DEVELOPMENT AND CHARACTERIZATION OF SAFFRON EXTRACT BASED POLYMERIC NANOPARTICLES FOR THE MANAGEMENT OF ARRYTHMIA AND HYPERTENSION

Suma U S[1], Senthil Prabhu Rajendran[2], Mamatha M K[3*], Shailaja Nare[4], Sathya Narayana Reddy B[5], Rajeev Ranjan[6], Pawar Shivkanya Sunil[7], Hupparage Vrushabh Balaso[8]

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Abstract

Introduction: Saffron is one of the oldest spices, its history going back to the highest antiquity. Biologically known as Crocus sativus belongs to the Iridaceae family. Traditionally Saffron is used in treatment of depression, Sexual dysfunction, Antioxidant, arrythmia and hypertension. Numerous investigations have shown that both arrythmia and hypertension. can be controlled by their entrapment in submicronic colloidal systems (nanoparticles).

Objectives: This research article aims to formulate Saffron extract based polymeric nanoparticles by the solvent evaporation method, Various Parameters like particle size, poly dispersity index, and zeta potential were calculated, Compatible studies between the nanoparticles and the loaded drug were analysed using FT-IR**,** invitro drug release studies were performed.

Areas covered: Polymeric nano formulations have gained considerable attention as effective carriers to enhance the bioavailability of drugs. This article covers an overview of saffron as potential source of arrythmia and hypertension, increase in bioavailability through nano formulations.

Conclusion: Saffron extract based polymeric nanoparticles by the solvent evaporation method were successfully prepared suggesting a comparatively suitable option for treatment of disease with fewer side effects and increased affinity of drug.Given the aforementioned information, formulated nano particles should be investigated for further in vivo studies.

Keywords: Arrythmia, Hypertension, Nano particles, Crocus sativus,saffron.

[1]Assistant Professor, Mallige College of Pharmacy, Silvepura, Bangalore. 560090
[2]Assistant Professor, Department of Pharmaceutics, College of Pharmacy , Madurai Medical College, Madurai, Tamil Nadu. 625020
[3*]Associate Professor, Mallige College of Pharmacy, Silvepura, Bangalore. 560090
[4]Assistant professor, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, Maharshtra. 416013
[5]Clinical Pharmacist, Venus Hospital, Surat, Gujarat. 335009
[6]Assistant Professor, Department of Chemistry, DSPM University, Morhabadi, Ranchi, Jharkhand. 834008
[7]Scholar, Dr NJ Paulbudhe College of Pharmacy, Jay Bhavani Nagar Kajabe Vasti Bhistabag Road, Savedi, Ahmednagar, Maharashtra. 414003
[8]Assistant Professor, Sharadchandra Pawar College of Pharmacy, Dumbarwadi, Otur, Khamundi, Junnar, Pune. 412409

Corresponding Author:
[3]Mamatha M K
[3]Associate Professor, Mallige College of Pharmacy, Silvepura, Bangalore. 560090

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1. Introduction:

Arrhythmia

An arrhythmia (also called dysrhythmia) is an abnormal heartbeat. Arrhythmias can start in different parts of your heart and they can be too fast, too slow or just irregular.

Normally, your heart beats in an organized, coordinated way. Issues with various parts of your heart — or even the blood your heart pumps — can affect your heart's normal rhythm. Having a normal heart rhythm matters because your heart supplies your whole body with nutrients and oxygen through the blood it pumps.

Types of Arrythemia

- **Supraventricular arrhythmias**: These begin in your atria (your heart's upper chambers). "Supraventricular" means above your ventricles or lower chambers of your heart.
- **Ventricular arrhythmias**: These begin in your heart's ventricles or lower chambers.
- **Bradyarrhythmias** and **junctional rhythms**: These can happen because of issues in your heart's conduction system, such as the sinoatrial (SA) node, atrioventricular (AV) node or His-Purkinje network.

Hypertension

Abnormally high blood pressure and a combination of high psychological stress are known as Hypertension. These patients suffering from this disorder will have their blood pressure reading greater than 140 over 90 mm.

