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Research Article

PREPARATION AND CHARACTERIZATION OF NARINGENIN GOLD NANO SUSPENSION FOR BREAST CANCER

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ABSTRACT

Breast cancer is world third leading cause of death in the world among women. Breast cancer is spreads from milk duct. Nano particle based formulation such as nano suspension enhance therapeutic effectiveness and reduce side effect of the drug payload by improving their bioavailability and improves the efficacy of cancer therapeutically. Naringenin is targets breast cancer cell by targeting estrogenic receptors. Bottom –Up approach was used. for preparation of naringenin gold nano suspension (NGNS).Gold has its own surface Plasmon resonance property, which targets the cell by combining with naringenin. Naringenin gold nano suspension solid particles were obtained by lyophilisation. Optimized batch of the NGNS shows the particle size by Dynamic light scattering 179.7±4.9nm with poly dispersity index of 0.28 ± 0.02.NGNS shows zeta potential -28.53±0.17. TEM image of the NGNS was show particle size of 160.7nm. The results suggested that precipitation was an effective way to prepare NGNS improves the dissolution rate for targeting cancer cells.

KEY WORDS: Naringenin, Gold nanoparticle, Precipitation method, Dissolution.

INTRODUCTION

A survey report represents world second most leading cause of death in the world. Breast cancer is originating from breast tissue, most commonly from milk duct or the lobules that supply the duct of milk.[1] Cancer cells are formed from normal cells due to modification /mutation in DNA or RNA. The rate of DNA and RNA mutations is higher under condition such as radiation, Chemical poor diet and menopausal women and with genetic defect. Chemotherapy is the use of anticancer drugs to treat cancerous cells.[2] Gold is newer approach for the treatment of cancer. Gold particles are smaller in size and penetrate throughout the body preferentially accumulates at the tumor site owing to EPR effect and importantly they can bind to many proteins and actively targeted to cancer cells and by over expressing cell surface receptor.[3] Gold has characteristics of surface plasmon resonance and as chemically inert for the proteins , antibodies and genes and also has higher biologically compatible and provide high surface area so large amount of drug can be loaded.[4] Natural phytoconstituent has now widely interested as having promising effect in development of new class of anticancer drugs since they have multiple targets in cancer cells with minimum toxicity of the constitute. Naringenin have ability to inhibits carcinogenesis has developed in mainly three stages tumor promotion, angiogenesis and tumor growth. Naringenin cause cytotoxic and apoptic effect in Naringenin derivatives N101-43 was found to induce apoptosis via upregulation of Fas/FasL expression, activation of caspase cascade and inhibition of phosphoinoaitide 3- kinase (PI3K)/protein kinase B (Akt) survival signaling pathway in the tested cells.[i5]The naringenin treated cells showed different cell cycle profiles with accumulation in G2/M Phase. Naringenin has higher affinity towards mutant H Ras studied from docking study. Naringenin inhibits the function of mutant H-Ras P21 protein which in turn arrest the process of cell growth [6]. Naringenin has been widely used as an anti-oxidant. Aglycone part of the naringenin has anti ulcer effect and dilatory effect as well as inhibits the proliferation of breast cancer and delay mammary tumor genesis[6]. Naringenin has the ability to alter signal transduction of protein kinase[7].

Nano suspension is prepared by various approaches like bottom-up technology, Top-down technology, micro emulsion and emulsion as template[8]. In the present research work nano suspension was prepared by bottom -up approach in which drug is dissolved in organic solvent which is then added to non organic solvent that cause precipitation of the nano particle and the system is stabilized by stabilizer or surfactant to prevent further accretion of particle[9].Bottom –up approach improves bioavailability by reducing particle size.[10,11]

MATERIAL AND METHOD

Gold chloride was procured from Astron Chemicals, Ahmedabad. Naringenin was procured from Tokyo Chemical Industry, Chennai, India. Poloxamer-188 was procured from Astron Research Center. Ahmedabad, Gujarat. PVP K-90 was procured from SD chemicals, Mumbai. Hydroxy propyl methyl cellulose was procured as gift sample from Cadila pharma, Gujarat India.

