ENRICHMENT OF IN VIVO EFFICACY OF CATECHIN RICH EXTRACT WITH THE APPLICATION OF NANOTECHNOLOGY

MONIKA P.1, BASAVARAJ B. V.2*, CHIDAMBARA MURTHY K. N.3, AHALYA N.1, BHARATH S.2

1Department of Biotechnology, M. S. Ramaiah Institute of Technology, MSRIT Post, Bengaluru 560054, India, 2Department of Pharmaceutics, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, MSRIT, Post, Bengaluru 560054, India, 3Division of Research and Patents, Central Research Laboratory, M. S. Ramaiah Medical College and Teaching Hospital, MSRIT Post, Bengaluru 560054, India

Email: bvbasu@rediffmail.com

Received: 09 Apr 2018 Revised and Accepted: 09 Jul 2018

ABSTRACT

Objective: The primary goal of this study was to convert a natural catechin-rich extract into nanoparticles by using a biodegradable and non-toxic polymer Eudragit L 100 to address the various biopharmaceutical problems of catechin.

Methods: Nanoparticles were prepared by emulsion solvent evaporation technique using Eudragit L 100 in increasing concentration. Optimization of processing conditions like a selection of organic solvents, diluent and surfactant concentrations, drug and polymer ratio and method of drying to increase the biological efficiency were duly attempted. Parameters such as dynamic light scattering, zeta potential, SEM and energy-dispersive X-ray spectroscopy were assessed for the evaluation of nanoparticles.

Results: The entrapment efficiency was found to be between 35-45 % with methanol compared to other organic solvents. The zeta potential values of all the formulations were in the range of±30 mV to±60 mV which confirms moderate to good stability. A rapid or ‘burst’ effect of the drug release in pH 6.8 buffer showing 92 % in the first 30 min which gradually decreased to 52 % by the end of 180 min but in the pH 7.4, the release was found to be moderate. SEM and DLS indicate nanoparticles were of spherical shape lying in a nanometer range of 100 to 200 nm with a proportional influence of polymer on the particles size.

Conclusion: Nanoformulations were found to be more stable and confirmed the presence of major elements such as carbon and oxygen. The findings collectively indicate that it may be worthwhile to apply nanotechnology for the design of an advanced oral dosage form for an enhanced bioavailability and biological efficacy.

Keywords: Catechin rich extract, Bioavailability, Bioefficacy, Pharmacokinetics, Eudragit L 100, Nanoformulation

INTRODUCTION

One of the important plant secondary metabolite belonging to a group of flavonoids with diverse health benefits in humans is catechin. They are polyphenolic compounds having three hydrocarbon rings consisting of six hydroxyl groups at different positions, and the position of hydroxyl groups is important for their antioxidant activities other biological activities [1, 2]. The prominent sources of catechin include apples, oranges, pears, black grapes, blackberries, cherries, raspberries, red wine and dark chocolate. Acacia catechu being one of the richest sources of catechin has been commercially exploited. Flavonoids have demonstrated the multitude of therapeutic effects due to their ability to interact with various biochemical activities. Some of the examples include, inhibiting enzymatic pathways and enzymes such as aldose reductase, cyclooxygenase, xanthine oxidase, lipoxygenase and phosphodiesterase. Catechin has gained an immense interest for its anti-inflammatory and antioxidant properties, it is also shown to improve blood flow and has potential benefits in cardiac health [3-7].

Chemoprevention using natural plant-based products or plant secondary metabolites are essentially important to reduce the cost of cancer therapy and mitigate the side effects of conventional cancer therapies. In order to understand the phenomenon of pharmacokinetics in human, it is essential to know and analyse the pharmacokinetic profiles. Bioavailability of the drug is an important criterion for assessing the pharmacokinetics in vitro and efficacy of any drug/compound. Catechin is known to regulate the cell signalling mechanisms due to their antioxidant activity and their capacity to interact with cell signalling proteins and membrane proteins, which in turn depends on bioavailability [8]. The absorption studies conducted on rats suggested that high quantity of oral dosage of catechin is required to achieve optimum serum concentration, which accounts to 100 folds more than intravenous dosage. Besides higher dosage, slow rate of absorption and rapid elimination from systemic circulation was also observed [9]. There are very few clinical studies, the very reason being its very low bioavailability which might be due to rapid elimination from the liver by biliary excretion [10] when administered in vivo [11].

