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CHRYSIN LOADED CHITOSAN NANOPARTICLE: FORMULATION AND *IN-VITRO* CHARACTERIZATION

K. S. Sridevi Sangeetha [*1], S. Umamaheswari [1], C. Uma Maheswara Reddy [1] and S. Narayana Kalkura [2]

Department of Pharmacology [1], Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai Tamilnadu, India.
Crystal Growth Centre [2], Anna University, Guindy, Chennai, Tamilnadu, India.

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Correspondence to Author:
K. S. Sridevi Sangeetha

Research Scholar
Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamilnadu, India.

E-mail: sangeethacologist@gmail.com

ABSTRACT: Flavonoids are natural products widely distributed in plant kingdom that gained lot of importance due to variety of biological effects relevance to numerous healthcare. It has been chosen as a drug molecule and gained attention in the area of novel drug delivery system because of their disease preventing property and therapeutic expediency in multiple biological effects. Chrysin (C) is one of the most utilized flavonoid having pharmacological effects such as anti-oxidant, anti-inflammatory, anti-cancer, antidiabetic and anti hypertensive actions. Even though it has potential therapeutic value and beneficial effects on human health, it possesses some disadvantages like poor solubility and low bio-availability, this limits its therapeutic usage of Chrysin. The present work designed to improve the solubility and the bioavailability of chrysin by the developing a chrysin loaded chitosan nanoparticles (NC). It was prepared by ionic gelation of chitosan and tripolyphosphate. The chrysin loaded chitosan nanoparticles were prepared in 5 batches and named as NC1, NC2, NC3, NC4 and NC5. The formulated nanoparticles were characterized by particle size analyzer, Zeta potential, Scanning Electron Microscopy, transmission Electron microscopy and Fourier transform infrared spectroscopy (FT-IR). The *in vitro* drug encapsulation efficiency and drug release were performed in the formulated nanoparticle. Among the different batches studied, NC1 batch showed lowest mean particle size and highest entrapment efficiency. Scanning Electron Microscopy of polymeric encapsulated chrysin nanoparticles morphology revealed that spherical in shape. *In vitro* drug release study showed the chrysin loaded chitosan nanoparticles were capable of releasing drug in sustained manner. It is concluded that, the developed chrysin loaded chitosan nanoparticles might be used as vehicle for the improved solubility and prolonged delivery of chrysin.

INTRODUCTION: Flavonoids are the polyphenolic compounds present abundantly in plants, more attention where paid towards research on natural flavonoids. They are found to have antioxidant property, one such flavonoid is Chrysin.

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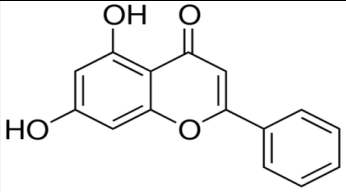
Chrysin 5, 7-dihydroxyflavone, **(Table 1)** is a flavone present at high levels in honey, propolis, blue passion flowers *passiflora caeulea*, *Passiflora incarnata* and in *Oroxylum indicum*. It is also found in *Pleurotus ostreatus* [1-5] (Mushroom).

TABLE 1: STRUCTURE AND CHEMISTRY OF CHRYSIN

Synonym: 5, 7-dihydroxyflavone Emprical formula: $C_{15}H_{10}O_4$ Molecular weight: 254.24	

Various research studies proved that chrysin having anti-oxidant, anti-inflammatory, anti-lipidemic, anxiolytic, anti-apoptotic effect, chemo-preventive effect, anti-hypertensive, anti-diabetic, anti-estrogenic, nephro-protective and hepato-protective activity [6-20].

Unfortunately, despite the wide spread therapeutic action of flavonoids, their poorly water soluble nature reduces their widespread use of flavonoids in the pharmaceutical field. Low drug solubility results in reduction in the amounts of drug absorbed and hence large dose is usually required for oral dosage regimens [20].The clinical use of chrysin is very less because of its hydrophobic nature, poor bioavailability, poor permeability, poor solubility, and the metabolism of chrysin occurs in a short time period without entering into the circulation.

In the present study, to overcome the disadvantages of chrysin, it is encapsulated in the chitosan nanoparticles.

