------------------|-----------------|--------------|--------------|----------------------|-----------------------------|
| -         | 250          | 80% acetone         | 20                | 100             | 25           | 25           | 500±20               | 60                          |
| Qu (50)   | 250          | 80% acetone         | 20                | 100             | 25           | 25           | 500±20               | 60                          |
| Ru (50)   | 250          | 80% acetone         | 20                | 100             | 25           | 25           | 500±20               | 60                          |
| Si (50)   | 250          | 80% acetone         | 20                | 100             | 25           | 25           | 500±20               | 60                          |
Figure 1: a) Particle size spectrum of plain; NPs b) Zeta potential spectrum of plain NPs; c) Particle size spectrum of Qu NPs; d) Zeta potential spectrum of Qu NPs; e) Particle size spectrum of Ru NPs; f) Zeta potential spectrum of Ru NPs; g) Particle size spectrum of Si NPs; h) Zeta potential spectrum of Si NPs
Table 4: characterization of flavono polymeric nanoparticulate system

| Method       | Formulation | Mean particle size (nm) | Polydispersity Index | Zeta Potential (mV) |
|--------------|-------------|-------------------------|----------------------|---------------------|
| Stirring     | Plain       | 128.30±0.72             | 0.184±0.16           | 27.3±2.70           |
| Approach     | Qu          | 84.40±0.86              | 0.268±0.22           | 10.2±0.82           |
|              | Ru          | 65.26±0.742             | 0.293±0.05           | 15.5±1.94           |
|              | Si          | 101.20±0.920            | 0.358±0.14           | 14.2±1.13           |

Qu: Quercetin; Ru: Rutin; Si: Silibinin; Qu-Ru:

Table 5: content of drug, Co-capsulation efficiency & drug loading

| Drugs     | W_{total} (mg) | W_{free} (mg) | W_{polymer} (mg) | Drug content in % | Encapsulation efficiency in % | Drug loading % |
|-----------|----------------|---------------|------------------|------------------|-----------------------------|---------------|
| Quercetin | 49.14 ± 0.16   | 1.67 ± 0.61   | 250              | 98.28 ± 0.46     | 96.60 ± 0.24                | 18.99 ± 0.21  |
| Rutin     | 49.09 ± 0.18   | 1.54 ± 0.85   | 250              | 98.18 ± 0.13     | 96.86 ± 0.31                | 19.02 ± 0.32  |
| Silibinin | 48.89 ± 0.17   | 2.00 ± 0.45   | 250              | 97.78 ± 0.14     | 95.91 ± 0.42                | 18.76 ± 0.42  |

Figure 2: Surface morphology a) plain NPs; b) Qu NPs; c) Ru NPs; d) Si NPs
meric nanoparticulate systems was engaged characterization studies like surface morphology using FESEM.

**Estimation of Drug loading, Encapsulation efficiency & Drug content**

The Concentration of drug in of synthesised flavono scaffold with polymeric nanoparticulate was estimated by using the pre developed HPLC method development for Qu, Ru, and Si (Li et al., 2009). Efficiency of encapsulation and loading of drug were quantified by approximation of free Qu, Ru and Si present in the prepared nanoparticulate system (Song et al., 2008).

**Estimation of encapsulation efficiency and drug loading**

Fabricated blank and flavono encompassed polymeric nanosuspension were separated by a cooling centrifuge at for 45 minutes 19,000 rotation per minutes at -20°C. The supernatant portion was collected and stored separately for further analysis (Pool et al., 2012; Jain et al., 2013; Dhanaraj et al., 2018). Approximately 1 ml of supernatant portion was mixed with Met-OH (1 ml) of, then it was mixed for near 15 min and filtered by membrane filter paper size is about 0.22 μm. approximate amount of free amount drugs were found as W_free. the Encapsulation efficiency (EE) and drug loading (DL) were estimated using suitable formula.

**FT-IR analysis**
Fabricated plain and flavonoid encapsulated polymeric nanoparticle system were taken in to cell FT-IR and analysed at 4000-400 cm\(^{-1}\) using FT-IR Spectroscopy.

**In-vitro drug release study**

Prepared Quercetin (Qu), Rutin (Ru), Silibinin (Si) loaded polymeric nanoformulations were designed to release nanosized Quercetin and natural bio-enhancers in gastric fluid. Hence, to evaluate the immediate liberation of Quercetin and rutin and silibinin act as bio-enhancer from the core of polymer, drug release in vitro study was performed. Designed duo scaffold encumbers polymeric nanosuspension which equivalent to 10 mg of Qu, Ru, Si were separately mixed with 900 ml of simulated gastric environment at 37±0.5ºC at a speed of rotation 100 rpm. Approximately 5 milli letter of samples was taken at the different time interval for every 5, 10, 20, 30, 45 and 60 minutes by reinstated with an same amount of blank. Consequently, samples were centrifuged using cooling centrifuge and portion of supernatant was carefully separated and using 0.22 μm filter paper.