Low bioavailability of catechin was found to be prominent in most of the in vivo research works, but the reason for this is still not clear and based on the existing literature, there are minimum efforts to address this issue. Hence, the existing problem of low bioavailability, slow absorption, and high first-pass effect necessitates the need to develop strategies that minimize the dosage and in vivo presentation of catechin to achieve maximum therapeutic activity with higher bioavailability. Several plant-based bioactive compounds are formulated into different dosage forms to overcome these drawbacks. Most of the recent studies employ nanotechnology approaches to improve the solubility, bioavailability and bioefficacy as it allows the use of biodegradable, non-toxic nanoparticles having a higher surface to volume ratio to attach or encapsulate natural plant products [12].

The main objective of the present research work was to assess nanotechnology as a tool to develop nanoparticles of catechin-rich extract with a biodegradable and non-toxic methacrylic acid co-polymer Eudragit L 100 to promote better delivery of catechin to the target site with sufficient residence time for enhanced bioavailability due to improvement in its physical properties.

MATERIALS AND METHODS

Catechin rich extract was a gift sample from Green Chem Herbal Pvt. Ltd., Bangalore, India. Sodium lauryl sulphate was procured from Hi Media, India. Eudragit L 100, Sodium Hydroxide and Potassium Dihydrogen Phosphate were procured from Yarrow Chem Products, India. All other chemicals and solvents used for the experiment were of analytical/HPLC grade obtained from Merck, Mumbai, India.
Size reduction of catechin-rich extract (CRE)

The Particle size of CRE was initially reduced by triturating the samples with pestle and mortar for a sufficient length of time ranging from 10 to 15 min. The triturated samples were then observed under an optical microscope (Olympus NFW, India). The particle size was measured for all the samples before and after particle size reduction [13].

Development of nanoparticles

Nanoparticles were developed by an emulsion solvent evaporation technique [15-17] with necessary modifications (table 1). CRE and the polymer Eudragit L-100 were finely triturated in a mortar to reduce the size to nano size. Drug and the polymers in the ratios of 1:1, 1:2 and 1:3 were dissolved in 15 ml of methanol, ethanol, acetone, methanol, propanol and an aqueous solution of 0.1N NaOH separately. These solutions were sonicated for 30 min using an Ultrasonicator (PCL, model 1.5 L 5 OH, Mumbai, India) following which they were filtered separately using a Whatman filter paper no.44 and mixed thoroughly using an electric stirrer (REMI Instruments, India) at 3000 rpm for 20 min. 5 mg of SLS was added to the solution. After the addition of SLS, 10 mg/ml aqueous solution of lactose monohydrate was added and continuously stirred. The obtained solution was dried in a hot air oven (Tempo Instruments, Mumbai, India) maintained at 40 °C±5 °C for 8 h.

Table 1: Different combinations of ingredients used for pre-formulation

| Code | CRE (mg) | EUD L 100 (mg) | SLS (mg) | Lactose (mg) | Alcohol (ml) | Acetone (ml) | Methanol (ml) | Propanol (ml) | 0.1N NaOH (ml) |
|------|---------|---------------|---------|-------------|-------------|-------------|-------------|-------------|--------------|
| PF1  | 100     | 100           | 30      | 100         | -           | -           | -           | -           | -            |
| PF2  | 100     | 100           | 30      | 100         | 30          | -           | -           | -           | -            |
| PF3  | 100     | 100           | 30      | 100         | 30          | 30          | -           | -           | -            |
| PF4  | 100     | 100           | 30      | 100         | 30          | 30          | 30          | -           | -            |
| PF5  | 100     | 100           | 30      | 100         | 30          | 30          | 30          | 30          | -            |

CRE: catechin rich extract, SLS: sodium lauryl sulphate, EUD: eudragit

Standardization of organic solvents for nanoformulation

Various organic solvents such as ethanol, acetone, methanol, propanol and an aqueous solution of 0.1 N NaOH were used for preparing nanoformulations (table 1). For the production of nanoparticles in bottom-up technology the drug was dissolved in a solvent, then added to the non-solvent that causes precipitation of the fine drug particles [14].

