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STUDY OF DEVELOPMENT OF STARCH NANOPARTICLES ENCAPSULATING CHLOROQUINE

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ABSTRACT

Chloroquine is extensively used anti-malarial drug, however resistance has been developed by the pathogen rendering it ineffective against the plasmodium strains.Chloroquine loaded starch nanoparticles were prepared by nanoprecipitation method.There was no appearance or disappearance of any characteristics peaks of pure drug or of polymer in chloroquine Starch loaded nanoparticles andthat there was no chemical interaction between chloroquine and starch.The results of the present investigation demonstrated the potential use of starch nanoparticles for effective delivery of chloroquine for treating malaria

Key words: Chloroquine**,** Nanoparticle, Nanoprecipitation,

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INTRODUCTION

Chloroquine is extensively used anti-malarial drug, however resistance has been developed by the pathogen rendering it ineffective against the plasmodium strains. This resistance is due to failure in achieving therapeutically effective chloroquine concentrating inside infected erythrocyte. Erythrocyte membrane contains glucose receptor (GLUT 1), which is utilized to transport glucose where as infected erythrocyte showed expression of new transporter called Plasmodial surface anion channel (PSAC). This novel ion channel induced on human erythrocytes infected with Plasmodium falciparum, mediates increased permeability to nutrients and presumably supports intracellular parasite growth. Hence it was reported that, glucose uptake by Plasmodium-infected erythrocytes is much higher compared to healthy

erythrocytes. The surface property of the nanoparticle can be functionalized to exploit the anionic conductance of newly generated PSACs and hence targeting the infected erythrocyte. This will reduce the toxic potential of chloroquine on healthy erythrocyte too. Therefore present study was designed to prepare starch nanoparticles encapsulating Chloroquine with surface functionalization and evaluate their potential in targeting PSACs transporters on infected erythrocytes. It is envisaged that, starch nanoparticle will concentrate chloroquine more towards infected erythrocyte thus stop heamolysis too in healthy erythrocyte.

MATERIALS AND METHOD

Materials

The drug and chemicals required for the work was procured from reputed firm. The details are furnished below:

Drug and Chemicals	**- Supplier/ Manufacturer**
Chloroquine	- Micro labs Pvt.Ltd. Hosur.
Starch	- Universal polymers, Cochin
Ethanol	- S.D Fine chem. Ltd, Mumbai.
HPLC water	- Merck Pvt, Ltd Mumbai.
Sodium Hydroxide	- Spectrum Reagents & Chemicals, Cochin
Potassium Dihydrogen Phosphate	- Spectrum Reagents & Chemicals, Cochin
Tween 80	- Loba chemicals, Mumbai.
Sodium Tri Poly Phosphate	- S.D Fine chem. Ltd, Mumbai
Dialysis membrane 11	- Himedia Laboratory, Mumbai

Instruments

Instruments	**Model/ Manufacturer**
FT-IR spectrophotometer	- Perkin Elmer Spectrum RX 1
Scanning electron microscope	- Joel model JSM 6400, Tokyo
UV-Visible Spectrophotometer	- Perkin Elmer Lambda 25
Single pan digital balance	- Shimadzu BL220H
Microscope	- Unilab
Digital pH meter	- Hanna instruments, Italy HI98
Magnetic stirrer	- Eltek MS 2012.
Sonicator	-BandelinSono plus Model HD, 2070
Freeze Drier	- Labconico, USA
Research centrifuge	- Hitachi Centrifuge USA

Differential Scanning Calorimetry - DSC DA 60 Shimadzu, Japan

Zeta potential analyzer - Zetasizer 3000HS,Malvern instrument, UK.

HPLC - Perkin Elmer USA

Methods

I. Compatibility Studies

Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Compatibility studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipient in the fabrication of a dosage form (Robert M,2005 ; Hobart H Willard,2010)

a) Fourier Transform InfraRed Spectroscopy (FTIR)

Compatibility study of pure chloroquine , starch polymer, chloroquine with starch polymer and chloroquine loaded starch nanoparticles (F-9) were determined by FTIR Spectroscopy using Perkin Elmer RX1. The pellets were prepared by gently mixing sample with potassium bromide at high pressure. The scanning range used is 450 to 4000 cm^{-1}. The pellets thus prepared were examined and the spectra of drug and the polymer in the formulations were compared with that of pure drug and polymer spectra.

b) **Differential Scanning Calorimetry (DSC)**

Differential scanning calorimetric curve of pure chloroquine, starch polymer, chloroquine with starch polymer and chloroquine loaded starch nanoparticles (F-9) were carried out by using thermal analysis instrument equipped with liquid nitrogen sub ambient accessory; 2-6mg samples were accurately weighed in aluminum pans hermetically sealed and heated at a rate of 10ºC per min^{-1} under nitrogen flow of 40 mL/ min.

