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NOVEL POLYSACHARIDE BASED NANOPARTICLE FOR IMPROVINGTHE PHAR MACOKINETICAND THERAPYUTICS APPROACHOF ANTIMALARIAL DRUG

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ABSTRACT:

The drug is dissolved, entrapped, encapsulated or attached to a nanoparticlematrix. The major goals in designing nanoparticles as a delivery system are to control particle size,surface properties and release of pharmacologically active agents in order to achieve the site- specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomehave been used as potential carriers with unique advantages which include protecting drugs from degradation, targeting site of action and reduction of toxicity or side effects, their applications arelimited, due to inherent problems such as low encapsulation efficiency, rapid leakage of water- soluble drug in the presence of blood components and poor storage stability.

The results of the present investigation demonstrated the potential use of starch nanoparticles for effective delivery of chloroquine for treating malaria. Starch based drug delivery system must overcome important physical barriers to reach the target cells. Different colloidal systems have been developed to solve these problems.Moreover starch nanoparticles can be easilyprepared under mild conditions, and can be incorporated in macromolecular bioactive compounds.

Keywords: Nanoparticle, Control Release, Chloroquinine, Antimalarial

INTRODUCTION:

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm[1]. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticlematrix.[2] The major goals in designing nanoparticles as a delivery system are to control particle size,surface properties and release of pharmacologically active agents in order to achieve the site- specific action of the drug at the therapeutically optimal rate and dose

regimen[3]. Though liposome have been used as potential carriers with unique advantages which include protecting drugs from degradation, targeting site of action and reduction of toxicity or side effects, their applications arelimited, due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability.[4] On the other hand, polymeric nanoparticles offer some specific advantages over liposome, for instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties[5.6]. **The advantages of using nanoparticles as a drug delivery system include the following:**

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration[7].
- They control and sustain release of the drug during transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.[8]
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction which is an important factor for preserving the drug activity.[9]
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.[10]
- The system can be used for various routes including oral, nasal, parenteral and intra-ocular administration.[11]

PREPARATION OF NANOPARTICLES

The selection of the appropriate method for the preparation of nanoparticles depends on the physicochemical characteristics of the polymer and the drug to be loaded[12]. On the contrary, the preparation techniques largely determine the inner structure, in-vitro release profile and the biological fate of this polymeric delivery system.[13]

Two types of systems with different inner structure are apparently possible, which are as follows:

- A matrix system consisting of an entanglement of oligomer or polymer units (nanoparticle/nanospheres).[14]
- A reservoir of the system comprised of an oily core surrounded by an embryonic polymeric shell

(nanocapsules).[15]

IV. Methodology: Preparation of chloroquine loaded starch nanoparticles

Chloroquine loaded starch nanoparticles were prepared by nanoprecipitation method as follow: different amount of native starch was dissolved in 70 ml distilled water containing 1.5 g NaOH. This solution was kept under high mechanical stirring for 30 min at 25°C. 0.4 g Tween®80dissolved in 20 ml distilled water containing drug, was slowly added, followed by the addition of10 ml H2O containing of STPP under continuous highly mechanical stirring, keeping in mind that the total volume of the reaction mixture is 100 ml. The reaction mixture was left to stand at room temperature for 2 h to effect crosslinking with constant agitation rate at 25°C. The resulting drug encapsulated cross-linked starch nanoparticles were subsequently precipitated by 100 ml of absolute ethanol. The resultant powder were purified by means of centrifugation and washing rinsed twice with 80/20 absolute ethanol/water to remove unreacted compounds and finally with

absolute ethanol. The resultant nanoparticles were then isolated by means of centrifugation for 1 h at 4500 rpm. At the end, the supernatant was taken for further analysis to determine the loss in the amount of drug and the supernatant was freeze-dried for 12 h and kept in closed containers for further analysis. The as described freezedried drug loaded cross-linked starch nanoparticles in thesolid state can be easily re-dispersed in distilled water by hand agitation before us.[16-17]

Table-1 Composition of chloroquine Loaded starch Nanoparticles

Formulation Code	Drug (mg)	Polymer (mg)	Tween 80 (%)	STPP (%)	Sonication Time (min)
F-1	350	150	0.5	0.25	0
F-2	350	150	0.5	0.25	5
F-3	350	150	0.5	0.25	10
F-4	350	250	0.5	0.25	0
F-5	350	250	0.5	0.25	5
F-6	350	250	0.5	0.25	10
F-7	350	350	0.5	0.25	0

F-8	350	350	0.5	0.25	5
F-9	350	350	0.5	0.25	10
F-10	350	450	0.5	0.25	0
F-11	350	450	0.5	0.25	5
F-12	350	450	0.5	0.25	10
F-13	350	550	0.5	0.25	0
F-14	350	550	0.5	0.25	5
F-15	350	550	0.5	0.25	10

RESULTS

Preformulation Studies i)Melting point determination:

Melting point of chloroquine was found to be in the range 87^{o}c to 89.5^{o}C, which compliedwith I. P. standards, indicating purity of the drug sample.

ii) Solubility:

Chloroquineis very slightly soluble in water.Soluble in dilute acids, chloroform and ether.

Compatibility Studies

a. Fourier Transform Infra Red Spectroscopy (FTIR)

There was no appearance or disappearance of any characteristics peaks of pure drug or of polymer in the physical mixture and chloroquine loaded nanoparticles, thus indicating absence of any physical interaction between the drug and polymer.

MolecularVibration	**Wave number in cm^{-1}**	
	Pure chloroquine	**Chloroquine loaded starchnanoparticles**
NH stretch in NH_2	3415.63	3389.12
P-H	2298.50	2930.67
C = C stretch	1612.72	1631.74
C-O Strech	1552.65	1553.53
CH_2& CH_3stretch	1457.97	1457.34
CH_3	1366.97	1341.34
C-N stretch	1212.31	1155.27
C-H BOND	821.16	860.72

MolecularVibration	**Wave number in cm^{-1}**
	Starch
OH -Stretch	3335.50
C-H Bond	2929.39
C-H Bond Strech	1018.82