A number of novel drug delivery systems have been investigated to overcome poor flavonoid dissolution. Polymers and surfactants were utilized in these formulations to facilitate solubilization of poorly soluble flavonoids. One such polymer is chitosan. Chitosan (CS) is a naturally occurring nontoxic, biocompatible, biodegradable, cationic polysaccharide obtained by partial deacetylation of chitin, the major component of crustacean shells. This hydrophilic polymer can easily cross-link with counter poly anions like TPP to control the release of drugs. CS based nanoparticles have provided the opportunities for the site-specific delivery of drugs because they can increase bioavailability and prolong time in blood circulation. Preparation of CS nanoparticles by ionic gelation has attracted much attention because the process is very simple and mild. Various work have been done in flavonoids like quercetin [21], rutin [22] and curcumin [23] using polymer as chitosan, so this method was utilized in this study.

MATERIALS: Chrysin, chitosan (High molecular weight) and sodium tri polyphosphate has been purchased from Sigma Aldrich. Tween- 80 and glacial Acetic acid was purchased from Thermo Fischer Scientific India Pvt. Ltd. All other reagents and solvents used were of analytical grade.

Compatibility Study: It was carried out to determine the possible interaction between the drug and polymer used. To investigate the compatibility, FTIR spectra of chitosan, chrysin and the physical mixture were recorded using FTIR spectrophotometer (Schimadzu IR) to characterize the possible drug and polymer interaction in the solid state. Samples of about 2mg were nicely ground and mixed with IR grade dry potassium bromide and then compressed at 10 tonnes under hydraulic pressure to form a thin disc. The spectra was scanned over the frequency range of 400 to 4000 cm^{-1}.

Preparation of Chrysin loaded Chitosan nanoparticles [24]: The chitosan nanoparticles were synthesized by ionic gelation method. Chitosan was dissolved in 0.1% acetic acid and kept under magnetic stirring for 24 hours. Few drops of 0.5% Tween-80 were added to the solution to prevent aggregation. To this solution 0.1% of sodium tripolyphosphate (TPP) was added in drops to the solution as a cross linking agent and the solution stirred continuously for two hours. The PH of the preparation was adjusted with sodium hydroxide. The final solution was centrifuged at 12000 RPM for 15 min.

The pellet formed was resuspended in water and again centrifuged to remove the solvent. The pellet was kept in -80°C for 12 hours and it was lyophilized. To prepare chrysin loaded chitosan nanoparticles (NC), Chrysin was dissolved in dimethyl sulphoxide (DMSO): water and added to chitosan in acetic acid. The drug was added before the addition of the Sodium Tri Poly phosphate (cross linking agent). The chrysin loaded chitosan nanoparticles were prepared in 5 batches, by changing the percentage of chitosan used as 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and named the formulation as NC_1, NC_2, NC_3, NC_4 and NC_5 respectively.

Characterization:

Drug encapsulation efficiency: The amount of chrysin encapsulated in chrysin loaded chitosan nanoparticles was determined by separating the unentrapped drug from the nanoparticle by

centrifugation at 12,000 rpm for 15 min. The supernatant obtained upon centrifugation was collected and measured for absorbance at 348 nm.

$$\% \text{Entrapment} = 100 \text{ X } \frac{\text{Total drug content} - \text{drug in the supernatant}}{\text{Total drug content}}$$

Particle size, poly dispersity index and zeta potential: Nanoparticles size distribution and zeta potential was determined using Malvern particle size analyzer. The size distribution analysis was performed at a scattering angle of 90° and at a temperature of 25°C using samples appropriately diluted with water. The poly dispersity index (PDI) indicates the broadness of a particle size distribution and can be used for the nanoparticle dispersion. The PDI range denotes the types of distribution of nanoparticle. The zeta potential was measured using a disposable zeta cuvette using samples appropriately diluted with double distilled water.

Morphological distribution: The morphology of nanoparticles was studied using a scanning electron microscope operated at an accerlating voltage of 3 kV. Samples were prepared by mounting powder on to a brass stub using graphite glue and coated with gold under vacuum before use. Transmission electron microscopy (TEM) was used to study the surface morphology of nanoparticles. A small aliquot of nanoparticles suspension were dropped onto the copper grids and viewed under.

***In vitro* drug release:** *In-vitro* drug release was studied using diffusion bag technique. Dialysis membrane, previously soaked in distilled water was tied to one end and 2 ml of formulation was accurately placed into it. Bag was tied at other end. Then dialysis bag containing nanosuspension was kept in100 ml freshly prepared phosphate buffer solution of PH 7.4 under stirring. 2 ml of solution was withdrawn at 15 min interval and each time fresh buffer was replaced. And absorbance was measured on UV spectrophotometer at 348 nm. Calculated by formula. All measurements were performed in triplicate (n=3) and SD was calculated.