Preparation of Gold Nanoparticle (GNP)

All glassware's were cleaned with an aqua regia solution (1:3 nitric acid /hydrochloric acid). 1mM solution of 250 ml of hydrogen tetrachloroaurate trihydrate was brought to a boil for

vigorous stirring. To this solution 25ml of 33.8 mM of sodium citrate was added. Sodium citrate is used as reducing agent. Gold solution was yellow at starting of reaction. Solution is allowed to boil. After adding sodium citrate act as reducing agent which sodium citrate turned to citric acid [12]. Conversion of Au^{+3} to Au^{0} yellow coloured solution become transparent and colorless and It changed to black than slowly to wine red. The reaction can be summarized as;

$$2HAuCl_4 + 3C_6H_8O_7 \rightarrow 2Au + 3C_5H_6O_5 + 8HCl + 3CO_2$$

Formulation

Nano suspension was prepared by "Bottom –Up" technology. In the first step of naringenin was dissolved in the acetone to prepare organic phase and solution was then filtered to clear impurities at room temperature[13]. Different concentration of surfactant was dissolved in water as anti solvent and to this equal quantities of gold nano particle was added in different batches[14]. In second step drug was dispersed in gold nano particle using overhead stirrer at 2000 RPM for 24 hour leading to precipitation of nano suspension of phytoconstituents. The solvent was allowed to evaporate from nano suspension[15].

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Drug (mg/ml)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Poloxamer -407(g/ml)	0.25	0.5	1	5	-	-	-	-	-	-	-	-	-	-	-	-
Poloxamer-188(g/ml)	-	-	-	-	0.25	0.5	1	5	-	-	-	-	-	-	-	-
PVP-K-90 (g/ml)	-	-	-	-	-	-	-	-	0.25	0.5	1	5	-	-	-	-
HPMC(g/ml)	-	-	-	-	-	-	-	-	-	-	-	-	0.25	0.5	1	5
Acetone (ml)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GNP (ml)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Water (ml)	05	20	25	50	05	20	25	50	05	20	25	50	05	20	25	50

CHARACTERIZATION OF PARTICLE SIZE

Particle Size and Polydispersity Index

Mean particle size of nano suspension and poly dispensability index of formulations were determined by Malveran Zetasizer using water as dispersion medium. The sample of different batches were scanned for determination of particle size. The samples of batches were measured after appropriate dilution with distilled water. Mean particle size and distribution of particle size in nanodispersion measured by dynamic light scattering. The zeta sizer measure particle in the range of 0.6 nm- 6µm. The reading of samples were taken at 90^0 angle of incident beam. The zeta potential was measured by laser doppler anemometer coupled with the same instrument. For accuracy the analysis of the sample was carried out in triplicate[16].

Zeta Potential

Zeta potential of the suspension is measured by Malvern zeta sizer. The zeta sizer is mainly consisting of the laser which is used to provide light source to illuminate particle within the sample. For measurement of zeta potential light splits into an incident and reference beam. The incident beam passes through the center of the sample cell. Zeta sizer produces a frequency spectrum from which the electrophoretic mobility hence zeta potential is calculated. The mobility and potential of nano dispersion particles within range of 3nm-10 µm.

Total Drug Content

An aliquot (0.5 ml) was evaporated to dryness. The residue was dissolved in acetone and filtered with 0.45µm filter paper. The sample was analyzed using UV spectrophotometer at λmax324 nm of naringenin. Total drug content (TDC) were calculated as below

Total Drug Content= (Total volume of nano suspension//Volume of aliquot) * Amount of drug in aliquot*100 [18].

Entrapment Efficiency

High concentration of the free drug present in the supernant after centrifugation can determined by entrapment efficiency.10 ml of the freshly prepared nano suspension was centrifuged at 10,000 rpm for 10 minutes using micro centrifuge. The supernant was collected and unincorporated drug measured by taking absorbance of super nant at 324 nm by using UV spectro photo metrically.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra were scanned in the range of 3600-400cm^{-1} at an ambient temperature. FT-IR was scanned in the sequence of naringenin, Polymer, gold nano particle optimized batch of formulation using KBr technique. The pellets were prepared using KBr hydraulic press under hydraulic pressure of 150 kg/m^2.

Differential Scanning Calorimetery

DSC of pure naringenin, Polymer, Gold chloride and naringenin gold nano suspension were studied using DSC (Shimadzu 60 with TDA trend line software, Shimadzu Co. Kyoto, Japan). In DSC analysis, the samples were weighed (5mg) hermetically sealed in flat bottom aluminum pans were heated over a temperature range of 50 °C to 300 °C at a constant increasing rate of temperature of 10°C/min. Nitrogen gas 50 mL/min. Calibration of instrument with respect to temperature and enthalpy was achieved[19].