Hypertension is diagnosed by measuring blood pressure. The Systolic pressure would be the first readings viz. a pressure by which the heart pumps blood through the body, and second readings would be the Diastolic pressure, meaning a pressure at which the heart relaxes and refills the blood.

Arrhythmia causes include:

- Coronary artery disease.
- Irritable tissue in your heart (due to genetic or acquired causes).
- High blood pressure.
- Changes in your heart muscle (cardiomyopathy).
- Valve disorders.
- Electrolyte imbalances in your blood.
- Injury from a heart attack.
- The healing process after heart surgery.
- Other medical conditions.

Types of Hypertension

When people talk about hypertension, they are usually referring to one of the two types, namely:

- Primary hypertension
- Secondary hypertension

Primary hypertension is also known as essential hypertension. This is the most prevalent form of hypertension and it has no identifiable cause.

Secondary hypertension is caused by an underlying disease or even medication. Thyroid dysfunction, sleep apnea and diabetes have been linked to secondary hypertension. Chemicals such as amphetamines, antidepressants and even caffeine can lead to hypertension.

Causes of Hypertension

Acute stress and unfavourable environmental factors are the main factors for increasing blood pressure in normal and healthy individuals. The increasing rate of the prevailing condition is mostly blamed on the lifestyle and dietary factors such as inactive habits, high diet sodium content from processed fatty foods, tobacco and alcohol use.

In the last few decades, nanotechnology has proven to be of significant importance owing to its application in optical, catalytic, electronic, and medicinal fields [1]. The application of nanotechnology in medicinal science is of most importance due to its beneficial impact on human as well as animal and plant health. Control drug delivery, tissue engineering, tumor detection and destruction, electroluminescent, drug and disease sensors, and diagnosis of cancer through MRI are some examples of nanoparticle application in the medical field [2,]. Currently, different techniques are applied for the synthesis of nanoparticles including microwave-assisted synthesis, chemical and photochemical synthesis protocols, reduction in solution, and electrochemical synthesis route [3]. Green synthesis of nanoparticles through ecofriendly synthesis methods is gaining attention among the researcher community because it does not require high pressure, temperature, and toxic chemicals but bacteria, fungi, and plant extract are used for the synthesis of green nanoparticles [4]. These biological systems can synthesize nanoparticles in a safe, easy, and economical way [5]. The formation of green nanoparticles is due to the strong reducing ability of these biological systems, and this reducing ability is attributed to the enzymes and/or biomolecules in plant cells [6]. The development of environment-friendly plant extract-based nanoparticles through green synthetic route has vast application in modern science owing to their efficient drug delivery model and less toxicity [7]. Green synthesis of nanoparticles developed using algae, plants, fungi, and bacteria is renowned as a safe, efficient, and environmentally friendly approach in drug discovery [8]. Around the globe, saffron is most commonly used as a traditional food spice and medicine. *Crocus sativus* L. (saffron) is a perennial herb that belongs to the family Iridaceae and is also known as red

gold. It is known to be the most expensive herb cultivated throughout the world [9].

Saffron is one of the oldest spices, its history going back to the highest antiquity. The earliest depiction dates from 1600 to 1700 BC[10]. Saffron is the dried stigmas of *Crocus sativus L*. *Crocus sativus L* belongs to the family of Iridaceae, the line of Liliaceae and is mainly cultivated in several countries of mild and dry climate.[11].

Saffron extract

Saffron's name is derived from the Arab word for yellow, a name reflecting the high concentration of carotenoid pigments present in the saffron flowers' stigmas which contribute most to the color profile of this spice[12]. In the traditional medicine, saffron is used as a diaphoretic, eupeptic, tranquilizer, expectorant, aphrodisiac, abortifacient, emmenagogue and in the treatment of hepatic disorders, flatulence, spasm, vomiting, dental and gingival pain, insomnia, depression, seizures, cognitive disorders, lumbago, asthma, cough, bronchitis, colds, fever, cardiovascular disorders, and cancer.[13,14].Saffron contains more than 150 volatile, non-volatile, and aroma-yielding compounds[15].Based on chemical analyses of dry stigma of saffron extracts, carotenoids, namely crocin and crocetin and the monoterpene aldehydes picrocrocin and safranal are the most important active carotenoid secondary metabolites of saffron.[16] Polymeric nanoparticles (NPs) are particles within the size range from 1 to 1000 nm and can be loaded with active compounds entrapped within or surface-adsorbed onto the polymeric core [17,18]The term "nanoparticle" stands for both nano capsules and nanospheres, which are distinguished by the morphological structure.Polymeric NPs have shown great potential for targeted delivery of drugs for the treatment of several diseases .