RESULTS AND DISCUSSION: FTIR study was done to find out the possible interaction between the chrysin and the polymer chitosan in the solid state. **Fig. 1** shows the IR spectra of chrysin, chitosan and physical mixture. The characteristic bands of chrysin were found for C= O at1710, C -H aromatic ring at 1670 and for C-C, C-O at 1226 and 988 respectively. The FTIR spectrum of physical mixture is seems to be the summation of chrysin and the polymer chitosan spectra. This showed that there is no interaction between chrysin and chitosan and hence they are compatible.

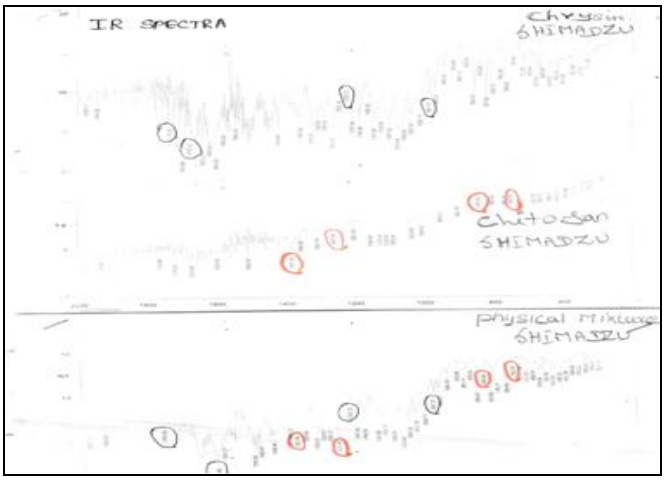


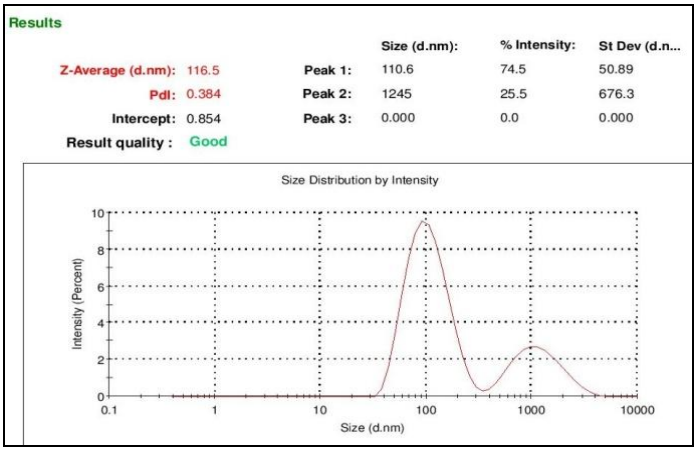
FIG. 1: IR SPECTRA OF CHRYSIN, CHITOSAN AND THEIR PHYSICAL MIXTURE.

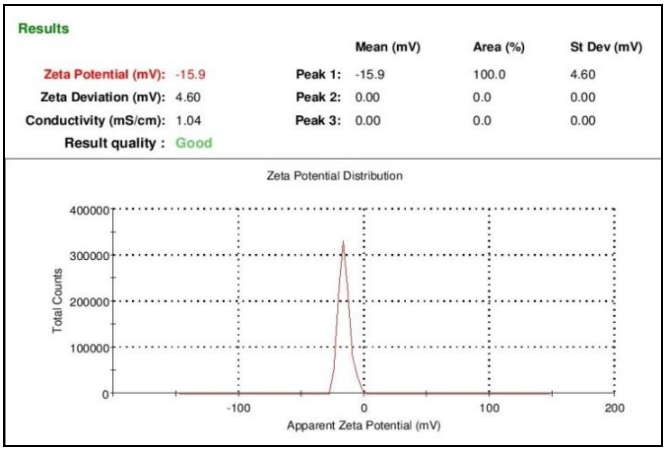
Preparation of chrysin loaded chitosan nanoparticle NC: Chrysin loaded chitosan particles were prepared in five different batches by changing the concentration of polymer to 0.1%, 0.2%, 0.3%, 0.4%, 0.5% respectively. The particle size determined in different experimental design was shown in **Table 2.** Particle size was determined based on the DLS technology which involves the non invasive technique that evaluate the variations of light scattering due to brownian motion of nanoparticle and that relates the particle size via an autocorrection function [24]. The particle size distribution pattern of nanoparticle NC_1, NC_2, NC_3, NC_4 and NC_5 were found to be 116.5, 262,175, 133 and 118.1 respectively. The polydispersity index ranges from 0.25 to 0.5, this shows that all the formulations are in broad distribution. Zeta potential indicates particle surface charge that in turn influence the performance in the blood circulation. When the zeta potential increases, the repulsive interaction will be larger, there by leading to the formation of more stable particles with more uniform distribution.