II Estimation OfPure Chloroquine

Chloroquine can be estimated spectrophotometrically at 510nm in the range of 2-18 µg/mL (Douglas A et. al.)

Preparation of Phosphate buffer pH 7.4

50 ml of 0.2M potassium dihydrogen phosphate was placed in a 200mL volumetric flask and added 39.1 mL of 0.2M sodium hydroxide and then distilled water to make up to 200m (IP, 1996)

Preparation of 0.2M potassium dihydrogen phosphate

27.218g of potassium dihydrogen phosphate was dissolved in distilled water and made up to 1000 mL.

Preparation of 0.2M sodium hydroxide

8g of sodium hydroxide was dissolved in distilled water and made up to 1000 mL.

Preparation of standard drug solution

Stock Solution

100mg of chloroquine was dissolved in 100ml of phosphate buffer pH 7.4 so as to get a stock solution of 1000 µg/mL concentration.

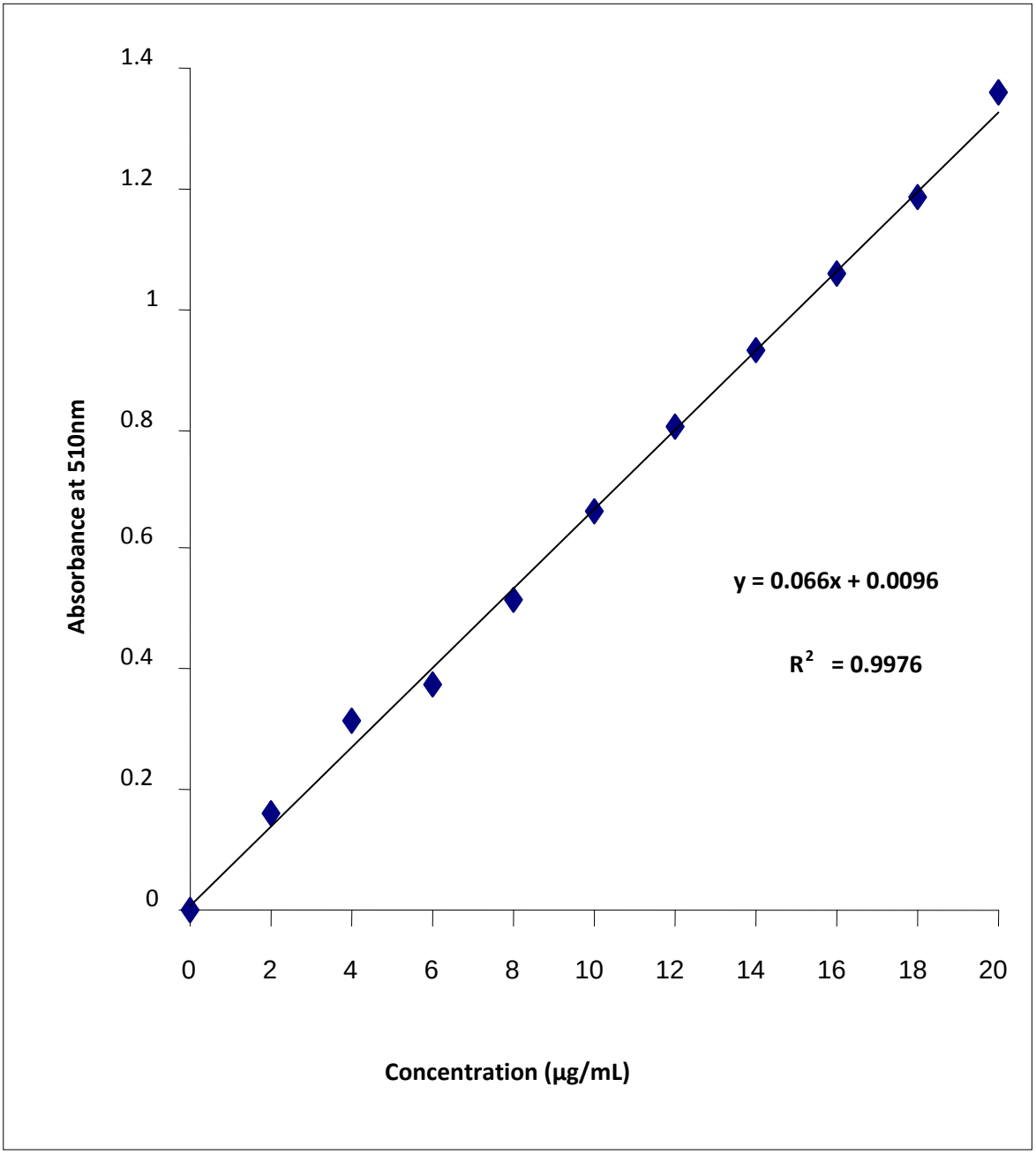
Standard Solution

2 mL of stock solution was diluted to 100mL with pH 7.4 phosphate buffer thus giving a concentration of 20 µg/mL of the drug. Aliquot quantities of standard drug solution ranging from 1mL to 9mL were transferred to 10mL volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Absorbance of each solution was measured at 253 nm against phosphate buffer pH 7.4 as a blank and the calibration curve was plotted.

Data for standard calibration curve of pure chloroquine

S.No	Concentration (µg/ mL)	Absorbance (253nm)
1	2	0.1634
2	4	0.3116
3	6	0.3773
4	8	0.5141
5	10	0.6610
6	12	0.8040
7	14	0.9318
8	16	1.0561
9	18	1.1861
10	20	1.3588
	Slope	**0.0660**
	Regression	**0.9976**

Standard calibration curve of pure chloroquine



III. Preparation of Cross-Linked Enset Starch.

Cross-linking reaction of enset starch was carried out following the procedures of Reddy and Seib [with slight modification. Enset starch (100 g, dry basis) was suspended in distilled water (150ml) which contains 3 g of dissolved NaCl and continuously stirred at 25° C. After adjusting to pH 10.0 with 1M NaOH, epichlorohydrin at different concentrations (3 to 16 g per 100 g of dry starch) was added directly to the slurry for low and high level of cross-linking, respectively, with stirring at 25 to 54° C for different cross-linking time (1 to 10 h),