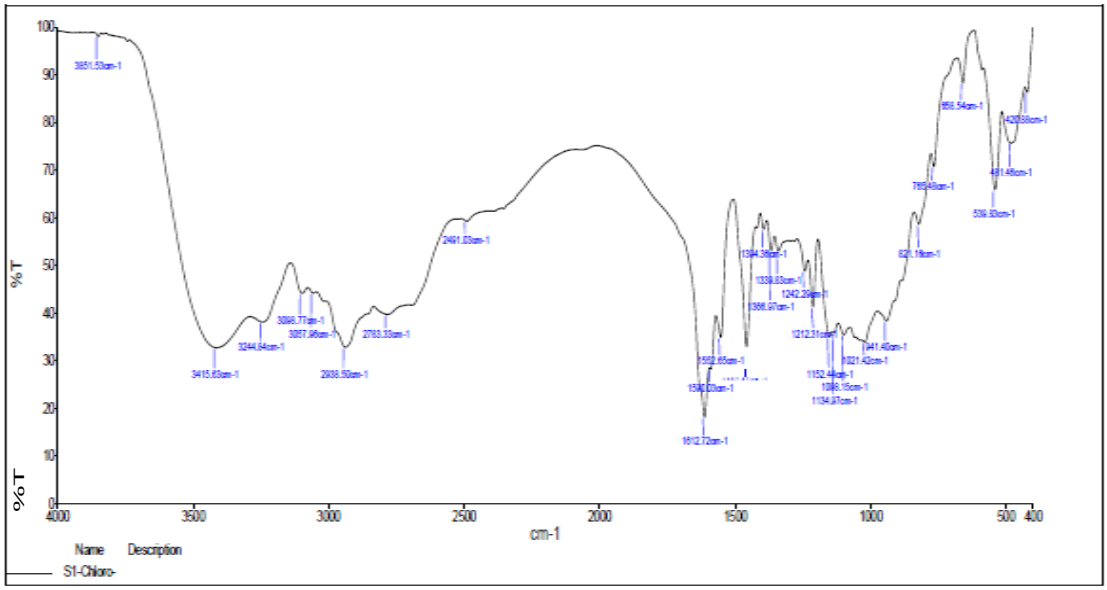


Fig-1A FTIR Spectrum of Chloroquine

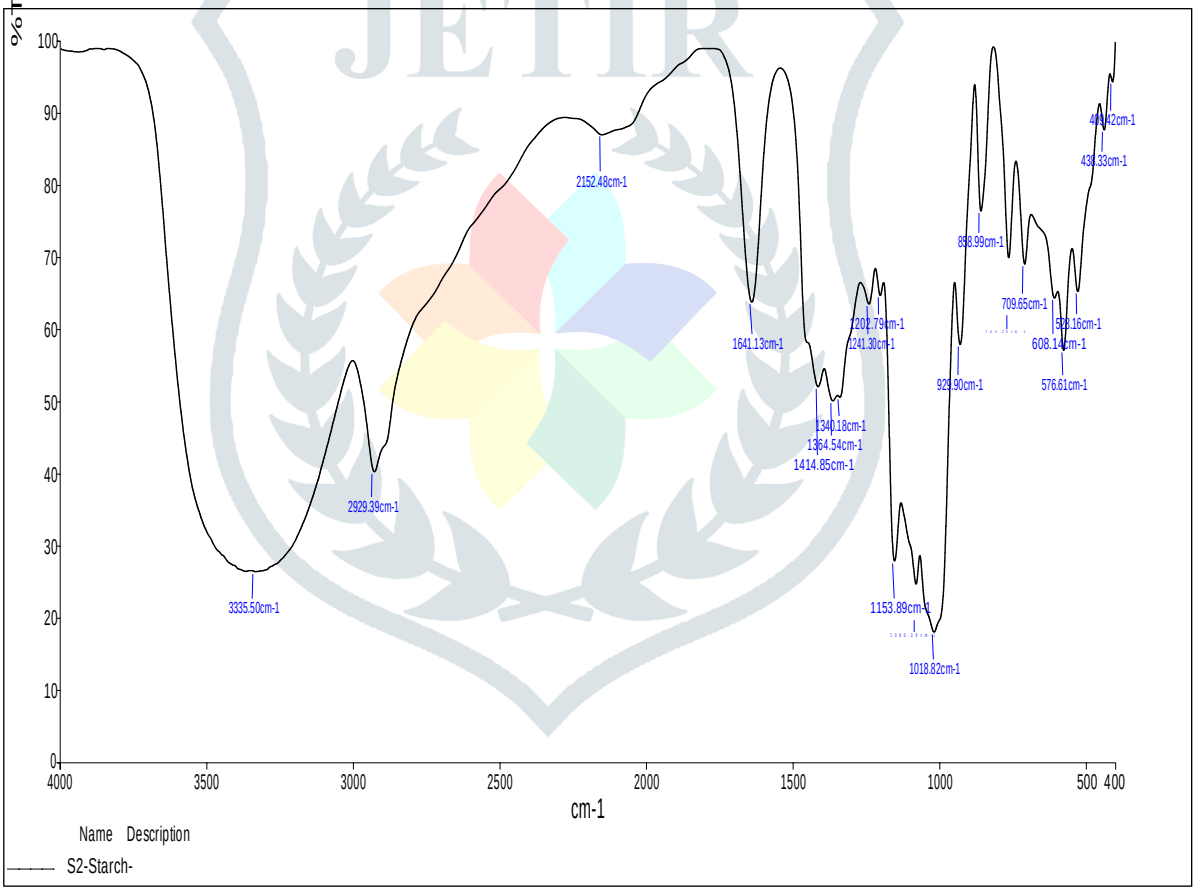


Fig-1B FTIR Spectrum of Starch

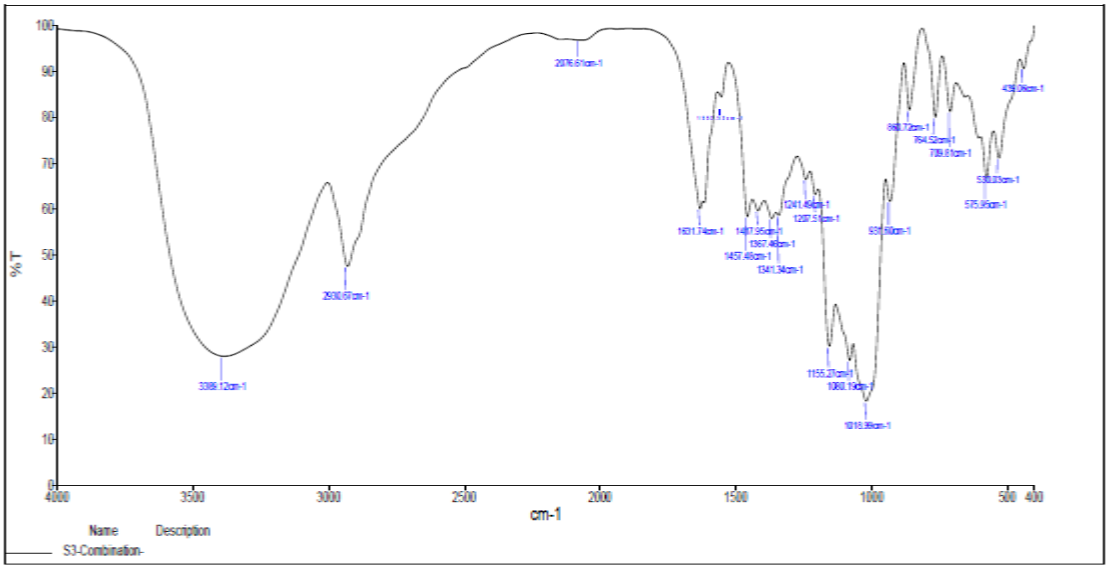
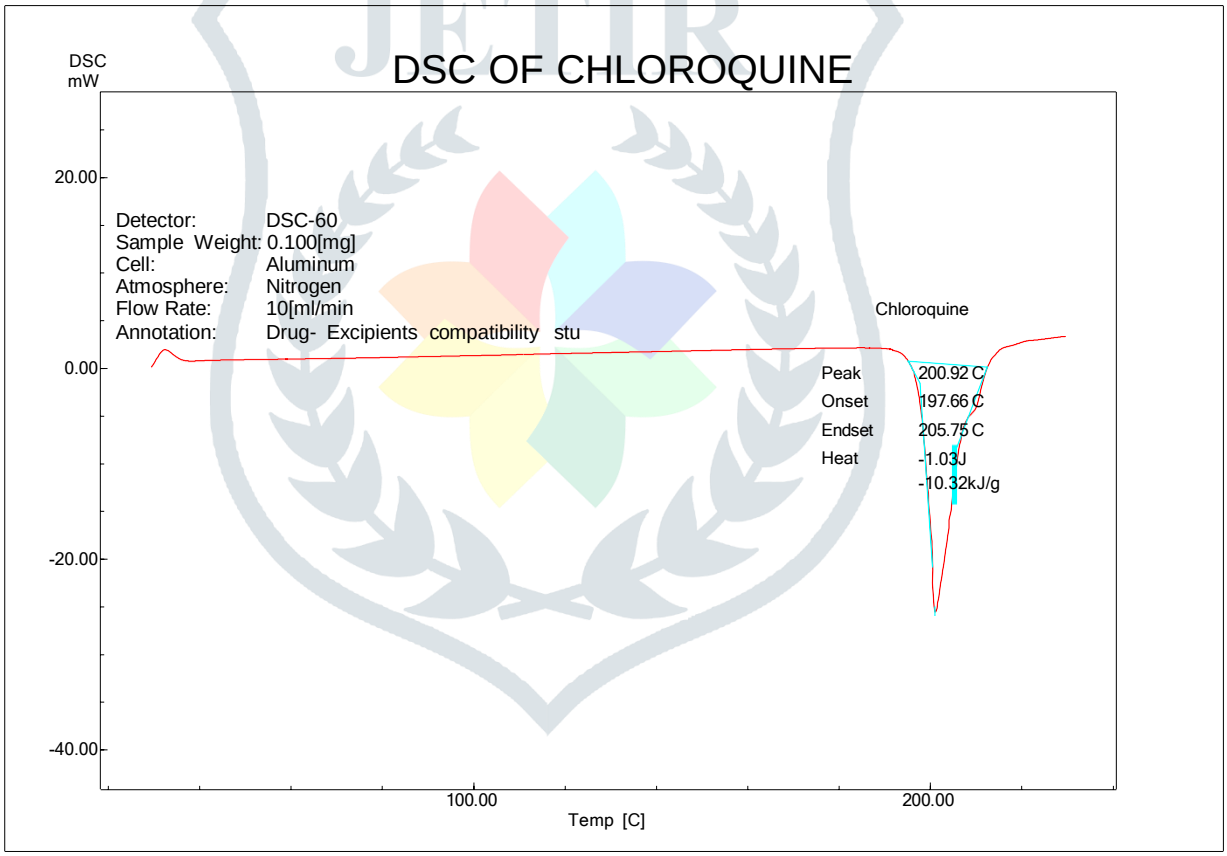


Fig-1C FTIR Spectrum of chloroquine loaded Starch Nanoparticles



b. Drug and carrier interaction by Differential Scanning Calorimetry

The results of the DSC study of chloroquine, starch and chloroquine loaded starchnanoparticles(F-9) are shown in Figs. 2A, 2B, and 2C. The DSC curve of chloroquine showed characteristic peaks at 200.92ºC, 197.66ºC and 205.75ºC. The DSC curve of starch showed characteristic broader peak at 50.81ºC. Fig-2A DSC Thermogram of chloroquine