Transmission Electron Microscopy

Transmission electron microscopy (TEM) studies were performed using transmission electron microscope (Philips Technai-2ath). The liquid nano suspension formulation was dropped on copper-gold carbon grid and allowed to dry. This grid was then mounted in the instrument and photographs were taken at various magnifications[20].

In vitro Drug Release Study

Dissolution studies were carried out in dissolution apparatus (TDT-08L, Electro lab) by using the USP apparatus 2 paddle method. The bath temperature and paddle speed were set at 37°C and 50 rpm respectively. The dissolution media include 900 ml of

distilled water and phosphate buffer at pH 7.4. Each dissolution media was maintaining the sink condition during dissolution process. An accurately weight bulk drug and nano suspension were dispersed to the vessels. 5 ml samples were withdrawn at 5,10,20,30 40, 50, 60 min from dissolution medium and replace with same buffer solution. The accumulation of dissolution amount of naringenin was determined by Spectro photo metrically.

RESULT AND DISCUSSION

Solvent precipitation method has been applied to produce nano suspension of naringenin Solvent to anti solvent ratio and concentration of polymer were selected based on change in particle size and zeta potential distribution over surface of charge particles. Stirring speed of overhead stirrer was remaining constant for 24 hours for all batches. Confirmation of formation of colloidal nano suspension can be visualized by bluish white transparent ring appearance at the top.

Determination of Particle Size

Particle size of different batches were taken are as below. Batch size from F1 to F16, maximum particle size was observed of batch F13 because of low concentration of polymers induce aggregation of particles. The optimized shows particle size 179.7 nm as shown below:

Table 1: Particle Size, Poly dispersity index and Zeta potential of different batches

BATCH NO	PARTICLE SIZE (nm) (MEAN±S.D)	PDI (MEAN±S.D)	ZETA POTENTIAL (mV) (MEAN±S.D)
F1	584.7±7.76	0.6±0.03	-12.42±0.16
F2	484.0±4.32	0.4±0.04	-23.54±0.37
F3	322.3±4.18	0.3±0.02	-26.46±0.41
F4	179.7±4.9	0.28±0.02	-28.53±0.17
F5	634.2±6.97	0.7±0.03	-9.70±00.70
F6	583±4.08	0.6±0.25	-15.45±0.41
F7	441.0±8.6	0.3±0.02	-21.49±0.44
F8	260.3±7.4	0.6±0.05	-27.37±0.26
F9	862.3±1.69	0.4±0.87	-11.61±0.48
F10	737.7±9.2	0.3±0.18	-12.79±0.46
F11	507.3±9.1	0.3±0.54	-14.89±0.31
F12	350.7±8.22	0.8±0.03	-21.63±0.39
F13	1041.7±4.12	0.8±0.18	-14.3±1.02
F14	912.7±9.46	0.7±0.57	-17.52±0.43
F15	780.3±3.68	0.4±0.33	-22.12±0.29
F16	583.0±3.74	0.3±0.05	-27.7±0.47

Determination of Poly Dispersity Index

Poly dispersity index of nano suspension batches were found to be in the range of o.8 to 0.28. F4 batch show the particle size distribution 0.28 which is most suitable for even distribution of particle size.

Determination of Zeta Potential

Zeta potential means the distribution of charges over dispersed particle of nano suspension. It indicates stability of nano suspension. Zeta potential of formulation batch of F1 to F16 were found to be -9.70 to -29.76. F4 batch of formulation show zeta potential of -28.53 was found to be stable naringenin gold nano suspension.