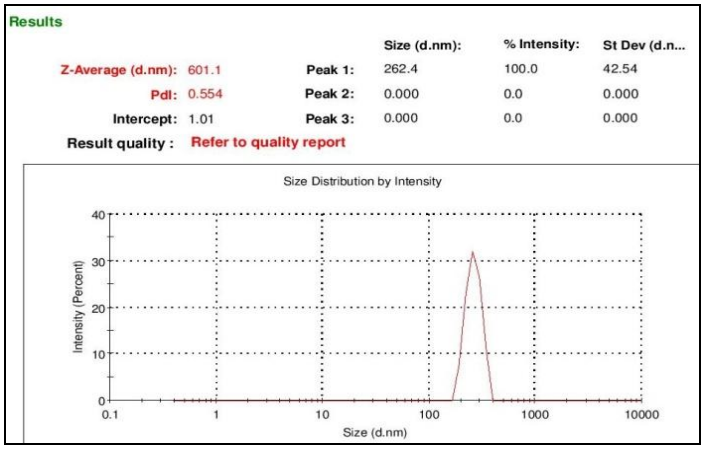
The prepared formulation showed the zeta potential in the range of -13.9 to -15.9. Zetapotential values from +30mV to -30mV is considered as a standard value [25, 26]. This proves that all the formulation NC1 to NC5 were stable. Entrapment efficiency of chrysin lodaded chitosan nanoparticle for batches NC_1 to NC_5 was given in **Table 2.** Entrapment efficiency and average particle size of NC_1 formulation was good i.e. 79.69% and 110 nm in size (**Table 2**) compared to other formulations.

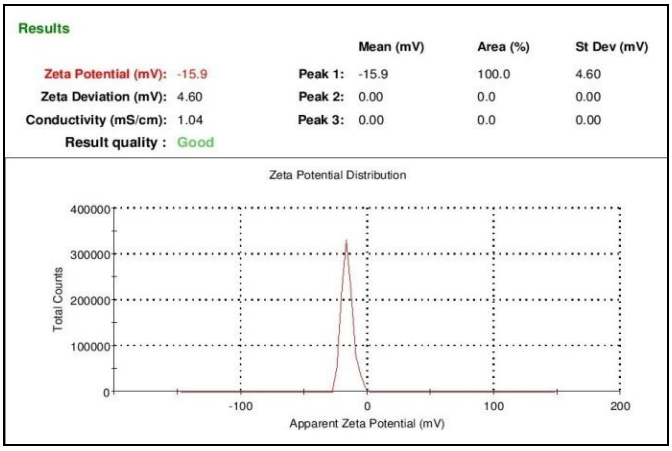
TABLE 2: PARTICLE SIZE, PDI, ZETA POTENTIAL AND ENTRAPMENT EFFICIENCY OF FORMULATIONS NC1-NC5

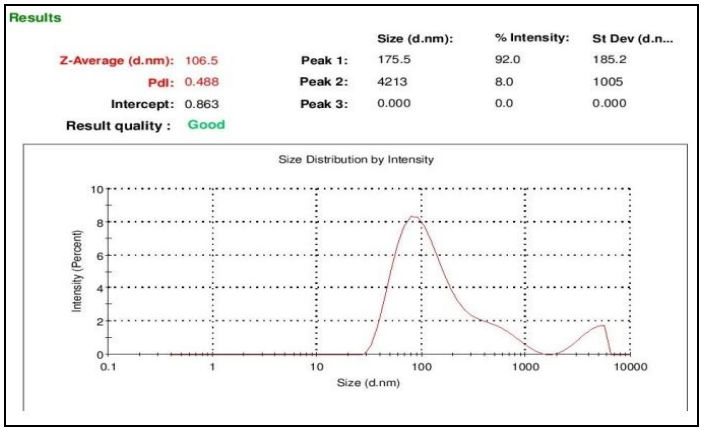
Nanoformulation	Particle size	Polydispersity Index	Zeta potential	Entrapment efficiency
NC1	110.6	0.384	-15.9	79.69±1.20
NC2	262	0.554	-15.9	78.18±0.76
NC3	175	0.488	-15.8	73.16±2.13
NC4	133.4	0.408	-15.3	74±0.97
NC5	118.1	0.270	-13.9	76.31±1.08