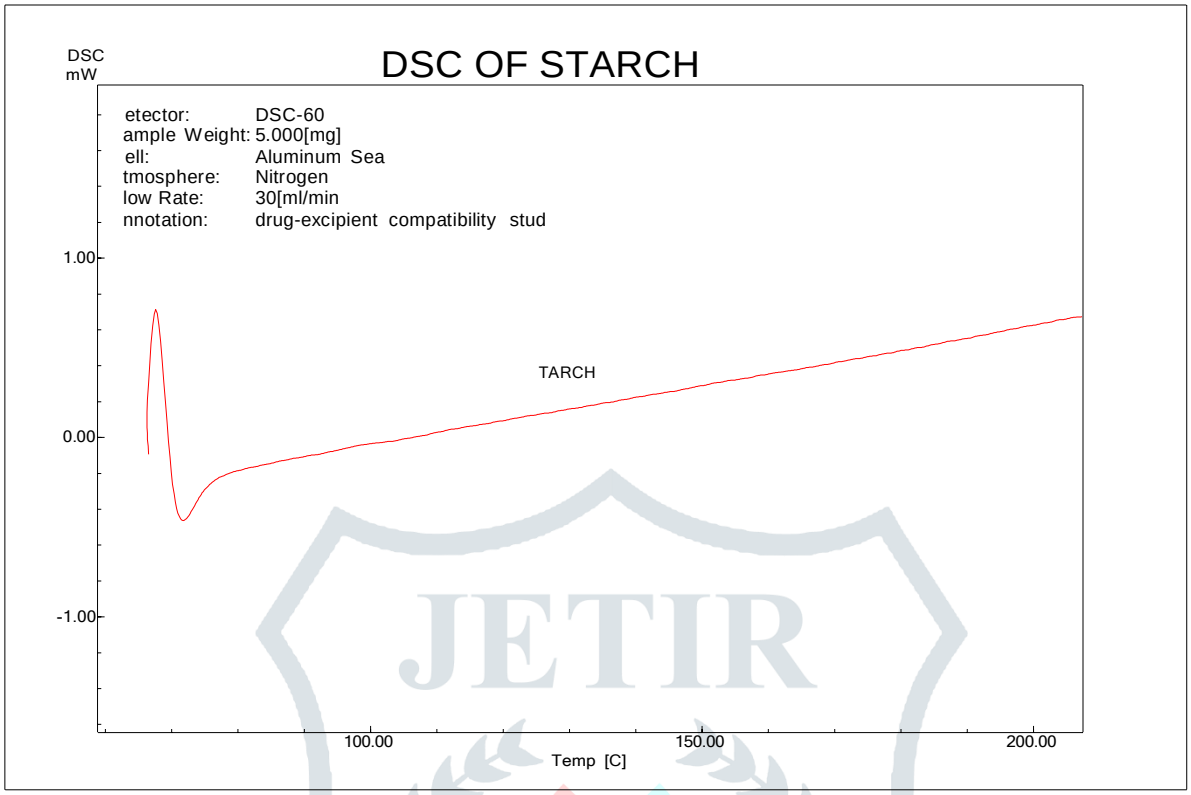


Fig-2B DSC Thermogram of Starch

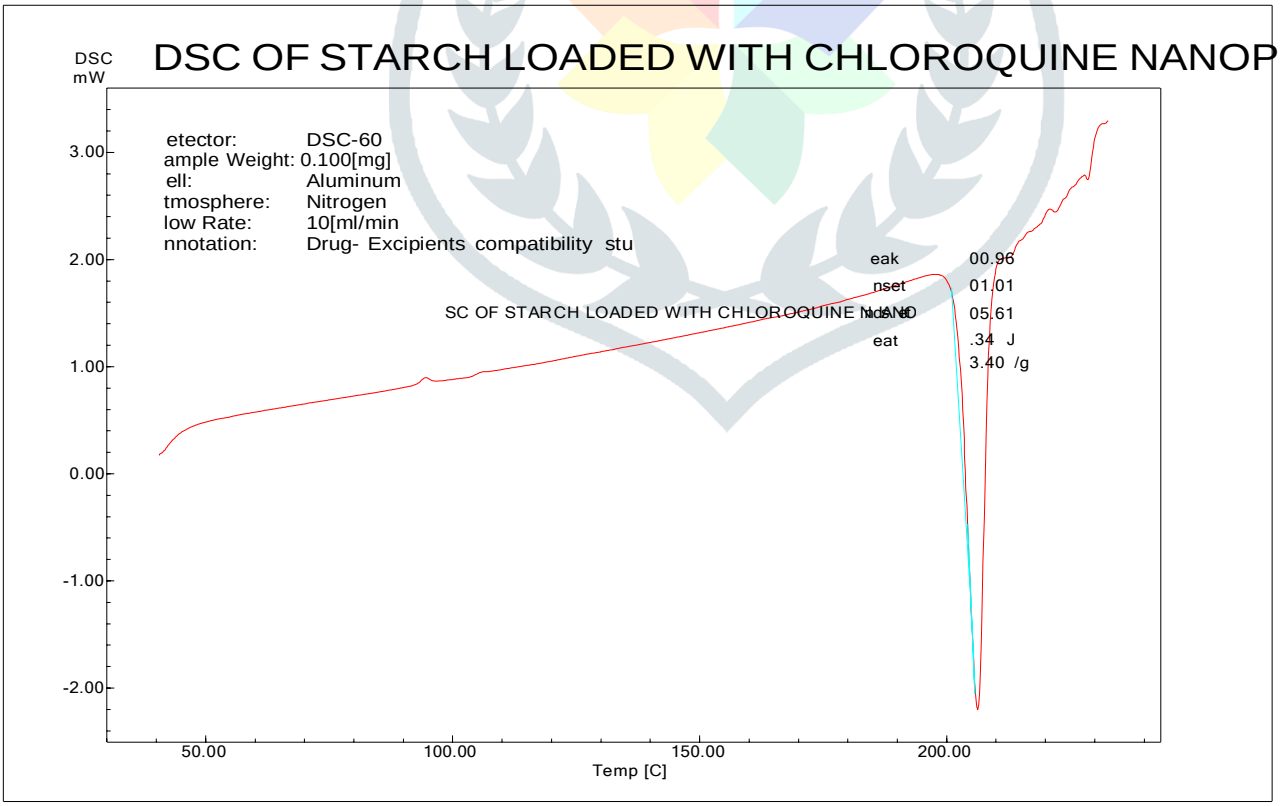


Fig-2C DSC Thermogram of chloroquine loaded starch nanoparticles

Particle size and Zeta potential of chloroquine loaded starch nanoparticles

The mean particle size and zeta potential of chloroquine loaded starch nanoparticles(F1– F15) are shown in