then adjusted to 6.0–6.5 with 0.2M HCl, and the crosslinked enset starch was isolated by centrifugation (3000×g, 15 min). After washing with distilled water, the sediment was then dried at 45° C for 48 h in a vacuum oven (MEMMER, GmbH D-91126, Schwabach, FRG, Germany)(Barry BW,2001)

IV. 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®80 dissolved in 20 ml distilled water containing drug, was slowly added, followed by the addition of 10 ml H_2O 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 drugencapsulated 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 inthe 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 the solid state can be easily re-dispersed in distilled water by hand agitation before use(I. Reddy and P. A. Seib,2000, Volpato NM et al,1998; Bouwstra JA, Honeywell-Nguyen PL,2002; Prausnitz MR, Langer R,2008; Richards DM,1983)

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 AND DISCUSSION

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 complied with I. P. standards, indicating purity of the drug sample.

II.Solubility:

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

III.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. The results show that the incorporation of the drug into the polymer did not change the characteristics of the drug. Observations of compatibility studies of infrared spectra for the pure drug, the mixture of chloroquine with starch and chloroquine loaded starch nanoparticles (F-9) are shown in Table-2. The IR spectra of starch,chloroquine and mixture of chloroquine withstarch and chloroquine loaded starch nanoparticles(F-9) are shown in Fig- 1A, 1B and 1C.