Evaluation

Determination of drug entrapment efficiency in the formulation

Using the standard calibration curves the drug entrapment efficiency of various pre-formulations (prepared using different organic solvents) was determined by accurately weighing 100 mg of formulation sample and dissolving it in a suitable buffer in which the drug has high solubility. Here, 100 mg of each formulation was weighed and dissolved in 100 ml of phosphate buffer pH 6.8 and pH 7.4 respectively in 100 ml volumetric flasks and the volume was made up to 100 ml with respective buffers to get (SS–I) of 1000 µg/ml and (SS–II) of 50 µg/ml which were spectrophotometrically analyzed at 276 nm [14].

\[
\% \text{ Drug content} = \frac{\text{Drug content (mg) × 100}}{\text{Label claim (mg)}}
\]

Standardization of concentration of diluent and surfactant for nanoformulation

Lactose was used as a diluent and sodium lauryl sulphate (SLS) as a surfactant in the preparation of nanoparticles. To determine the effect of diluent and surfactant used in the pre-formulation samples, dissolution studies were carried out in phosphate buffers pH 6.8 and pH 7.4. The pre-formulation ingredients used for standardized formulation PF3 is mentioned in table 2.

Table 2: Standardization of concentration of diluent and surfactant for PF3 formulation

| Pre-formulation ingredient quantity (mg) |
|-----------------------------------------|
| Catechin rich extract (CRE)              | 100 |
| Eudragit L 100                           | 100 |
| Sodium lauryl sulphate (SLS)             | 30  |
| Lactose (aqueous solution)               | 100 |
| Methanol                                 | 30  |

In vitro dissolution studies

In vitro dissolution of nano tablets was carried out for 12 h in pH 6.8 and pH 7.4 phosphate buffer of 900 ml volume in dissolution apparatus (USP XXII (paddle), Lab India, DS 8000, Mumbai, India) at 50 rpm maintained at 37 °C±0.5 °C. Sample absorbance was measured at 276 nm by using a UV/Visible Spectrophotometer (UV-1600, Shimadzu, Japan). Percentage drug release was calculated using the equation obtained from a standard curve [13]. The final standardized nanoformulation ingredients are as shown in table 8.

Residual moisture content analysis

The moisture content of nanoformulation after freeze-drying was analyzed by Karl Fischer Titration using a Auto-Titrator (Titrasyx 352, Systronics, India). The solvent dimethyl sulphoxide (DMSO) was standardized with the help of Karl Fischer reagent to determine and eliminate moisture content. The nanoformulation was solubilised in the DMSO. To the standardized solution, a fixed volume of the sample solution was added and titrated with Karl Fischer reagent [19]. The percentage moisture content was determined by the formula given below.

\[
\text{Moisture Content (¢)} = \frac{\text{KF Factor } \times \text{ KF reagent required (ml)}}{\text{Volume of sample solution (ml)}} \times 10.1556
\]

Particle size distribution and polydispersity by dynamic light scattering

The particle size distribution and polydispersity were analyzed by a dynamic light scattering (Brookhaven Instruments, Zetapals, Germany) [16]. Before measurement, a small number of nanoparticles were suspended in distilled water maintained at 25 °C,
the suspension was then dispersed by ultrasonic waves with power 120 W for 1 min. Particle size distribution and polydispersity of NF1, NF2 and NF3 nanoformulations were then assayed by the analytical software. The measurement was performed in triplicates.

Zeta potential measurements

Zeta potential of all standardized nanoformulations was measured to determine the stability of nanoformulations using Zeta Potential Analyzer (Brookhaven Instruments, Zetapals, Germany). A sample was dispersed in distilled water at 25 °C for a period of 30 sec in a quartz cuvette with the electrode dipped inside the distilled water. Each sample was run 5 times, and the zeta potential graph was obtained using zetapals software of the Smoluchowski model [16].

Surface morphology by scanning electron microscopy

Surface morphology and particle size measurement of the nanoparticles was determined by scanning electron microscopy (SEM) (Zeiss Ultra 55, Germany) [17-24]. In the analysis, the samples were firstly attached to a small piece of the electro-conductive silicon chip; then gold sputtered using a vacuum gold sputter coater.

Elemental analysis of nanoformulation by energy-dispersive x-ray spectroscopy

Various elements present in the nanoformulation were determined by energy-dispersive x-ray spectroscopy (EDX), which was attached to SEM (Zeiss Ultra 55, Germany). The sample analyzed using EDX was NF1. A small part of the sample attached to carbon tape was selected by viewing under SEM. This selected portion of the sample was analyzed for its elemental composition. Weight (%) and Atomic (%) for all the elements present was determined [25].