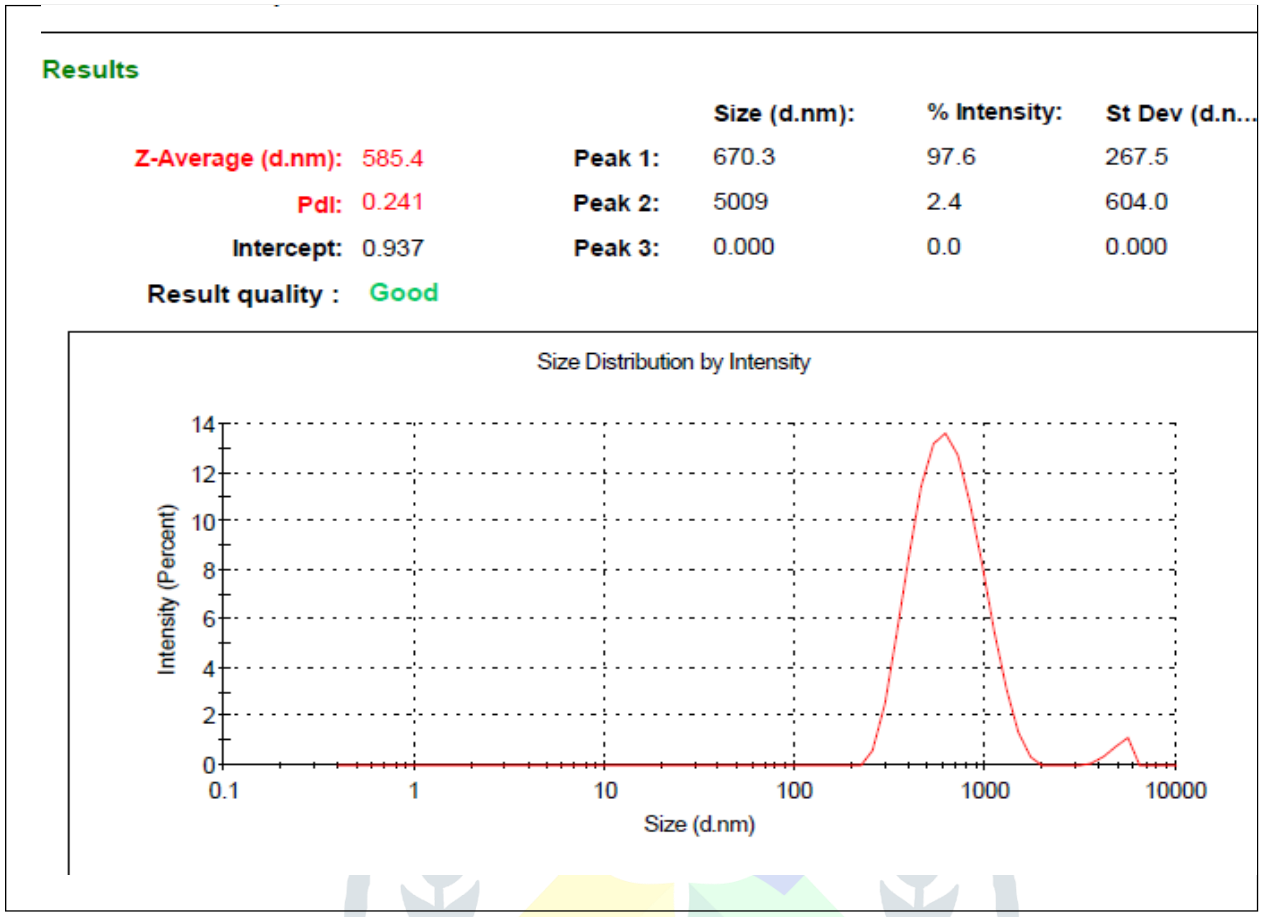


Table-3 and figure 3A.

Fig-3A Particle Size of formulation F-9

The zeta potential values of all the chloroquine loaded starch nanoparticlesdisplayed a negative surface charge ranging from -2.89 to -4.85 mV.

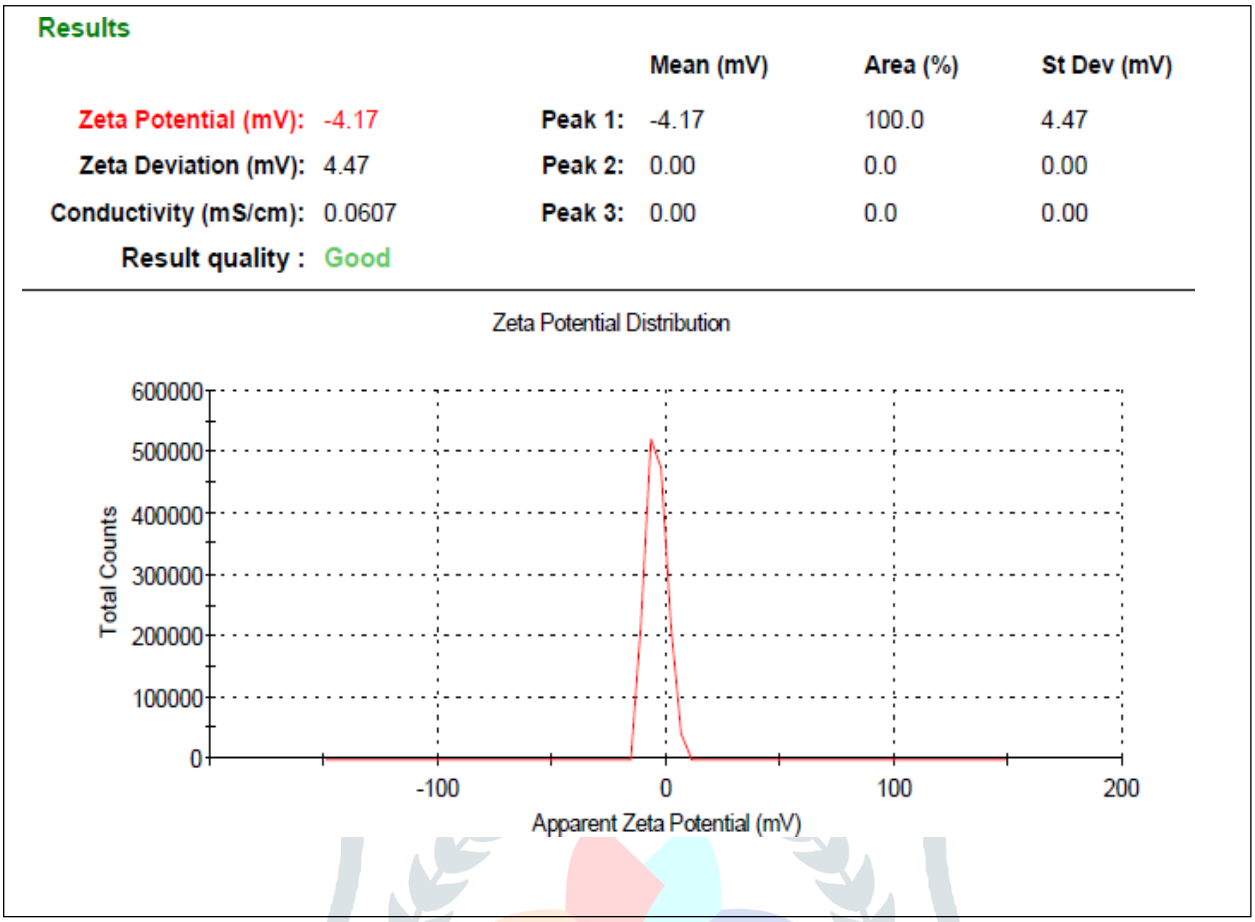


Fig-3B Zeta Potential of formulation F-9

Table-3 Particle size and Zeta potentialofstarch nanoparticles

Formulation Code	MeanParticle size (nm)	Zeta potential (mV)
F-1	411.7	-2.89
F-2	398.1	-3.31
F-3	377.9	-3.37
F-4	517.0	-3.52
F-5	492.3	-3.56.
F-6	426.2	-3.71
F-7	596.3	-3.96
F-8	591.5.	-4.00
F-9	585.4	-4.17
F-10	717.3	-4.35
F-11	681.5	-4.40
F-12	670.4	-4.48
F13	720.6	-4.61
F-14	710.3	-4.72
F-15	708.8	-4.85