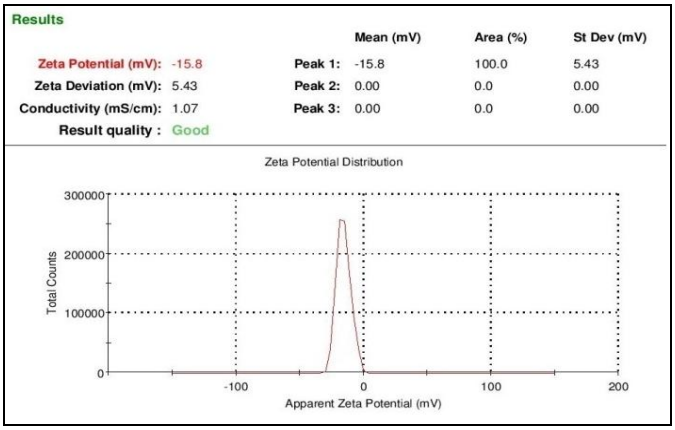












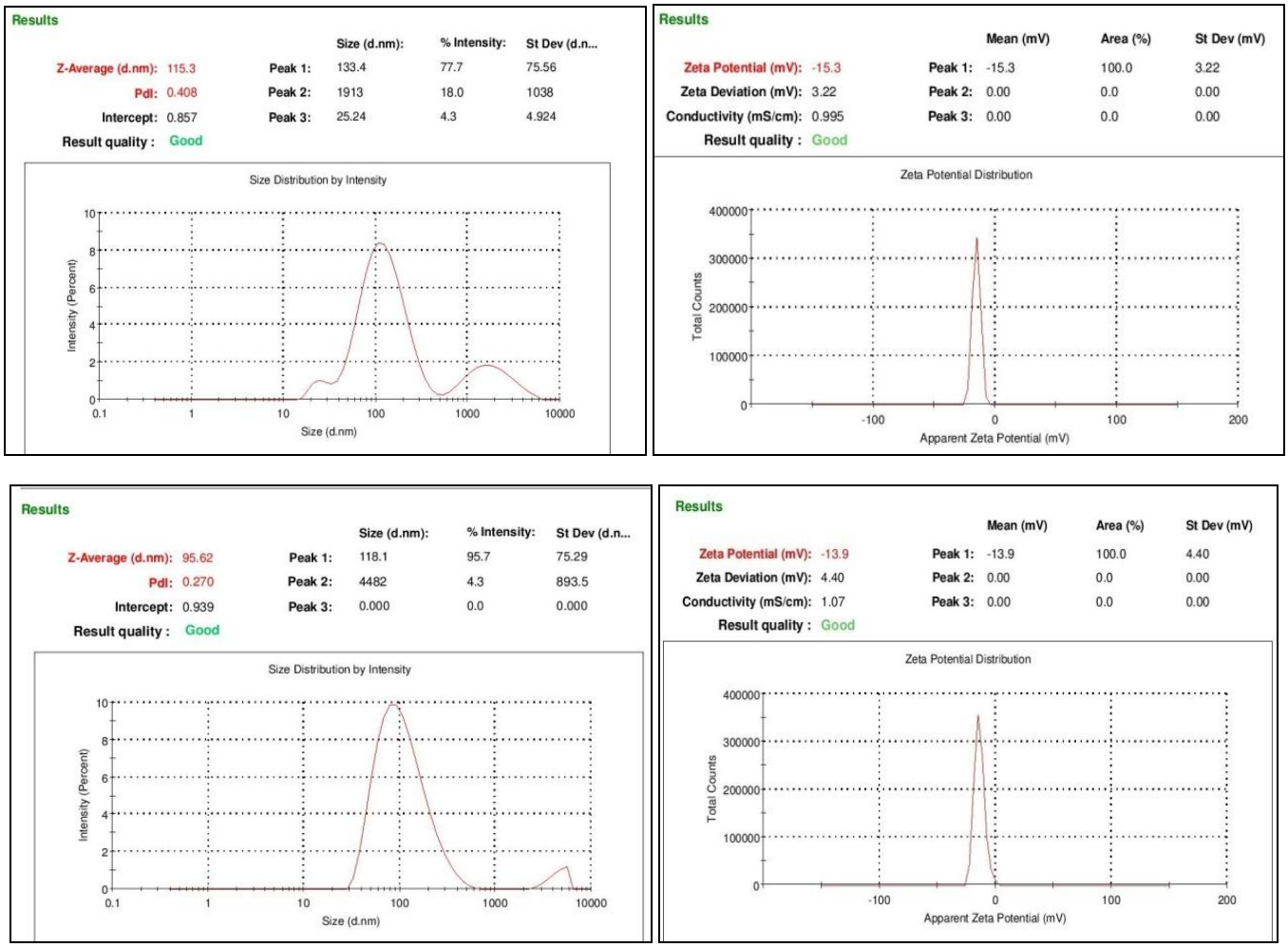


FIG. 2: PARTICLE SIZE AND ZETA POTENTIAL VALUES OF NC_1, NC_2, NC_3, NC_4 AND NC_5

***In vitro* release study:** The result obtained from in-vitro drug release study was fitted to mathematical equations of different kinetics model such zero-order (cumulative of drug release versus time), first-order (log cumulative percentage of drug remaining versus time), higuchi (cumulative percentage of release versus square root of time) and Korsmeyer-Peppas (log cumulative percentage of drug released versus log time) equation models to know the mechanism of drug release. The equation with the high regression coefficient (r^2) for formulation will be the best fit of release data [27]. The calculated regression coefficient for all the formulation was tabulated and given in **Table 3**. This shows that the formulation NC1 having greater regression coefficient value and follows zero order kinetics.

TABLE 3: *IN VITRO* RELEASE; REGRESSION COEFFICIENT FOR THE FORMULATION NC1-NC5

Formulation	R^2 value			
	Zero order kinetics	First order kinetics	Higuchi release	Korsmeyer pappa's plot
NC1	0.991	0.947	0.961	0.938
NC2	0.978	0.922	0.978	0.904
NC3	0.961	0.901	0.985	0.966
NC4	0.956	0.914	0.990	0.955
NC5	0.957	0.900	0.984	0.920