Zeta potential measurements

Zeta potential of all standardized nanoformulations was measured to determine the stability of nanoformulations using Zeta Potential Analyzer (Brookhaven Instruments, Zetapals, Germany). A sample was dispersed in distilled water at 25 °C for a period of 30 sec in a quartz cuvette with the electrode dipped inside the distilled water. Each sample was run 5 times, and the zeta potential graph was obtained using zetapals software of the Smoluchowski model [16].

Surface morphology by scanning electron microscopy

Surface morphology and particle size measurement of the nanoparticles was determined by scanning electron microscopy (SEM) (Zeiss Ultra 55, Germany) [17-24]. In the analysis, the samples were firstly attached to a small piece of the electro-conductive silicon chip; then gold sputtered using a vacuum gold sputter coater.

Elemental analysis of nanoformulation by energy-dispersive x-ray spectroscopy

Various elements present in the nanoformulation were determined by energy-dispersive x-ray spectroscopy (EDX), which was attached to SEM (Zeiss Ultra 55, Germany). The sample analyzed using EDX was NF1. A small part of the sample attached to carbon tape was selected by viewing under SEM. This selected portion of the sample was analyzed for its elemental composition. Weight (%) and Atomic (%) for all the elements present was determined [25].

Table 3: Particle size measurements before and after particle size reduction of catechin rich extract

| Trial no. | Particle size before triturating (µm)±SD | Particle size after triturating (µm)±SD |
|-----------|----------------------------------------|----------------------------------------|
| 1         | 218.07±2.049                           | 41.62±0.832                           |
| 2         | 261.19±1.028                           | 32.45±0.330                           |
| 3         | 181.91±1.579                           | 54.58±1.176                           |
| 4         | 244.85±0.652                           | 33.93±2.773                           |
| 5         | 182.67±1.233                           | 34.82±0.268                           |
| 6         | 174.40±1.287                           | 44.32±1.940                           |
| 7         | 171.24±1.037                           | 52.9±0.952                            |
| 8         | 185.60±0.756                           | 55.33±2.253                           |
| 9         | 129.38±1.338                           | 38.17±1.216                           |
| 10        | 198.64±0.664                           | 43.94±0.721                           |
| mean±SD  | 198.04±1.104                           | 41.62±0.832                           |

The values are expressed as mean±SD; n=3

The particle size of the formulations containing different proportions of organic solvents was measured using an optical microscope and results are shown in Table 3. The mean particle size of ethanol, acetone, methanol, and propanol were found to be drastically reduced to as low as 40 µm. Table 4 shows that ethanol sample has the highest particle size with the mean particle size of 4.98 µm followed by propanol, methanol and acetone samples having a mean particle size of 3.02, 2.98 and 2.95 µm respectively. The order of the mean particle size for different organic solvents was in the follows Ethanol>Propanol>Methanol>Acetone. Based on the above results, propanol, methanol and acetone pre-formulation samples were considered for further evaluations. The uniform distribution of particles of PF1 (Ethanol), PF2 (Acetone), PF3 (Methanol) and PF4 (Propanol) was determined by an optical microscope. The pictures of ethanol, acetone, methanol and propanol pre-formulations captured by the camera fitted to the optical microscope are shown in fig. 1-4. It was observed that acetone, methanol and propanol pre-formulations showed lesser particle size. Methanol and propanol pre-formulations had uniformly distributed particles without any aggregation. Thus acetone, methanol and propanol pre-formulations were subjected for the assay to determine drug entrapment efficiency in pH 6.8 and pH 7.4 phosphate buffers.

Entrapment efficiency

Based on the results obtained, acetone, methanol, and propanol were selected as efficient organic solvents for nanoformulation development. Selection of a final organic solvent was done based on the evaluation of drug entrapment efficiency in these solvents. Methanol pre-formulation showed highest drug entrapment efficiency in pH 6.8 and pH 7.4 phosphate buffers than in acetone and propanol pre-formulations (Table 5). It was also observed that an increase in pH of the media from 6.8 to 7.4 increased the solubility of the drug with higher drug entrapment efficiency. Therefore methanol was selected as a choice of an organic solvent for preparation of all nanoformulations. The percentage entrapment efficiency mainly depends on the nature of the drug and polymer.
Table 4: Particle size measurement of the pre-formulations