Molecular Vibration	**Wave number in cm^{-1}**	
	Pure chloroquine	**Chloroquine loaded starch nanoparticles**
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

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

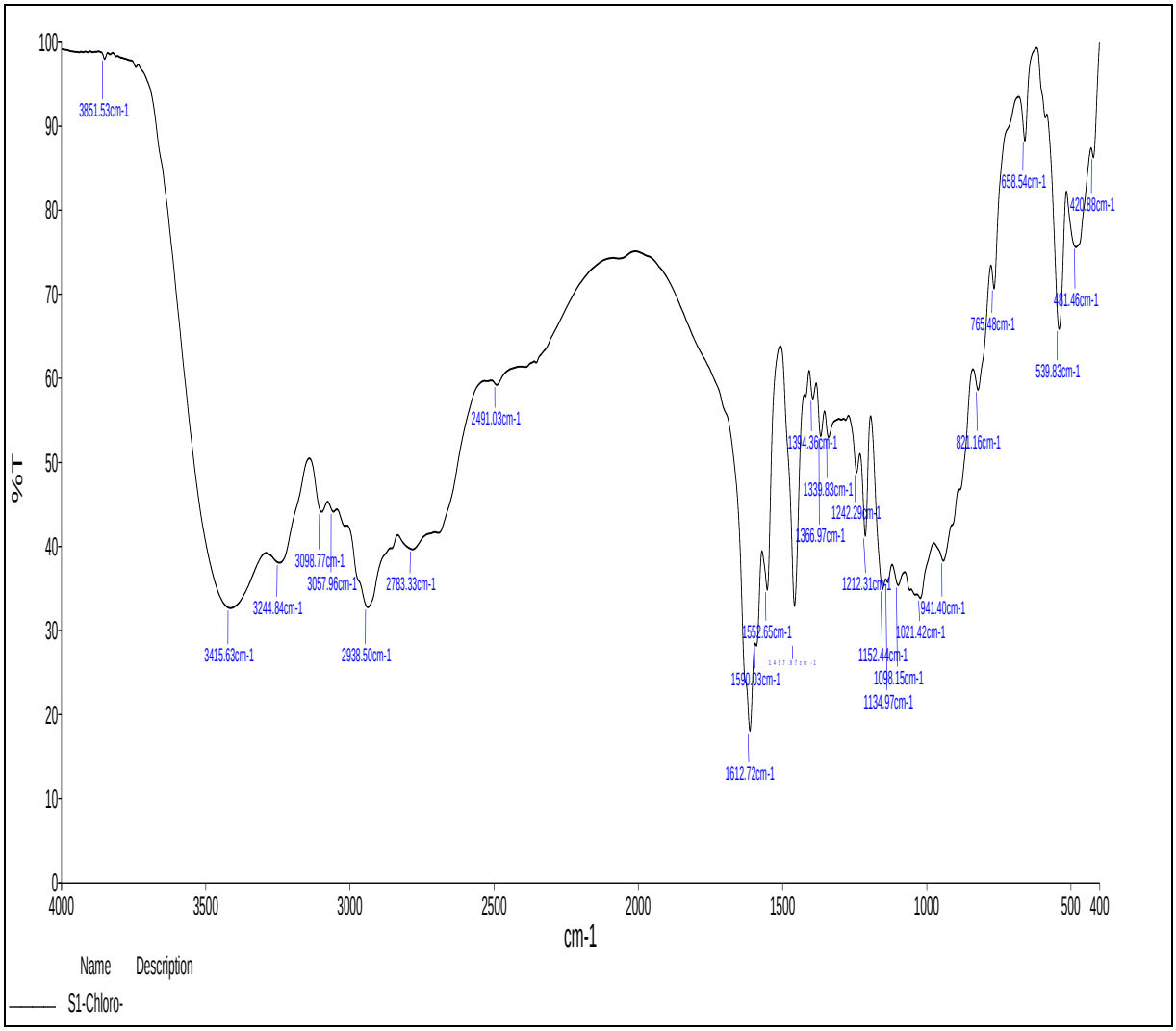


Fig-1AFTIR Spectrum of Chloroquine

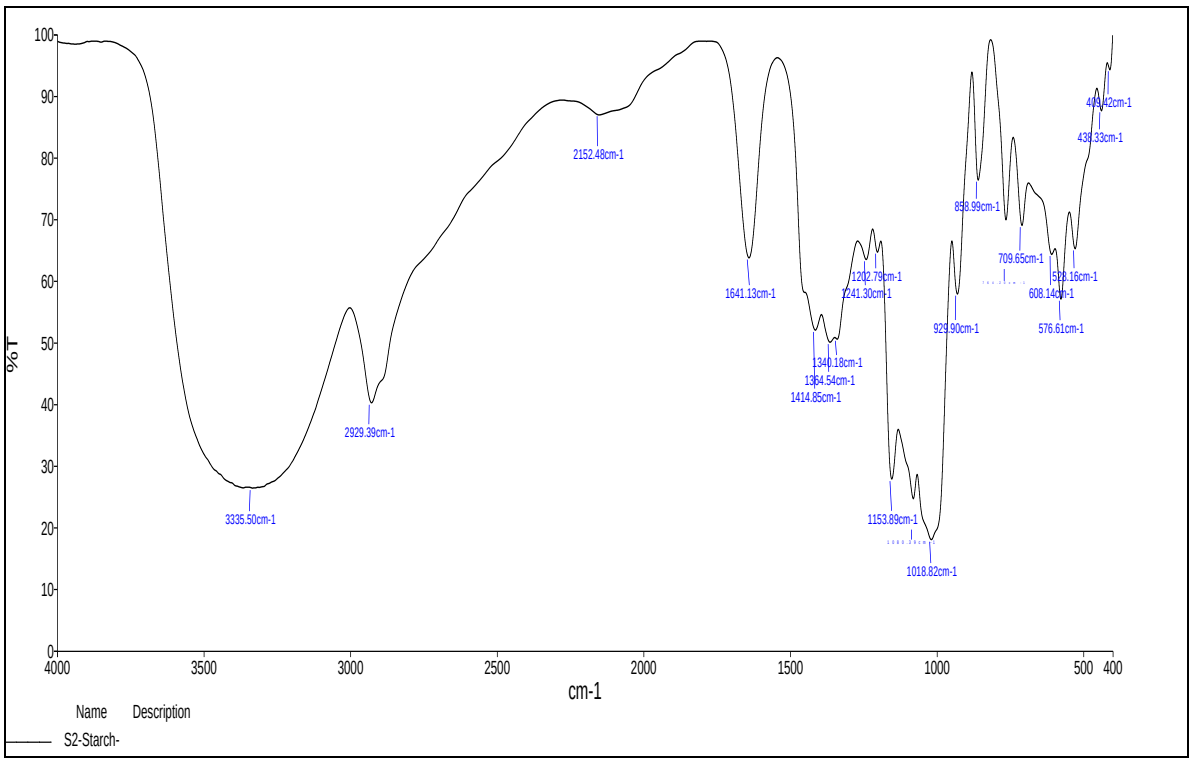