Surface Morphology by scanning electron microscopy

According to morphological evaluation analysis by scanning electron microscopy all the prepared chloroquine loaded starch nanoparticles(F1-F15) seemed to have a similar spherical shape. The sizes of nanoparticles of all the formulations were in nanometer. The morphological characters of chloroquine loaded starch nanoparticles(F3, F6, F9, F12 and F15) are shown in Fig-3A, 3B, 3C, 3D and 3E.

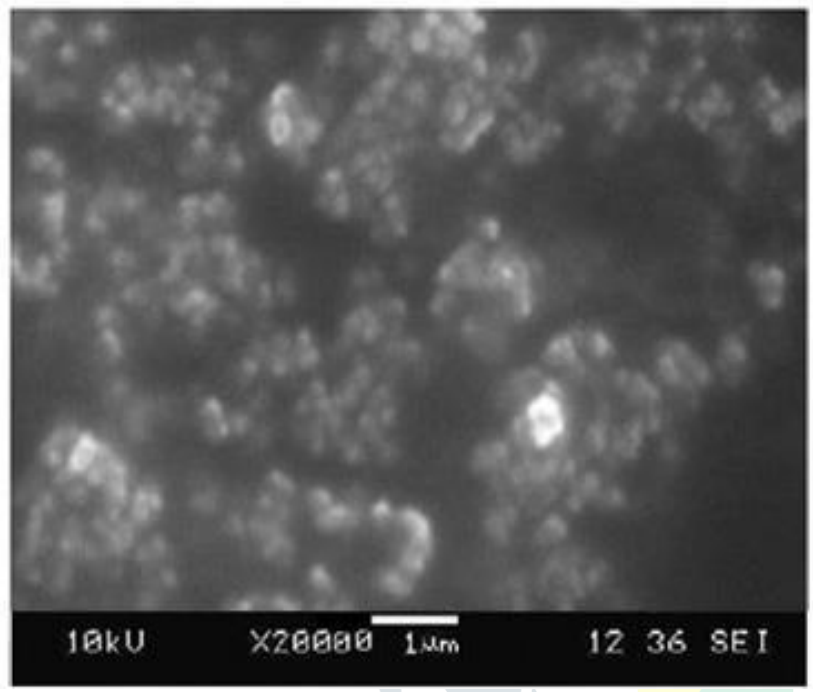


Fig-4A SEM Photograph of formulationF-3

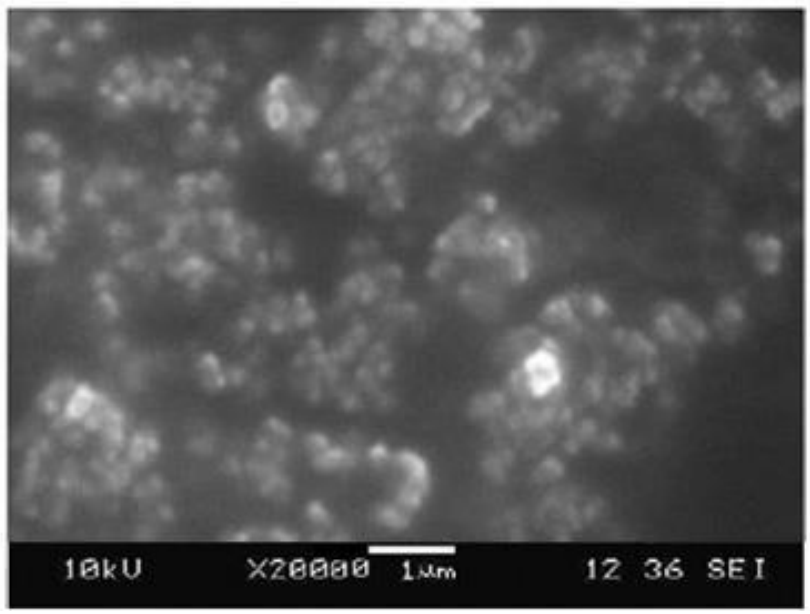


Fig-4B SEMPhotograph of formulation F-6

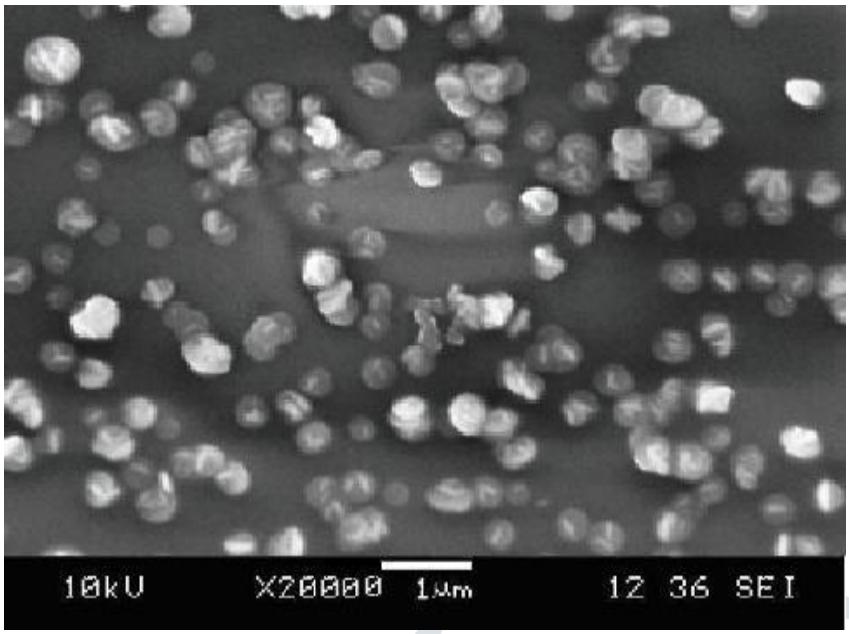


Fig-4C SEM Photographof formulation F-9

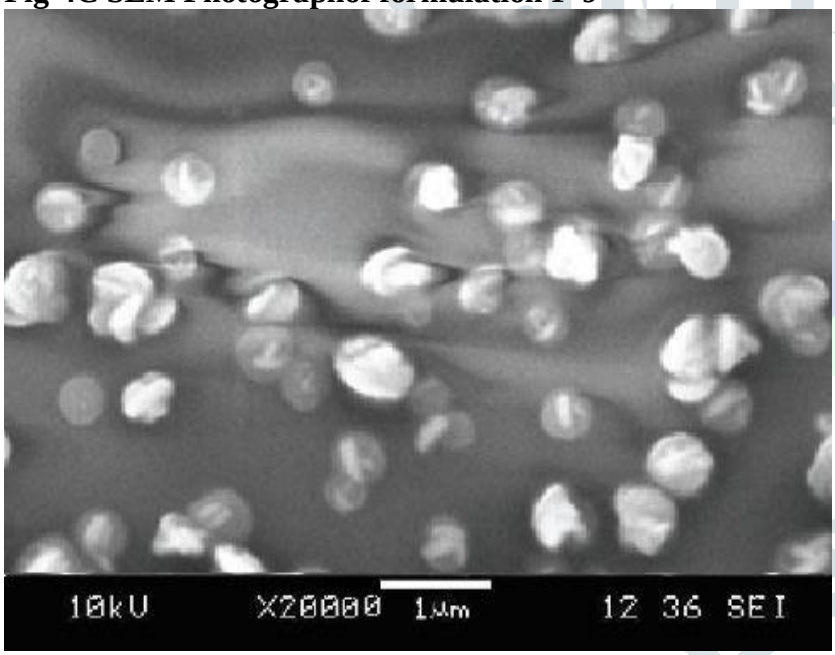


Fig- 4D SEM Photograph of formulation F-12

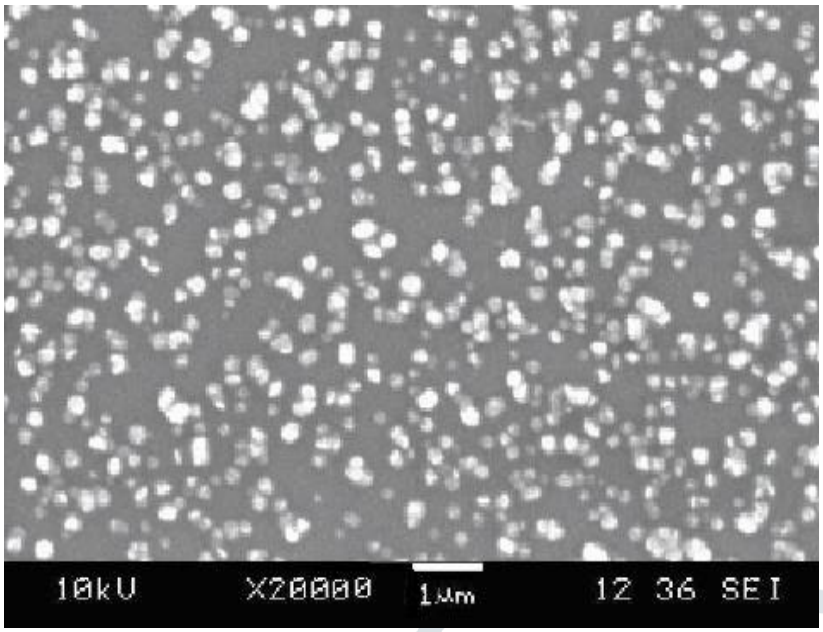


Fig-3E SEM Photograph of formulation F-15

Chloroquine encapsulation efficiency and loading capacity

The encapsulation efficiency and loading capacity of chloroquine loaded starchnanoparticles (F1– F15) are shown in Table-4.

Table-4 Encapsulation efficiency and Loading capacity of the nanoparticles

Formulation Code	Encapsulation efficiency (%)	Loadingcapacity (%)
F-1	70.7	50.8
F-2	71.5	50.6
F-3	71.8	50.3
F-4	74.3	40.58