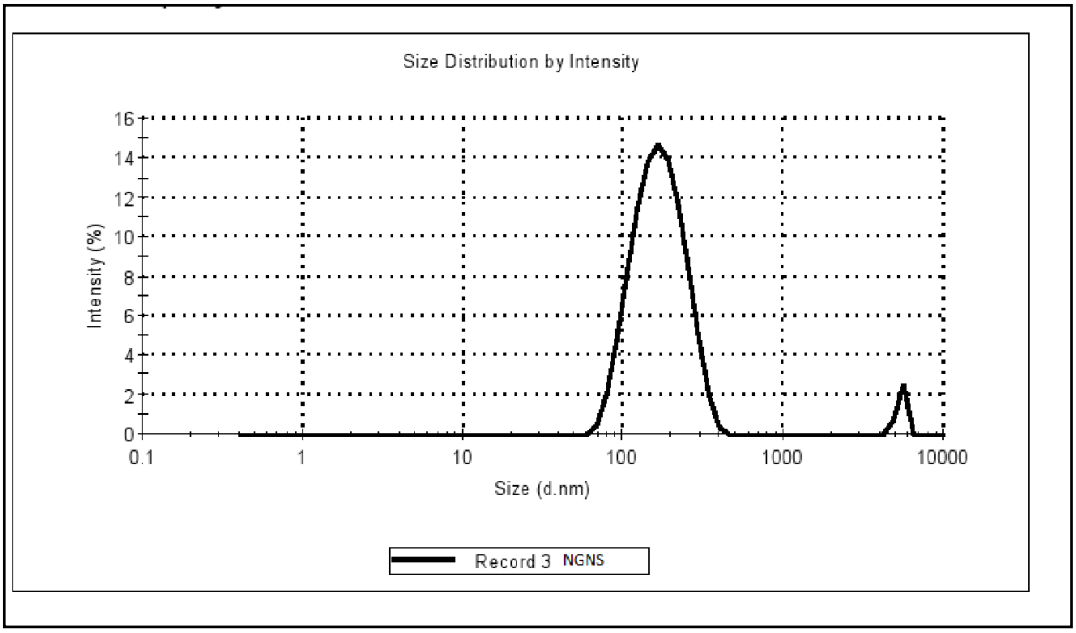
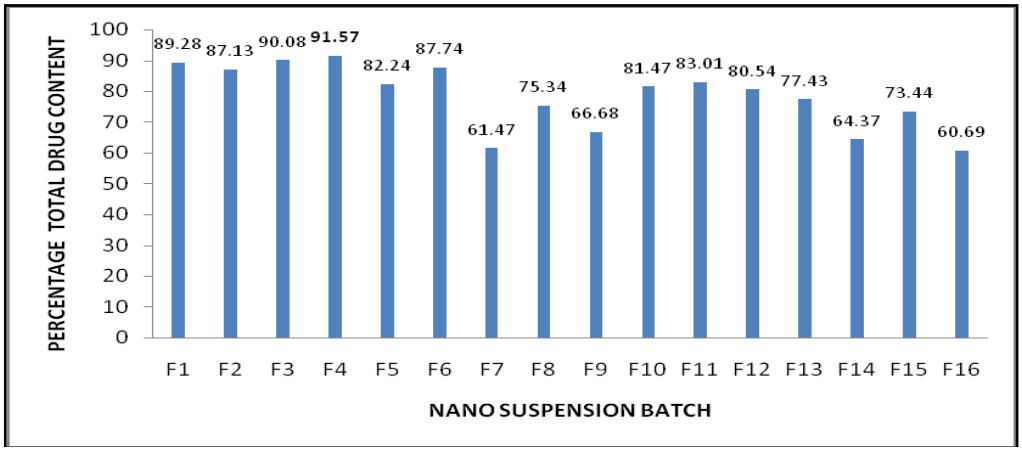


FIGURE 1: Particle size graph of optimized batch of NGNS

Determination of Total Drug Content

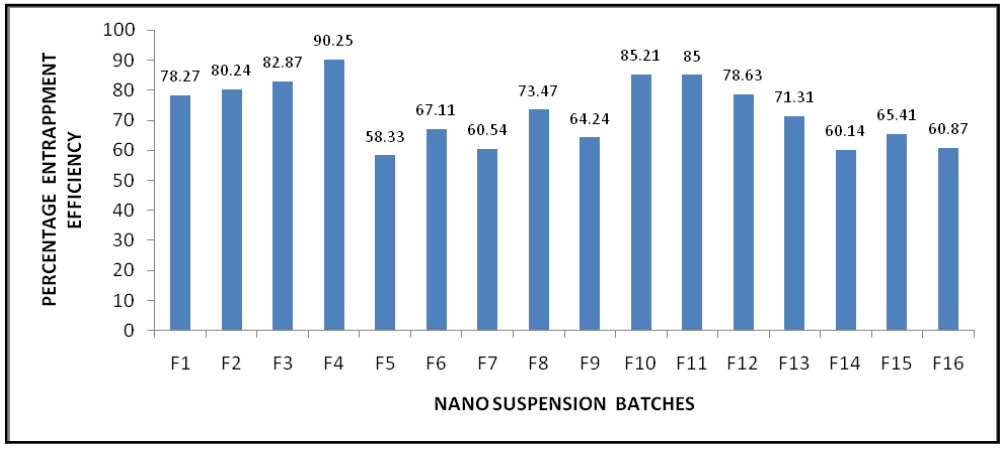
Drug content of all NGNS formulation was found to be greater than 80% except some of batches indicates suitability of these method for particle size reduction. The optimized batch F4 shows 91.57% of total drug content as shown.