| Trial no. | Sample size (µm) | Ethanol sample (PF1) | Acetone sample (PF2) | Methanol sample (PF3) | Propanol sample (PF4) |
|-----------|------------------|----------------------|----------------------|-----------------------|-----------------------|
| 1         | 6.40             | 2.23                 | 4.01                 | 2.00                  |
| 2         | 6.08             | 4.24                 | 3.63                 | 2.00                  |
| 3         | 3.16             | 4.12                 | 3.84                 | 1.41                  |
| 4         | 4.12             | 2.82                 | 2.82                 | 2.01                  |
| 5         | 4.10             | 3.13                 | 3.32                 | 3.10                  |
| 6         | 5.83             | 2.23                 | 4.12                 | 3.12                  |
| 7         | 5.65             | 4.12                 | 3.16                 | 3.16                  |
| 8         | 6.02             | 1.20                 | 2.23                 | 3.16                  |
| 9         | 6.70             | 2.82                 | 1.00                 | 4.12                  |
| 10        | 2.22             | 2.82                 | 3.00                 | 3.23                  |
| mean±SD  | 4.98±0.331       | 2.95±0.226           | 2.90±0.127           | 3.02±0.214            |

The values are expressed as mean±SD; n=3

Fig. 1: Optical microphotograph of ethanol pre-formulation
Fig. 2: Optical microphotograph of acetone pre-formulation
Fig. 3: Optical microphotograph of methanol pre-formulation
Fig. 4: Optical microphotograph of propanol pre-formulation

Table 5: Drug entrapment efficiency of CRE pre-formulation samples in various organic solvents

| Medium          | Drug entrapment efficiency (CRE) (%) | Acetone | Methanol | Propanol |
|-----------------|--------------------------------------|---------|----------|----------|
| pH 6.8 buffer   | 21.21                                | 35.96   | 24.96    |
| pH 7.4 buffer   | 34.92                                | 47.42   | 44.32    |

CRE-catechin rich extract

Standardization of concentration of diluent and surfactant for nanoformulation

Lactose was used as a diluent and sodium lauryl sulphate was used as a surfactant in preparing the formulation by emulsion solvent evaporation technique (table 6). In vitro dissolution studies were carried out two different dissolution solvents pH 6.8 and pH 7.4 phosphate buffers separately to ascertain the effect of diluent and surfactant used in the pre-formulation samples, (table 7 and 8).

Every ingredient used in the preparation of nanoformulation has an effect on the dissolution rate and mechanism of drug release. The pre-formulation ingredients used for the nanoformulations areas mentioned in table 5. It can be observed that percentage drug release was decreasing with increase in time (fig. 8). Also, there was a rapid or ‘burst’ effect of the release of drug in pH 6.8 buffer showing 92 % in the first 30 min which gradually decreased by the end of 180 min (table 7). Almost all the drug content was released in the initial 30 min and did not show a sustained release pattern.
Similarly in case of pH 7.4 phosphate buffer the drug was released in an increasing order from 0 min to 120 min and reached a maximum drug release at the end of 120 min after which the % drug release decreased (table 8). Sustained release pattern was not observed in case of pH 7.4 phosphate. The faster dissolution rate of the Eudragit L 100 coated pre-formulation in the initial periods of time was found to be due to higher SLS concentration (30 mg/100 mg drug). SLS is a surfactant known to increase the dissolution rate significantly which can be observed as an initial 'burst' effect and subsequent decrease in the percentage drug release (fig 5). The rate of release was increased with increasing concentration of surfactant. This could be due to the emulsification effect of the surfactant after the hydration of the nanoparticles by the dissolution medium. Based on the obtained results, the concentration of SLS was significantly reduced to obtain an optimum sustained release pattern and a final concentration of 5 mg/100 mg of the drug was used for further nanoformulations.