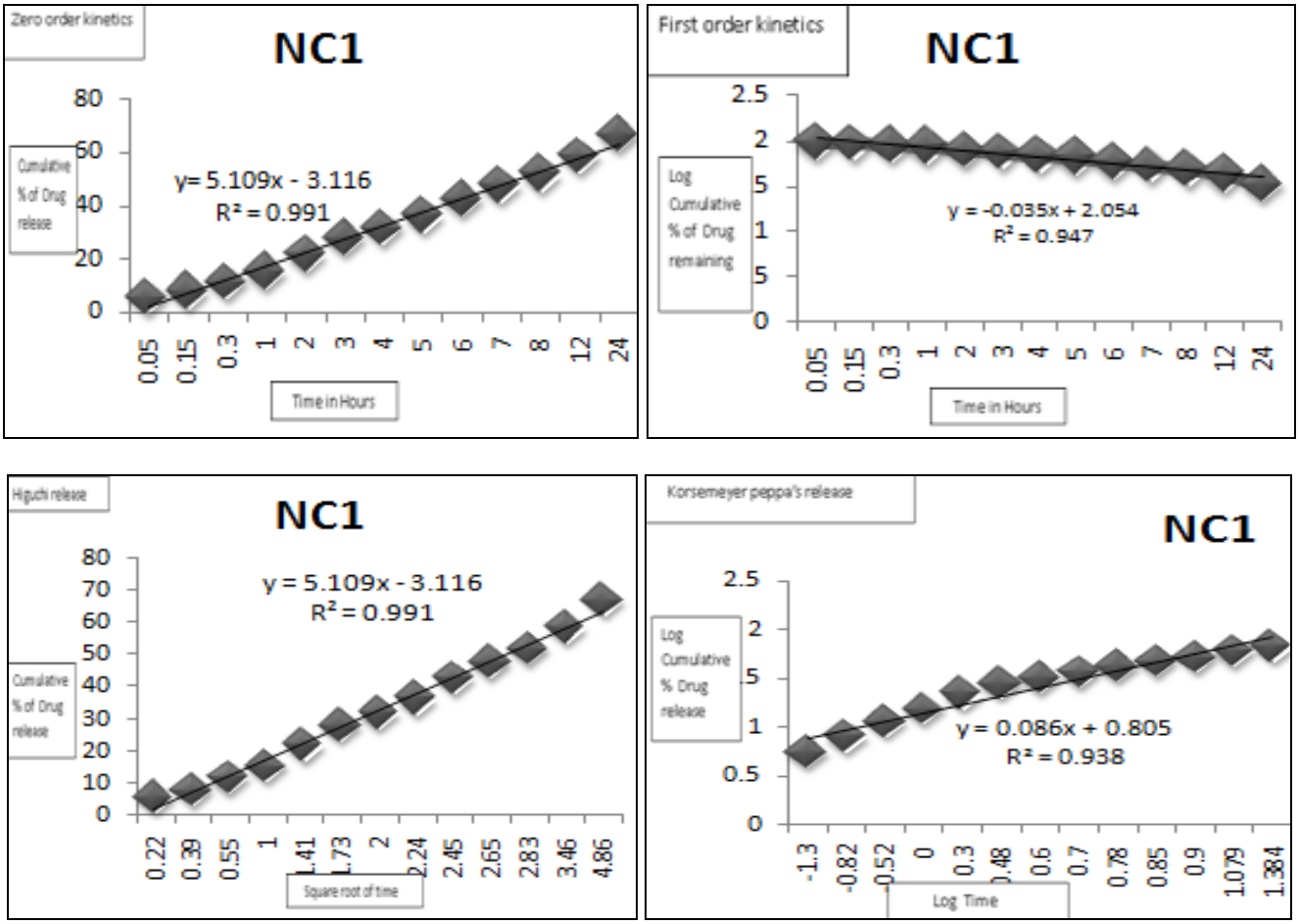


FIG. 3: *IN VITRO* RELEASE FOR THE FORMULATION NC1

Morphology of the nanoparticle: As it is shown in **Fig. 4**, chrysin loaded chitosan nanoparticles are spherical, dense and highly unsized. The mean particle size in the SEM was found to be within the range of 100 nm. The particle size analyzer showed