Graph 1:Percentage total drug content of different NGNS batches

Determination of Entrapment Efficiency

Entrapment efficiency all the batches were found to be greater than 60% except some of batches. The optimized batch F4 shows entrapment efficiency 90.25% as shown in fig as below:



Graph 2: Percentage entrapment efficiency of different NGNS batches

Fourier Transform Infrared Spectroscopy

From FTIR Spectrum of naringenin, polymer and nano suspension were taken to determine physicochemical property and intermolecular bonding of drug and polymer. The FTIR spectra of pure naringenin and naringenin gold nano suspension curves were well matched. FTIR spectra shows O-H stretching was shift from 3172 cm^{-1} to 2968 cm^{-1} indicates intermolecular bonding. Broadening of peak was due to loading of naringenin with gold and due to formation of hydrogen bond.The FTIR spectra of naringenin and Poloxamer 188 shows no interaction between them.

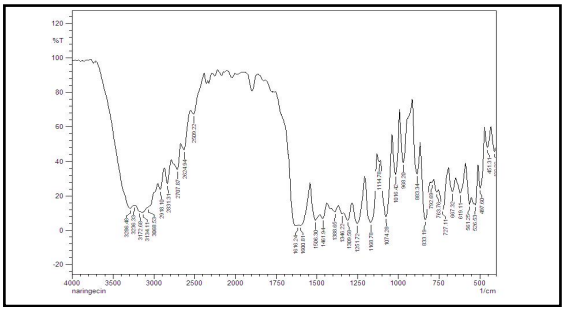


Figure 2: FT-IR spectra of pure Naringenin

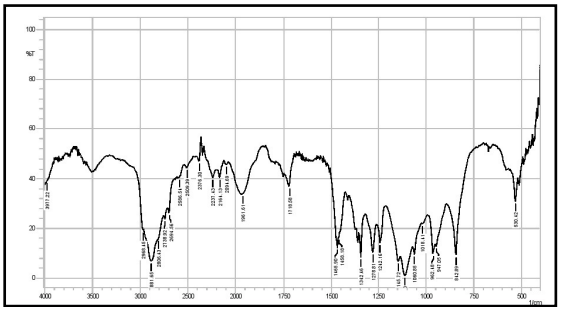


Figure 3: FT-IR spectra of Poloxamer 188

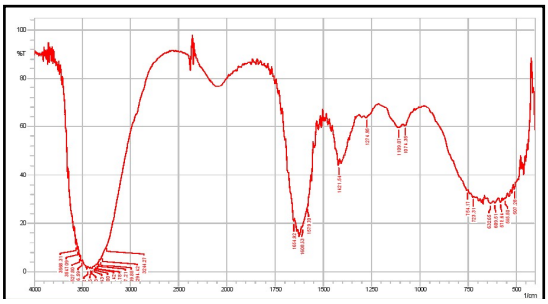


Figure 4: FT-IR spectra of Gold Nanoparticle

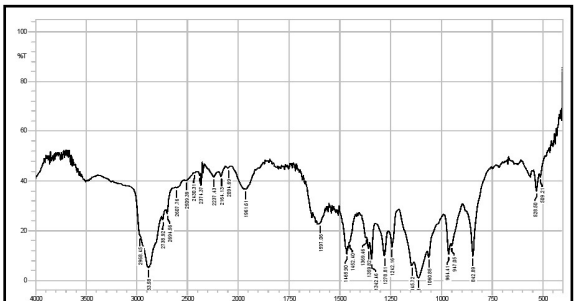


Figure 5: FT-IR spectra of Naringenin gold nano suspension

Differential Scanning Calorimetry

DSC was used to elucidate the physical state of the drug within the formulation. DSC was carried out to determine molecular state of the drug. The DSC curve of naringenin, Poloxamer 188 and naringenin gold nano suspension was carried out. Naringenin exhibit endothermic peak at its melting point at 252.39 ℃ indicates the crystalline nature of the drug. Naringenin gold nano suspension showed melting peak indicates the absence of the crystalline nature of the drug. Reduction in height of the peak these changes indicates as result of reduction of particle size. This indicates changes in nature of drug giving a more amorphous type as the product this may increase rate of dissolution of NGNS.