| Table 6: Standardized nanoformulation ingredients and their quantity |
|---------------------------------------------------------------|
| Nanoformulation ingredients                                  | Quantity (mg) |
|                                                              | NF1 | NF2 | NF3 |
| Catechin rich extract                                         | 100 | 100 | 100 |
| Eudragit L 100 (polymer)                                     | 100 | 200 | 300 |
| Sodium lauryl sulphate                                       | 5   | 5   | 5   |
| Lactose (aqueous solution)                                   | 10  | 10  | 10  |
| Methanol (ml)                                                | 30  | 30  | 30  |
| Drug: Polymer ratio                                          | 1:1 | 1:2 | 1:3 |

| Table 7: Dissolution of PF-3 in pH 6.8 buffer |
|-----------------------------------------------|
| Code  | Media  | Time (min) | % Drug release* |
|       |        |            |                |
| PF3   | pH 6.8 | 30          | 92.12±7.31     |
|       |        | 60          | 65.65±9.08     |
|       |        | 90          | 80.12±7.66     |
|       |        | 120         | 65.98±4.44     |
|       |        | 150         | 59.99±8.84     |
|       |        | 180         | 51.94±6.88     |

*The values are expressed as mean±SD

| Table 8: Dissolution of PF-3 in pH 7.4 buffer |
|-----------------------------------------------|
| Code  | Media  | Time (min) | % Drug release* |
|       |        |            |                |
| PF3   | pH 7.4 | 30          | 71.59±4.44     |
|       |        | 60          | 71.75±6.66     |
|       |        | 90          | 78.04±8.52     |
|       |        | 120         | 78.21±9.88     |
|       |        | 150         | 72.25±5.88     |
|       |        | 180         | 58.09±6.52     |

n=3 *The values are expressed as mean±SD; n=3

![In vitro drug release of PF-3](image)

**Fig. 5: In vitro dissolution profile of PF3 in pH 6.8 and 7.4 phosphate buffer**

Based on previous results, standardized nanoformulation ingredients are as shown in table 8. For all the further evaluations NF1 with the drug: polymer ratio of 1:1, NF2 1:2 and NF3 1:3 respectively, were considered.

**Development of optimized nanoformulation**

Nanoformulations of CRE were developed by emulsion solvent evaporation technique using Eudragit L 100 as an enteric polymer for encapsulation of the drug. Drug and polymer in the ratios of 1:1, 1:2 and 1:3 were prepared and evaluated as NF1, NF2 and NF3 respectively throughout the study (table 6). Nanoformulations were developed by an emulsion solvent evaporation technique. The standardized nanoformulation ingredients and their quantity are given in table 8. Nanoformulations were developed by a simple and cost-effective method i.e., emulsion solvent evaporation technique using an enteric and biodegradable polymer Eudragit L 100.

**Residual moisture content analysis**

The residual moisture content was determined by Karl fischer titration. The residual moisture content of the freeze-dried nanoformulation (NF1) was found to be 0.00549 using the equation as mentioned in section materials and methods. The negligible moisture content as determined indicates that there was very negligible moisture content in the nanoformulation. Thus the nanoformulation was efficiently dried using lyophilizer. Hot air oven method was easier and quicker method compared to lyophilization, but the standardization of method of drying was carried out based on the evaluation of several parameters such as percentage yield, particle size distribution and drug entrapment efficiency in pH 7.4 phosphate buffer (table 9). The method of drying of nanoformulation was standardized by evaluating parameters such as percentage yield, the particle size measured by SEM and drug entrapment efficiency (table 9).
Table 9: Standardization of method of drying

| Code | Media/Buffer | Method of drying | Percentage yield (%) | Particle size±SD (nm) | Drug entrapment efficiency (mg) |
|------|--------------|------------------|-----------------------|-----------------------|--------------------------------|
| NF1  | pH 7.4       | Hot air oven     | 78                    | 241±46.87*            | 30                             |
|      |              | Lyophilization   | 74                    | 550±272.7**           | 20                             |

* The values are expressed as mean±SD; n=3, ** The values are expressed as mean±SD; n=5

Based on the results, hot air oven method of drying was found to be the most suitable and efficient compared to lyophilization (table 9). NF1 dried by hot air oven was found to have higher percentage yield content and drug entrapment efficiency of 78 % and 30 mg respectively than NF1 dried by lyophilization. Most importantly, a freeze-dried sample showed a lot of aggregation having to mean particle size of 550 nm which is much greater than NF1 dried by hot air oven having a mean particle size of 241 nm. This might be due to high vacuum used for freeze-drying to dry the moisture content via sublimation process. Finally, it dries the sample leaving intact particles which were agglomerated. Thus, hot air oven method of drying was standardized as the method of drying for preparation of nanoformulations.