F-5	75.2	40.67
F-6	74.8	40.54
F-7	81.4	35.90
F-8	80.8	34.89
F-9	81.1	33.87
F-10	86.3	30.90
F-11	86.9	30.84

F-12	87.0	29.76
F-13	90.5	25.4
F-14	90.7	25.2
F-15	90.9	25.0

The encapsulation efficiency was increased by increasing the concentration of polymer. The encapsulation efficiency ranged between 70.7 to 90.9%. The maximum entrapment was found in F-15 (90.9%) and lowest entrapment in F1 (70.7

***In-vitro* drug release**

Diffusion of chloroquine from the starch nanoparticles i.e the drug leakage was monitoredfor 24 hrs as shown in Fig- 4. From the *in-vitro* drug release data for F1-F15, it was observed thatincrease in the polymer concentration delays the drug release due to increased particle size and reduced surface area available for drug release. The drug release data was found to increase after 10mins sonication with increased polymer concentration. Based on this, five formulations F-3, F-6, F9, F12 and F-15 were chosen for *in-vitro* drug release study. In the first hour, drug released was 12.0%, 12.5%, 12.8%, 13.8%, 14.71%, 16.62%, 14.56% and 15.85% for F-3, F-6, F-9, F-12 and F-15 respectively. The cumulative percentage of drug released for F3, F6, F9, F12 and F15 after 24 hrs was 76.14%, 85.28%, 90.10%, 82.30% and 80.40% respectively.