the particle sizes above 100 nm it may due to the aggregation of the particle during the storage period.

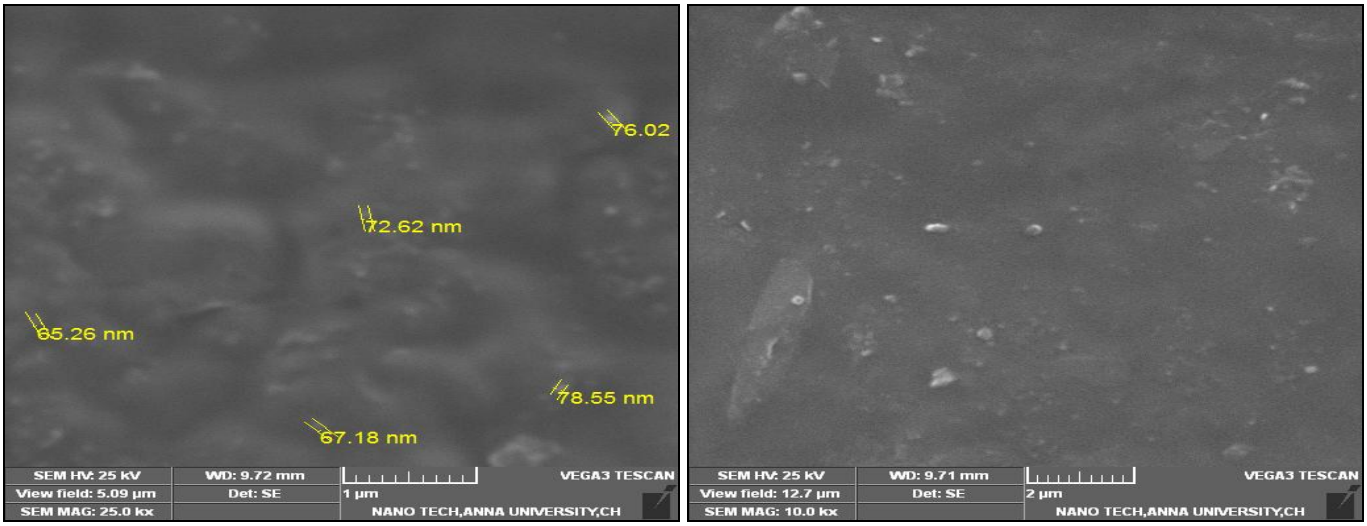


FIG. 4: SEM IMAGES OF CHRYSIN LOADED CHITOSAN NANOPARTICLE NC1

Visual examination of the TEM images (**Fig. 5**) indicated that the chrysin lodaded chitosan nanoparticle were spherical in shape.