Particle size distribution and polydispersity by dynamic light scattering

Particle size distribution and polydispersity of NF1, NF2, NF3 was determined by DLS and are shown in table 10. Nanoformulations had a mean particle size ranging from 200 nm to 400 nm and with an increase in polymer concentration the particle size increases due to the coating of Eudragit L 100 around the drug in multiple layers which in-turn depends on polymer concentrations. Hence, the particle size is directly proportional to polymer concentration. From table 10 it can be observed that all nanoformulations have low polydispersity index with uniformly dispersed nanoparticles.

Surface morphology by scanning electron microscopy

The scanning electron microscopy revealed the formation of well recognizable nanoparticles with uniformity in distribution (fig. 6). The examined nanoparticles appeared almost spherical with sharp boundaries and a smooth outer surface. Sphericity can be attributed to the presence of surfactants due to minimization of surface free energy (26). Based on the particle size measurement, nanoformulations were found within the nanometer range of 100 nm-200 (fig. 6, 7). SEM also revealed the agglomeration of nanoparticles which might be due to the adhesive nature of the polymer and drying process of the sample before SEM analysis.
Zeta potential measurements

Zeta potential determines the physical stability of nanoparticles in a suitable carrier. It is an indirect measurement of the thickness of the diffusion layer, i.e., it can be used to predict the long-term stability of the nanoformulations. In order to obtain an electrostatically stabilized nanosuspensions. In order to obtain an electrostatically stabilized nanosuspension exhibiting good stability, a minimum zeta potential of ±20 mV is required whereas, in case of combined electrostatic and steric stabilization, a minimum zeta potential of ±20 mV is desirable.

![Table 1: Comparison of particle size distribution, polydispersity and zeta potential measurements of standardized nanoformulations](image)

| Nano formulation code | Particle size distribution (nm) ±SD | Polydispersity ±SD | Zeta potential (mV) ±SD |
|-----------------------|-----------------------------------|--------------------|------------------------|
| NF1                   | 24±4.6.8                          | 0.005±0.042        | -38.36±2.01            |
| NF2                   | 33±4.7.4                           | 0.005±0.045        | -46.96±1.14            |
| NF3                   | 36±8.7.3                           | 0.015±0.046        | -45.84±2.00            |

*The values are expressed as mean±SD; n=3

CONCLUSION

In the present study, Eudragit L 100 was used as a pH sensitive, biodegradable and non-toxic polymer. In order to develop a biologically efficient nanoformulation, few vital parameters like the selection of organic solvents, diluent and surfactant concentrations, drug and polymer ratio, a method of drying were standardized. DLS data proved that particle size distribution of the nanoparticles increases with the increase in the concentration of the polymer. Analytical characterization proved the occurrence of the formulations in the nanoscale range with optimum stability and drug release. At last, it could be reemphasized that the nanoformulation serves as an effective tool in the improvement of in vivo efficacy of herbal extracts with bioavailability problems and solubility limitations.

ACKNOWLEDGMENT

Authors would like to acknowledge the support and motivation from Gokula Education foundation and its institutions, specifically, Central Research Lab of M. S. Ramaiyah Medical College and Teaching Hospital, M. S. Ramaiyah Institute of Technology and Faculty of Pharmacy, RUAS. I would like to acknowledge the support of Vision Group of Science and Technology, Department of Science and Technology, Government of Karnataka for financial support for the project through Technology Related Innovative Projects (TRIP-2013-14). Authors would like to thank Dr. Rajendra, Groen Chem Herbal Pvt. Ltd., Bangalore, for providing Catechin Rich Extract for the study.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Authors have no conflict of interest