Fig-1B FTIR Spectrum of Starch

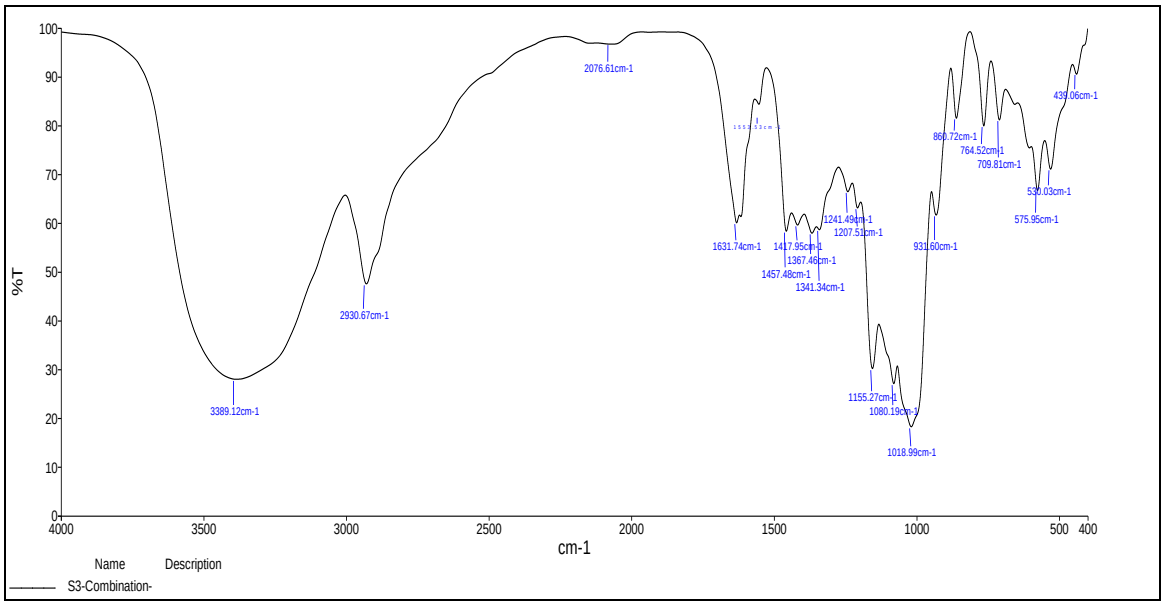


Fig-1C FTIR Spectrum of chloroquine loaded Starch Nanoparticles

b. Drug and carrier interaction by Differential Scanning Calorimetry

The results of the DSC study of chloroquine, starch and chloroquine loaded starch nanoparticles(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. The