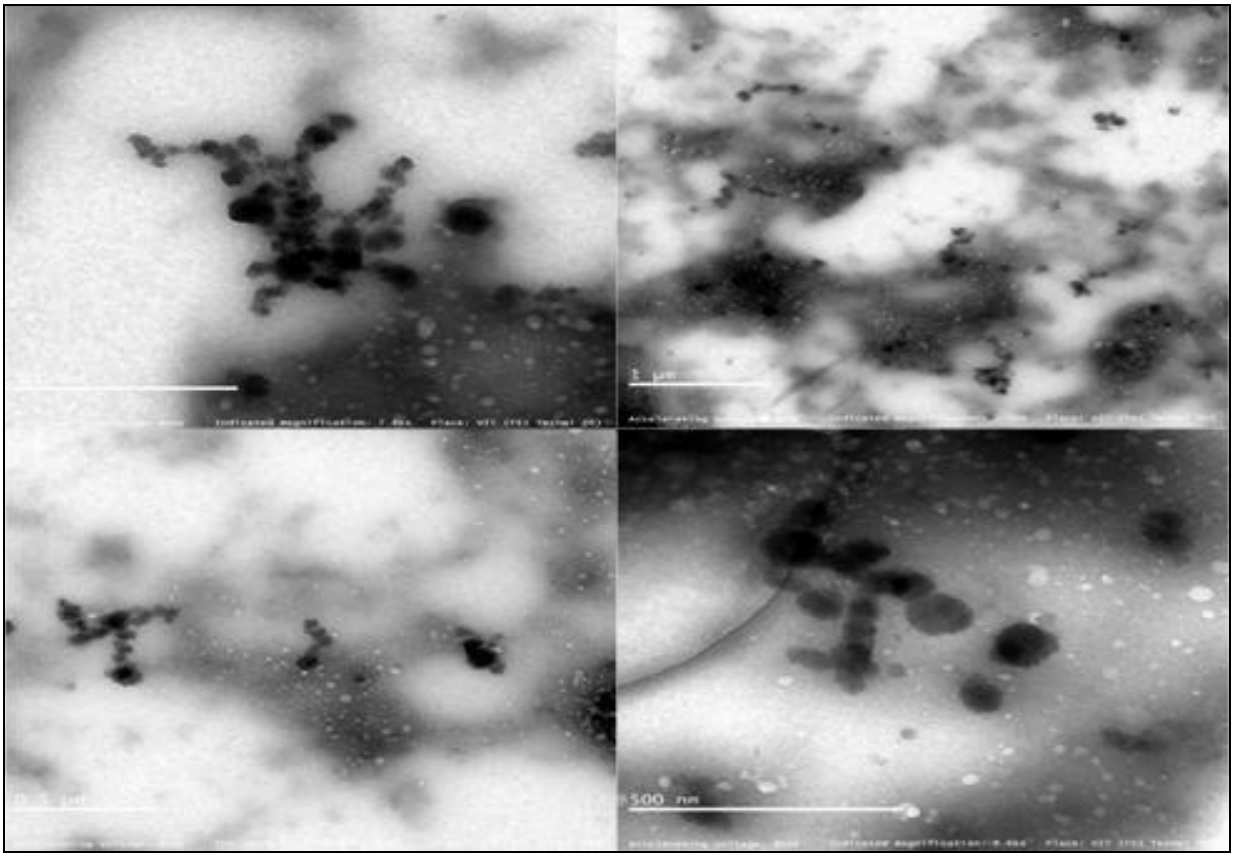


FIG. 5: TEM IMAGES OF CHRYSIN LOADED CHITOSAN NANOPARTICLES

CONCLUSION: Chrysin lodaded chitosan nanoparticle was prepared successfully using ionic gelation method. The percentage of the polymer used was found to influence the percentage entrapment efficiency and particle size. All results of this research work provide useful information for future studies aiming at development of drug delivery formulation consisting of chitosan nanoparticles.

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