Saffron contains a host of plant compounds that act as powerful antioxidants and protect cells against free radicals and oxidative stress that can lead to cancer. Crocin and crocetin are antioxidants that are responsible for saffron's vibrant red color. These compounds are thought to have antidepressant effects, protect brain cells against damage, decrease inflammation, and reduce appetite.

Safranal is what gives saffron its distinct taste and smell. Evidence points to its ability to help improve mood, memory, and protect the brain against oxidative stress.[4] Test-tube studies have shown that saffron and its antioxidant compounds kill different types of cancer cells or suppress their growth. While these studies are promising, much more research is needed.

Studies also show that saffron may help treat symptoms of premenstrual syndrome (PMS), which is the physical and psychological symptom that may occur before starting a menstrual period. One study showed that women who took 30 milligrams of saffron daily decreased irritability, headaches, cravings, and pain. Additionally, saffron was more effective than taking the placebo.

Additionally, animal and test-tube studies have indicated saffron's efficacy in reducing the risk of heart disease by lowering cholesterol and preventing clogged blood vessels. Saffron may also lower blood sugar and increase insulin sensitivity, as shown in mice studies.

While these results are promising, more research needs to be done on humans before recommending saffron supplementation for certain health conditions. However, there are some older studies that indicate that the antioxidants in saffron tea can help reduce the risk of cardiovascular diseases. Researchers also note that the flavonoids found in saffron also can provide protection.

Mental Benefits

Saffron is known as the "sunshine spice," not only due to its bright yellow color, but also for its effects on helping to improve mood. One review study showed that taking saffron supplements was significantly more effective than placebos at treating mild to moderate depression symptoms.

Older studies have shown that taking 30 milligrams of saffron daily was as effective as taking conventional medications for depression and those individuals experienced fewer side effects. Always speak with a healthcare provider if you are considering using saffron as an adjunct to or in addition to other forms of mental health treatment, though.

Saffron has also shown to have aphrodisiac properties and may be particularly effective in individuals taking antidepressants. One study demonstrated that men with antidepressant-related erectile dysfunction had improved function after 30 milligrams of saffron daily over a 4-week period [19, 20].

This article presents a comprehensive methodology for the development of Saffron extract based polymeric nanoparticles aimed at managing thearrythmia and hypertension [21]. Nanoformulations like polymeric nano particles offer a promising approach to overcome these limitations by enhancing drug solubility, stability, and absorption. This methodology outlines the key steps involved in the formulation development process, including preformulation studies, formulation design, optimization, and characterization techniques. Furthermore, it highlights the importance of in vitro studies to assess the performance of the nanoformulation in terms of drug release, permeability, pharmacokinetics, and therapeutic efficacy [22,23].

2. Methodology:

2.1 Materials:

Quercetin dihydrate & poly caprolactone sample sigma Aldrich, Polyvinyl alcohol, Gelatin, Acetone, Tween 80, span 80 was found to be Himedia. Dichloromethane, poloxamer-188, ethanol was found to be Hayman.

2.2 Preparation of Saffron extract

Five grams of dried saffron stigmas was completely ground with a porcelain mortar. Then, the ground stigmas were extracted by methanol/water (50:50) with stirring under nitrogen for 5 h at 250 rpm. The obtained extracts were sonicated using a probe sonicator at 40 kHz and 40% of full power for 3 min, filtered, the solvent was completely removed using rotary evaporator and transferred to Petri dishes. The latter was let to freeze at -20 ◦C for 24 h and then freeze-dried by using a LyoAlfa 6-50 freeze-dryer (Telstar, Terrassa, Spain) for 24 h.