REFERENCES

1. Jin Ze Xu, Sai Ying Venus Yeung, Qi Chang, Yu Huang, Zhen-Yu Chen. Comparison of antioxidant activity and bio-availability of tea catechins with their epimers. Br J Nutr 2004;91:873–81.
2. Chidambaram Murthy KN, Kim J, Vikram A, Patil BS. Differential inhibition of human colon cancer cells by structurally similar flavonoids of citrus. Food Chem 2012;132:27-34.
3. Agrawal AD. Pharmacological activities of flavonoids: a review. Int J Pharma Sci Nanotech 2011;4:1394-8.
4. Vanna Samna, Imtiaz, Siddiqui A, Mario Sechi, Hasan Mukhtar. Nanof ormulation of natural products for prevention and therapy of prostate cancer. Cancer Lett 2013;334:142–51.
5. Fraga Cesar G, Oteiza Patricia I. Dietary flavonoids: the role of (−)-epicatechin and related procyanidins in cell signalling. J Free Radical Bio Med 2011;51:813-23.
6. Zhu Min, Chen Yu, Ronald C Li. Oral absorption and bioavailability of tea catechin. Plant Med 2000;66:444-7.
7. Kobri T, Nano F, Suzuki M. Synthesis of (−) [4-3H] epigallocatechin gallate and its metabolic fate in rats after intravenous administration. J Agric Food Chem 2001; 49:1042-8.
8. Tasmina Khushud, Shaker A, Moussa. Potential role of naturally derived polyphenols and their nanotechnology delivery in cancer. Mol Biotech 2013;55:78-86.
9. McNeil SE. Nanotechnolog for the biologist. J Leukocyte Bio 2005;78:585-94.
10. AdlInjno Nesalin J, Anton Smith A Preparation and evaluation of chitosan nanoparticles containing zidovudine. Asian J Pharma Sci 2012;7:80-4.
11. Ashwini Kumar. Enhancement of solubility and dissolution rate of irbesartan by solid dispersion technique. Asian J Pharm Clin Res 2011;4:36-40.
12. Prasanna Lakshmi, Giddam Ashwini Kumar. Nanosuspension technology: a review. Int J Pharm Pharm Sci 2010;2:35-40.
13. Monika P, Ahalya N, Basavaraj BV, Chidambaram Murthy KN. Development and characterization of catechin-rich extract nanoparticles. Int Rev Appl Biotech Biochem 2014;2:85-93.
14. Iai Prakash B, Gaja, Sayyad FJ. Preparation and evaluation of nanodispersions of telmisartan by various techniques. Inventi Impact NDD 2013;2:151-9.
15. Nagavarma BVN, Hemant KS Yadav, Ayaz A, Vasudha LS, Shrivakumar HG. Different techniques for preparation of polymeric nanoparticles-a review. Asian J Pharm Clin Res 2012;5:16-23.
16. Zu Y, Wang D, Zhao X, Jiang R, Zhang Q, Zhao D. A novel preparation method for camptothecin (CPT) loaded folic acid conjugated dextran tumor-targeted nanoparticles. Int J Mol Sci 2011;12:4237-49.
17. Kunal A Gaidhani, Mallinath Harwalkar, Deepak Rambhane, Pallavi S Nirgude. Lyophilization/Freeze drying–a review. Int J Novel Trends Pharm Sci 2015;4:516-43.
18. Bharathi M, Sarat Chandra Prasad M, Latha Eswari R, Wasim Raja S, Ravi Teja Allena, Brito Raj S. Preparation and in vitro and in vivo characterization of valsratan loaded eudragit nanoparticles. Der Pharm Sin 2012;3:516-25.
19. Momin Mohammed, Abdul Motalib, Afroze Afgina, Khan Mohammed Salahuddin, Mashud SM. Method validation for the determination of water content of metered dose inhaler by Karl Fischer coulometer. Int J Phar 2012;3:144-7.
20. Hu Daode, Liu Liang, Chen Wenjuan, Li Sining, Zhao Yaping. A novel preparation method for 5-aminosalicylic acid loaded eudragit S100 nanoparticles. Int J Mol Sci 2012;13:454-68.
23. Sudheer Nadendh, Snehalatha, Nagaraja TS, Bharathi. Effect of various polymers on swelling and in vitro release of ramipril in the effervescent system. Int J Pharm Life Sci 2013;4:2621-5.
24. Kalam MA, Humayun M, Parvez N, Yadav S Garga. Release kinetics of modified pharmaceutical dosage forms: a review. Cont J Pharm Sci 2007;1:30-5.
25. Sharma A, Shukla T, Indira M. Design, development and evaluation of aceclofenac sustained release matrix tablet. Int J Drug Res 2011;3:307–12.
26. Bharate SS, Bharate SB, Bajaj AN. Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review. J Excep Food Chem 2010;1:3–26.
27. Ahmed M Samy, Afaf A, Ramadan, Amal SM. Abu el-enin, Yasmin IM Mortagi. Formulation and optimization of itraconazole pro-niosomes using box behnken design. Int J Appl Pharm 2018;10:41-51.