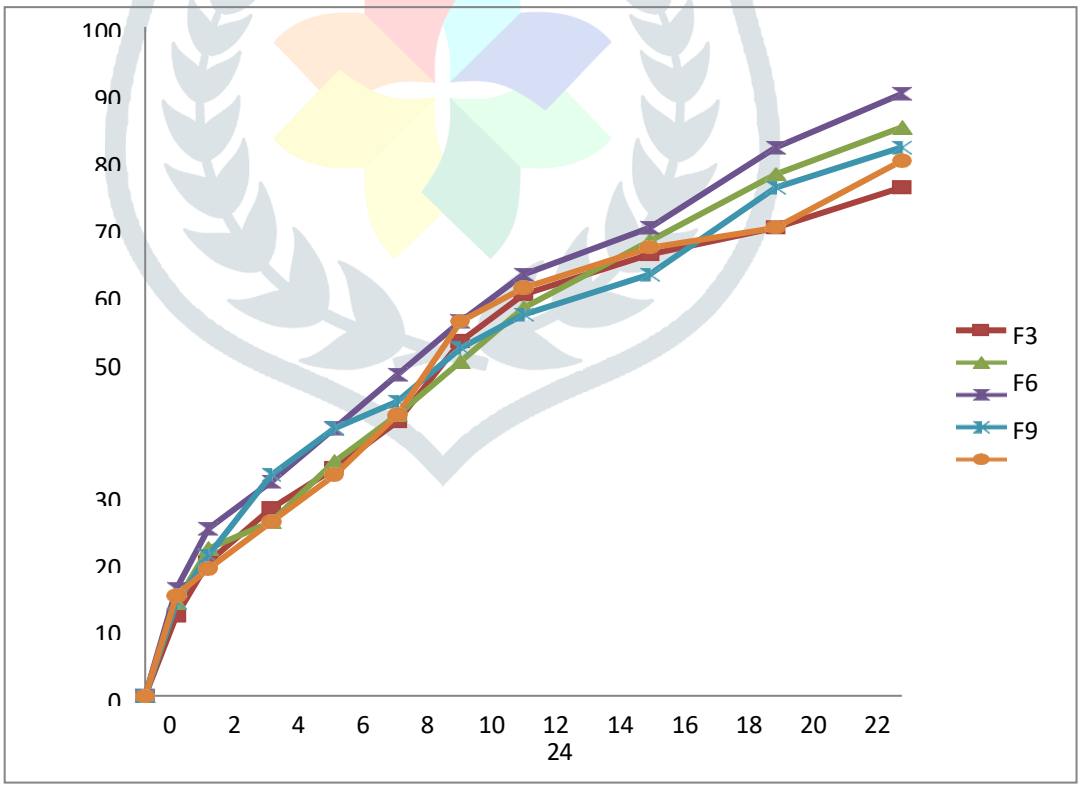


Fig-4 Comparative *in-vitro* drug release profile of F-3, F-6, F-9, F-12 and F-15

Maximum drug release was found in F-9 (90.10. %) and minimum was found in F-1 (70.2%). Out of all the formulations F-9 was selected as optimized formulation due to its desirabledrug release in 24hrs.

DISCUSSION

The results of the present investigation demonstrated the potential use of starch nanoparticles for effective delivery of chloroquine for treating malaria. Starch based drug deliverysystem must overcome important physical barriers to reach the target cells. Different colloidal systems have been developed to solve these problems.Moreover starch nanoparticles can be easilyprepared under mild conditions, and can be incorporated in macromolecular bioactive compounds. Among the various methods developed for preparation of nanoparticles, nanoprecipitationmethod is simple to operate and also to optimize the required particle size of the drug that can penetrate the ocular surface. From the IR spectral analysis and DSC study, it was found that IR spectrum and thermogram of pure chloroquineand combination of pure drug with polymer like starchand prepared nanoparticles showed all the characteristic peaks of Chloroquineconfirming the physical and chemical compatibility of the pure drug and polymer (Table-2 and Fig-2A, 2B, 2C, 2D and 2E). pH values for all the formulations are within acceptable range 6.6-7.4 and hence would notcause any irritation upon administration of the formulation. It was also observed that increase in Starchpolymer causes a slight increase in pH for formulations. The mean particle size of the chloroquine loaded starch nanoparticles(F1-F15) were increased by increasing the polymer concentration (Table-3).The maximum size of nanoparticles was observed in F-15 as compared to other formulations and the least size was seen in F-1. Thus the results indicated that the size of the nanoparticles varied with the polymer concentration. Previously it has been reported that the particle size is dependent on the starchconcentration, the minimum size corresponding to the lowest starch concentration. The presence of a nonionic surfactant is very important for the so-called “long-term” stability of the nanosphere colloidal suspension, which is determined by the adsorption ofhydrophilic macromolecules on the nanosphere surface, thus increasing the steric repulsion between particles. The presence of hydrophilic macromolecules on the surface of nanosphere leads to a change of the surface properties (zeta potential) of the colloidal carrier. Considering these factors the non-ionic surfactant Tween 80 (0.05%) was used to stabilize the formulation. The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential values of all the

chloroquine loaded starch nanoparticles displayed a positive surface charge ranging from -2.89 to -4.85 mV (Table-4). The zeta potential values increased as the concentration of polymer increased. All formulations showed zeta potential above -30 mV indicating that the formulations are moderately stable. The present study demonstrated increase in zeta potential as the concentration of starch increased which is in line with earlier findings of the ammonium glycyrrhizinate (model drug) loaded starch nanoparticles. The SEM of the chloroquineloaded starch nanoparticles showed that the nanoparticles have a solid dense structure with smooth spherical shape. The encapsulation efficiency of the nanoparticles ranged from 70.7 to 90.9% and the loading capacity ranged between 25.00 to 50.8% as shown in Table- 4. The encapsulation efficiency was increased by increasing the concentration of polymer. Conversely the loading capacity of the nanoparticles decreased as the concentration of polymer increased. The results suggested that the encapsulation efficiency and loading capacity of the nanopartcles depend on theconcentration of the polymer used in the preparation. The *in-vitro* diffusion of chloroquine from the nanoparticles was studied by monitoring the drug leakage for 24hrs. The chloroquine release profile from starch nanoparticles is characterized by an initial rapid release followed by a sustained release of the drug over a period of 24hrs (Fig-4). The initial rapid release can be due to the burst effect resulting from the release of the drug encapsulated near the nanosphere surface and thereafter the slow release of chloroquinefrom the starch nanoparticles is possibly the consequence of the release of the drug fraction encapsulated inthe core of the nanospheres. The cumulative percentage drug released for F3, F6, F9, F12 and F15 after 24hrs was 76.14%, 85.28%, 90.10%, 82.30% and 80.40% respectively. Maximum drug release was found in F-9 (90.10. %) and minimum was found in F-1 (70.2%). Among all the formulations F-9 was selected as an optimized formulation due to its desirable drug release during24hrs.

CONCLUSION: Chloroquine loaded starch nanoparticles exhibited excellent capacity for the association of chloroquine. The mean particle size, morphological characteristics, surface property, encapsulation efficiency and loading capacity of the nanoparticles appear to depend on the concentration of polymer. The results demonstrated the effective use of chloroquine loaded starch nanoparticles as a controlled release preparation for treatment of malarial infections.

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