2.3 Preparation of polymeric nanoparticles

In this the nanoparticles are prepared by solvent evaporation method [24], Solvent evaporation was the first method developed to prepare polymeric NPs from a preformed polymer the preparation of an oil-in-water (o/w) emulsion is initially required [25]Initially a weighed quantity of poloxamer-188 is added to double distilled water with magnetic stirring. The solution was maintained at 50-600 C and the PCL was dissolved in acetone with mild sonication [26]. Then organic solution was added to the aqueous solution slowly using micro pipette to disperse the organic solution. Immediately on addition of PCL to aqueous solution it forms a bluish tinge which indicates the formation of nanoparticles. This solution is then stirred for about 2 hrs at the same temperature. Then the nanoparticles are recovered by centrifugation at low pressure above 10,000 rpm. The formed sediment is then lyophilized to form nanoparticles [27].

2.4 Preparation of Saffron extract based polymeric nanoparticles

This follows the same method as that of empty nanoparticles along with addition of drug into organic solution with PCL. The formed saffron nanoparticles are then recovered by centrifugation at low pressure and 10,000 rpm. The different formulations of Sf loaded PC

2.5 Physicochemical characterization of Nano particles

2.5.1. Measurement of particle size, polydispersity index, and zeta potential

The particle size analysis was performed using dynamic light scattering (DLS) technique, which is based on Brownian motion of molecules, dispersed in liquid and relates this to the size of the particles by illuminating the particles with a laser light and analysing the intensity fluctuations in the scattered light [28]. The particle size along with zeta potential analysis of optimised nanoparticles was done on Malvern Zetasizer (Nano ZS). Zeta potential (ZP) shows the electro phoretic particle velocity in an electrical field where the particle obtains a charge due to the dissipation of the counter ions on the surface of molecule.

Characterization of nanoparticles

Particle size (PS), Poly dispersibility index (PDI) and Zeta potential (ZP) analysis Average particle size (PS) of optimised ETP loaded chitosan nanoparticles was 94.61 nm which was further confirmed by TEM. The PDI score of the same was recorded as 0.25 ± 0.06 showing good homogeneity and dispersibility of nanoparticles in the solution (Figure 1) Whereas, saffron extract based nano particles Zeta potential of formulation was -1.80.41

± 0.27 mV (Figure 2) indicating the surface electrical charge (negative) due to ionization or dissociation of surface groups (carboxyl and/or amino and phosphate groups) along with total molecular charge (positive or negative).Therefore, from the recorded observations, it can be suggested that the optimised formulation is highly stable with less ionic charge.

2.5.2 Transmission electron microscopy(TEM)

Transmission electron microscopy (Morgagni 268D) analysis was done to find out the morphology of the nanoparticles and to confirm the size range of the drug loaded nanoparticles. The optimised ETP loaded nanoparticle was further diluted (1: 50) by distilled water and ultrasonicated for 15 minutes. It was then stained with 2% phosphotungstic acid and a drop of sample was then fixed on 300 mesh carbon-coated copper grid. The images of representative areas were taken at suitable magnifications (200nm)

2.5.3Scanning electron microscopy (SEM)

Scanning electron microscopy (ZEISS EVO 40) was done to study the topographical and compositional arrangements

2.5.4 FT-IR Spectral analysis

Fourier transform infrared spectroscopy (IR-810) spectra of saffron polymeric nanoparticleswere scanned. The samples were prepared by potassium bromide disc method and scanned for absorbance from the range of 400 – 4000 cm-1

2.5.5Rheological parameters

Different physico-chemical parameters like pH, conductivity and density of optimised nanoparticle were measured. pH and conductivity of the samples were measured using pH meter (Thermo Orion 420A+) whereas, density was measured using specific density bottle (Borosil).The viscosity was measured with aBrookfield rotational viscometer (LVDV, Brookfield Inc., USA). The measurement was done at 30°C at 5 rpm viscosity.