**RESULTS AND DISCUSSION**

**Formulation of plain, Quercetin, Rutin, Silibinin polymeric nanoparticles**

Fabricated Plain, single and duo encompassed with polymeric system of nanoparticulate were deliberate using final optimization formula from Plackett-Burman factorial design. In the region of 250 mg of polymer with and without 50 mg of Qu/Ru/Si were perched with 20 milliliter of 80 fraction of acetone. The prepared organic solution was poured in to 100 mL of distilled water in a beaker, and then 250 mg of Poloxamer 188, and 25 mg of Poloxamer 407 under mechanical stirrer. Then the fabricated nano particulate system engaged further characterization study

**Characterization study of plain, Quercetin, Rutin, Silibinin polymeric nanoparticles**

**Particles size, Zeta potential and Polydispersity Index analysis**

After fabrication, Plain, Quercetin, Rutin, Silibinin, loaded polymeric nanoparticles were stored at room temperature for one month to identify any agglomeration and degradation of post-formulation state. Then the fabricated Plain, Qu, Ru, Si encompassed with polymeric nano particulate system was characterized for size of particle, zeta potential and polydispersity index. All the results were showed in the Table 4 and characterization spectrums were demonstrated in Figure 1 a, b, c, d, e, f, g, h.

The result revealed that plain polymeric nanoparticulate had shown an mean particle size of 128. 30 nm with PDI of 0.1841 and zeta potential of 27.3 mV. Qu polymeric nanoparticles has made known a mean particle size of 84.4 nm with PDI of 0.268 and zeta potential of 10.2 mV. A Ru polymeric nanoparticle has revealed a mean particle size of 65.26 nm with PDI of 0.293 and zeta potential of 15.5 mV. Si polymeric nanoparticles had exposed an average particle size of 101.2 nm with PDI of 0.358 and zeta potential of 14.2 mV. Among all the formulation Ru polymeric Nanoformulation shows significant size reduction.

**Surface morphology Analysis**

Surface morphology come to a decision the basic purpose of particles, decrement, release of active drug from polymer coat, helps to absorbed the particles in side the body, EPR effect \( \beta \) internalization of drug. Nano fabrications of Plain, Qu, Ru, Si, coca-epsulated polymeric nanoparticles were imaged using FE-SEM images were displayed in Figure 2 a, b, c, d.

Synthesized Plain, Quercetin, Rutin, Silibinin co-capsulated polymeric nanoparticles were sphere-shaped. Hence, all the nano formulation scaffold in the polymer core will also be in round shaped and which was expected to enhance therapeutic efficacy.

**Estimation of Nano particulate system**

The quantity of Quercetin and bio-enhancer (Rutin and Silibinin) encompassed in polymeric nanoparticulate system determines the therapeutic efficacy prepared nanoformulations. consequently, content of drug, Co-capsulation competence and estimation of drug loading were performed. All the results were summarized in Table 5.

The result of drug content of all the nanoformulation was the range of 97.78 - 99.28%, which result revealed that there was no drug degradation or drug loss after the formulated nanoformulation. In stirring method, encompassed efficiency of the quercetin was in the ranging from 95.91 - 96.37 % and loading of drug were in the ranging from 18. 76 - 19.02 %. Stirring approach significantly produced excellent co capsulation, loading of drug and only insignificant amount of Quercetin and bio enhancer were displayed as free drug. Hence, the designed Quercetin, Rutin, Silibinin scaffold with polymeric nanoparticulate system was expected to display superior therapeutics and pharmacological activities.

**FT-IR analysis**

Prepared Plain, Qu, Ru, Si, Qu-Ru, Qu-Si loaded polymeric nanoformulations were scanned using Spectroscopic method FT-IR, and the FT-IR spectrum
were displayed in Figure 3 a, b, c, d.

Study of In-vitro drug release

Among all nanoformulations shown >40% release of flavonoids within 5 mins, >60% release of flavonoids within 10 mins, >85% release of flavonoids within 20 mins, >92% release of flavonoids within 30 mins and >95% release of drugs within 45 mins. Hence, released nanosized quercetin and bio-enhancers are predictable to demonstrate enhanced aqueous solubility and its permeability. Hence, the movement of undissolved quercetin to the intestine will be prevented thereby hydrolytic degradation of quercetin in the intestine will also be prevented.

CONCLUSIONS

All prepared nanoformulations exhibit excellent characterization and drug releasing pattern within 45 mins. Hence, released nanosized quercetin and bio-enhancers are expected to display enhanced aqueous solubility and permeability. Hence, the kinesis of quercetin to the intestine will be prevented; in this manner hydrolytic degradation of quercetin in the intestine will also be put off. This novel approach of nano co-capsulation with anionic polymer may provide a unique system for the fortification and delivery numerous a ranges of biopharmaceutical class system IV (BCS -IV) drugs and hydrophobic chemicals in specific target drug delivery.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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