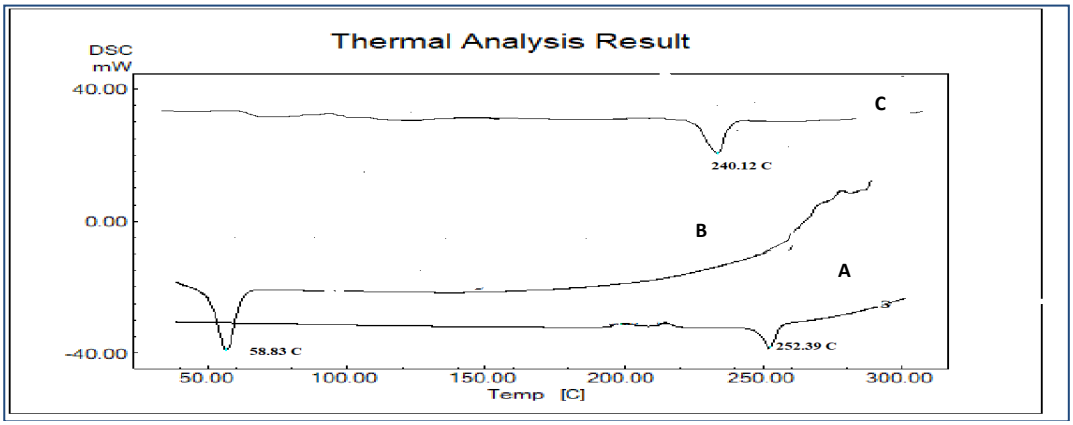


FIGURE 6: DSC Thermogram of A) Naringenin B)Poloxamer -188 C) NGNS

Transmission Electron Microscopy

TEM image of naringenin gold nano suspension indicate that naringenin particle exhibit spherical shape with a diameter of about 160.7 nm. It shows uniform dispersion of particles. The results suggested that naringenin nano particles were well stabilized by precipitation method.

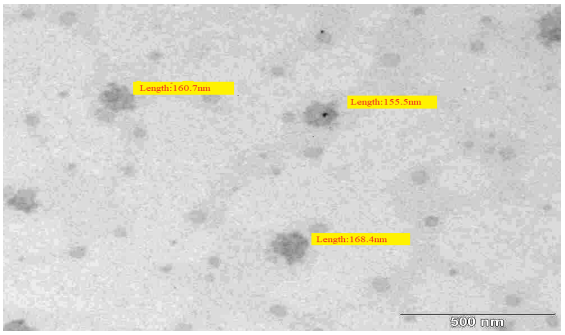
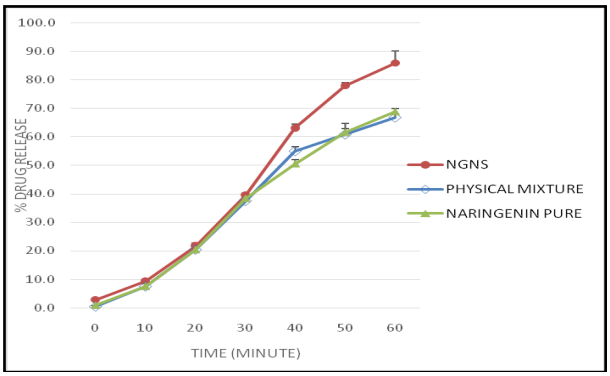


FIGURE 7: TEM image of optimized formulation batch NGNS

In Vitro Drug Release

Most important feature of nano particle is the increase in dissolution of formulation with decreasing in particle size. As decrease in particle size increase surface area for absorption because of increase saturation solubility. *In vitro* drug release from the nano suspension were carried out for 60 min. It was graphically represented as percentage drug release versus time in minutes. The percentage of drug release was carried out of pure drug, physical mixture formulation batch of NGNS of F4.From study reveals that F4 batch shows faster release of drug as compare to pure drug and physical mixture. Optimized formulation batch was enhanced dissolution by 85 % in 60min as compared to pure naringenin showed 68%. So as improves the drug release as well by decreasing particle size. That improves absorption targeting action.



Graph 3: In vitro drug release study

CONCLUSION

Naringenin gold nano suspension was precipitated by solvent – anti solvent precipitation method. "Bottom –Up" approach was used as key approach.Gold was used in combination with naringenin to target breast cancer cells. Different surfactants were used for various batches. Among them optmized formulation batch F4 showed particle size of 179.7 nm and with uniform dispersion of particle in nano suspension**.** Naringenin combination with gold it targets cancer cells more and improves dissolution rate as compare to pure phytoconstituent naringenin. Naringenin gold nanosuspension reduces the dose with surface plasmon properties nano particle based formulation enhance surface area of the absorption at targeted site.

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