2.5.6 Invitro release studies

In vitro drug absorption and permeability analysis was performed by using Franz diffusion cell with pretreated dialysis membrane (Sigma9777). This was mounted between donor and receiver compartments held together on both sides with a clamp. The receiver compartment was completely filled with PBS buffer (pH 7.2) and kept under continuous stirring whereas,nanoparticle solution was filled in donor compartment to get diffused through the semi permeable dialysis membrane. Diffused nanoparticle samples (1 ml) were collected at predetermined time intervals (0 – 24 hours) from the sampling port and reloaded it with equal volume of PBS (1 ml) again.The collected samples were then analysed at 238nm.

3. Results And Discussion

3.1 Measurement of particle size, polydispersity index, and zeta potential

Particle size (PS), Poly dispersibility index (PDI) and Zeta potential (ZP) analysis Average particle size (PS) of optimised ETP loaded chitosan nanoparticles (A3) was 94.63 ± 1 nmThe PDI score of the same was recorded as 0.25 ± 0.06 showing good homogeneity. Whereas, Zeta potential was -1.8 ± 0.41 mVindicating the surface electrical charge (negative) due to ionization or dissociation of surface groups (carboxyl and/or amino and phosphate groups) along with total molecular charge (positive or negative)

Group	Formulation	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)	LC (%)
I	Saffron extract-basednanoparticles	94.61 ±1.25	0.25 ± 0.06	-1.8 ± 0.41	96.45 ± 2.18	3.61

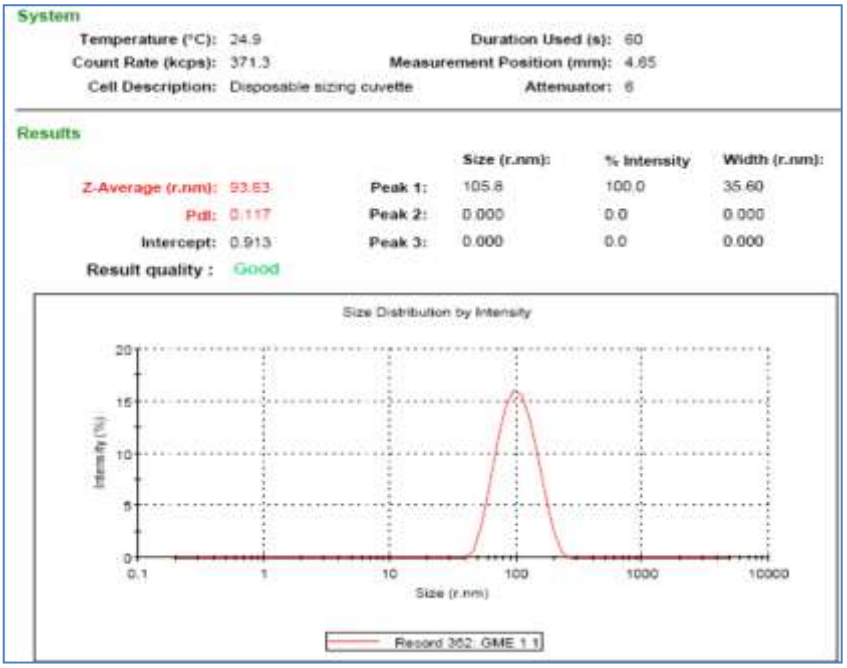

Figure.1 saffron extract based nano particles Particle Size of optimized nanoparticles was 94.61nm

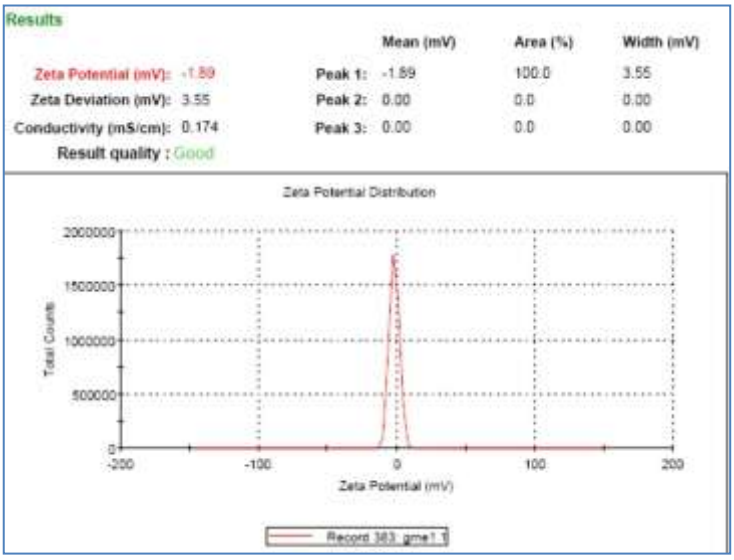

Figure.2 saffron extract based nano particles zeta potential of optimized nanoparticles was -1.80.41 mV with PDI 0.25.

3.2Transmission electron microscopy (TEM)

The TEM micrograph obtained from the imaging showed that the droplet size of the samples was in nanometric range (60 - 115nm in diameter) as shown in figure 3

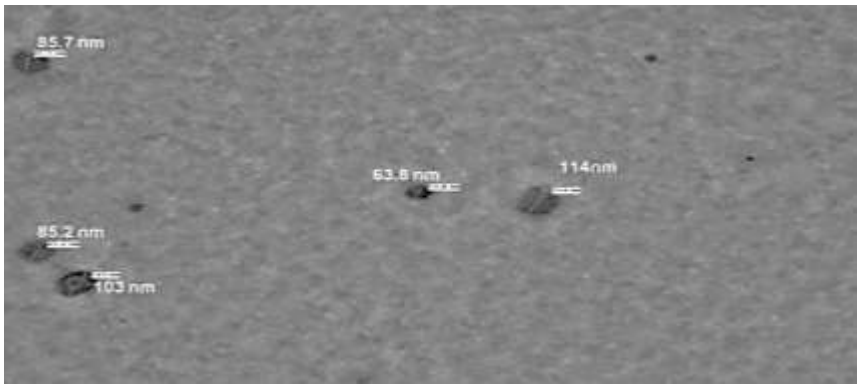

Figure.3 Transmission electron microscopy of Saffron extract based polymeric nanoparticles

3.3. Scanning electron microscopy (SEM)

SEM technique is used for the morphological characterization of particles, thereby using a high energy electron beam to scan over the surface. The results obtained from SEM indicates the almost spherical and smooth morphology of nanoparticles when observed in the scale of 200 nm although it appears to be irregular when observed in the scale of 2 µm so it can be concluded that morphologically the particles are almost smooth and spherical as shown in the figure 2

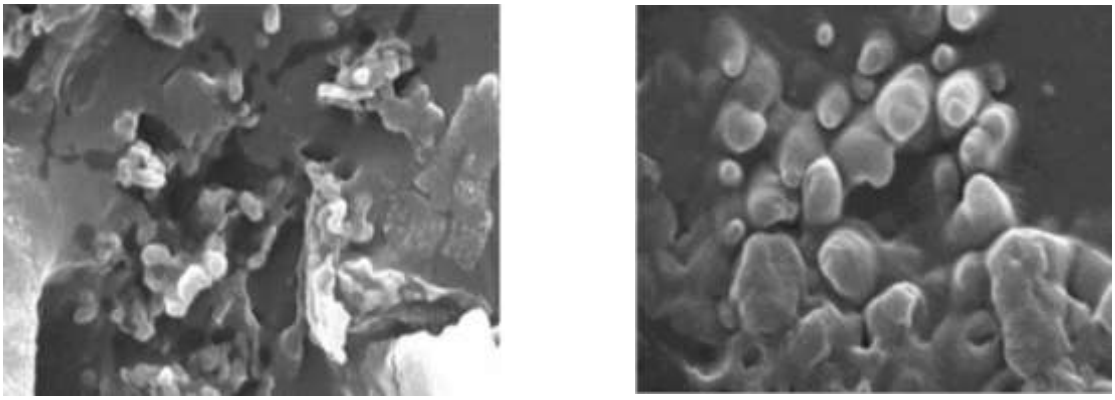

Figure.4 SEM analysis of polymeric nanoparticles

3.4FT-IR Spectral analysis

In addition to the same, emergences of all the prominent peaks as shown in the figure 3 in Saffron extract NP's were quite similar with nanoparticles without drug, suggesting less possibility of Saffron extract available on surface of NP's.

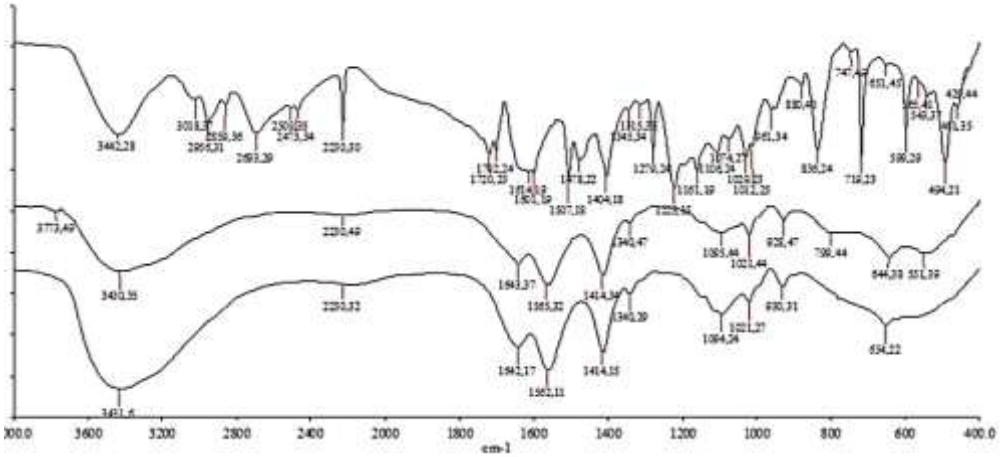

Figure 5 - FTIR of drugs, blank and loaded nanoparticles

3.5Rheological parameters

The pH of the nano particles was recorded as 6.5 showing proximity with neutral (pH-7.3), similarly conductivity of the same was 0.155 mS/cm which is quite less than the conductivity of blood (blood plasma conductivity 12mS/cm), hence allowing the nanoparticles to easily flow through blood vessels without any repulsion. The density observed was 0.952g/ml, equivalent to that of water (1g/ml) and is suitable administration through any of the delivery route whereas, the obtained viscosity of the sample (0.867cP) is reported to show good flowability and can easily pass through any biological barrier.

Sample	**pH**	**Density (g/ml)**	**Conductivity (µS/cm)**	**Viscosity(cP)**
Saffron extract-based nanoparticles	6.5	0.952g/ml,	0.155 µS/cm	0.867cP

3.5 Invitro release studies

In vitro drug release pattern of Saffron extract-based nanoparticles was studied to check permeability of pure drug (saffron extract) and its optimized nanoparticles through dialysis membrane, it showed 74.6 ± 2.6% release (24 hours) of drug in receptor compartment whereas, for saffron extract loaded nanoparticles it was 98.4 ± 1.07% release in 24 hours, proposing a typical linear diffusion profile of nanoparticles through the dialysis membrane. The expected characteristic of nanoparticles of sustained release was verified. Results further propose sustained release of drug molecules using nanoparticle system.

3. Conclusion:

This study concludes that saffron extractpolymeric nanoformulations offer a promising approach for enhancing the activity of asarrythmia and hypertension.Formulated nano particles upon determination of particle size showed a range of 94.61 ±1.25**nm**,The TEM micrograph obtained from the imaging showed that the droplet size of the samples was in nanometric range **(60 - 115nm in diameter)**. SEM analysis results indicates that the almost spherical and smoothmorphology of nanoparticlesNo peak shifting or drug peak loss is visible in the FT-IR spectra of the drug, blank nano particles and Saffron extract loaded nanoparticles concluding that there is no interaction between the medicine and the solid lipid component of nano particles ,based on the results, formulated nano particles showeda promising increase in bioavailability and cutting-edge scientific methodologiesmust be used for further studies.

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