thermogram of chloroquine loaded starch

nanoparticles exhibited same characteristic peaks of chloroquineat 200.96ºC, 201.01ºC and 205.61ºC. The results of the thermogram suggested that there was no chemical interaction between chloroquine and starch.

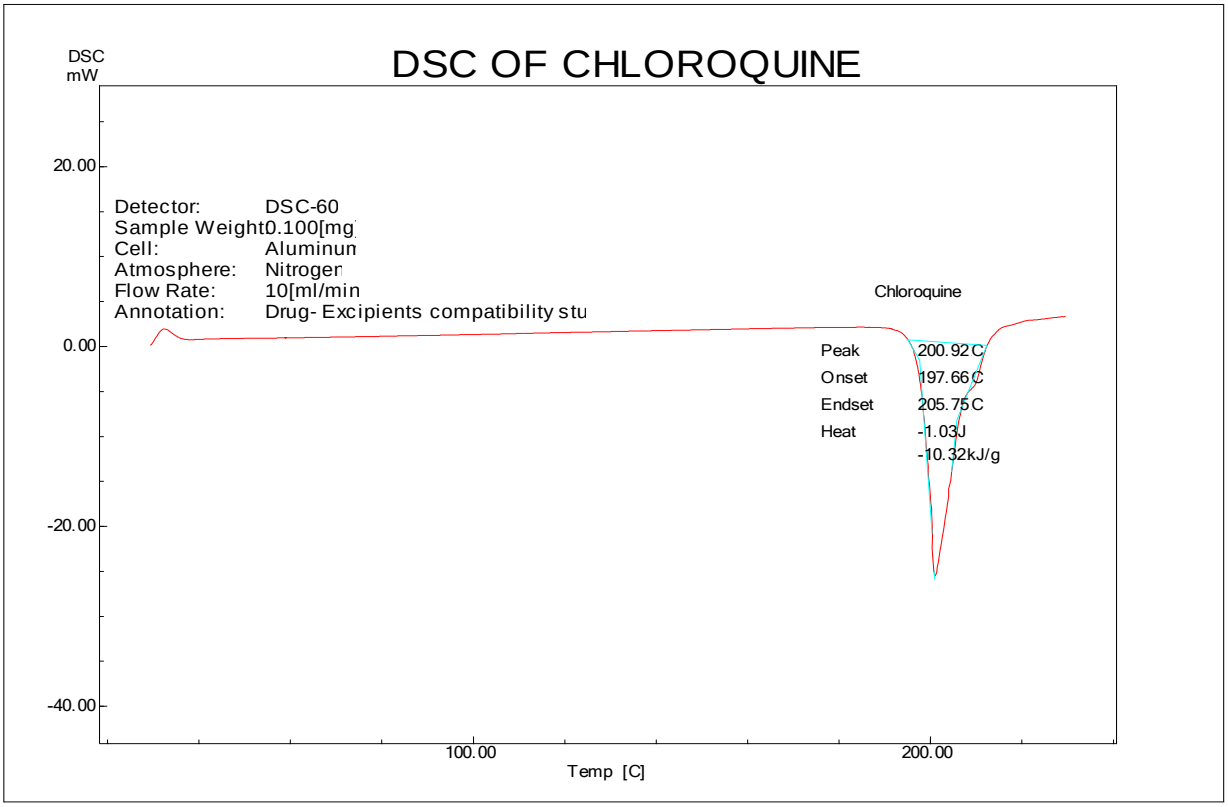


Fig-2A DSC Thermogramof chloroquine

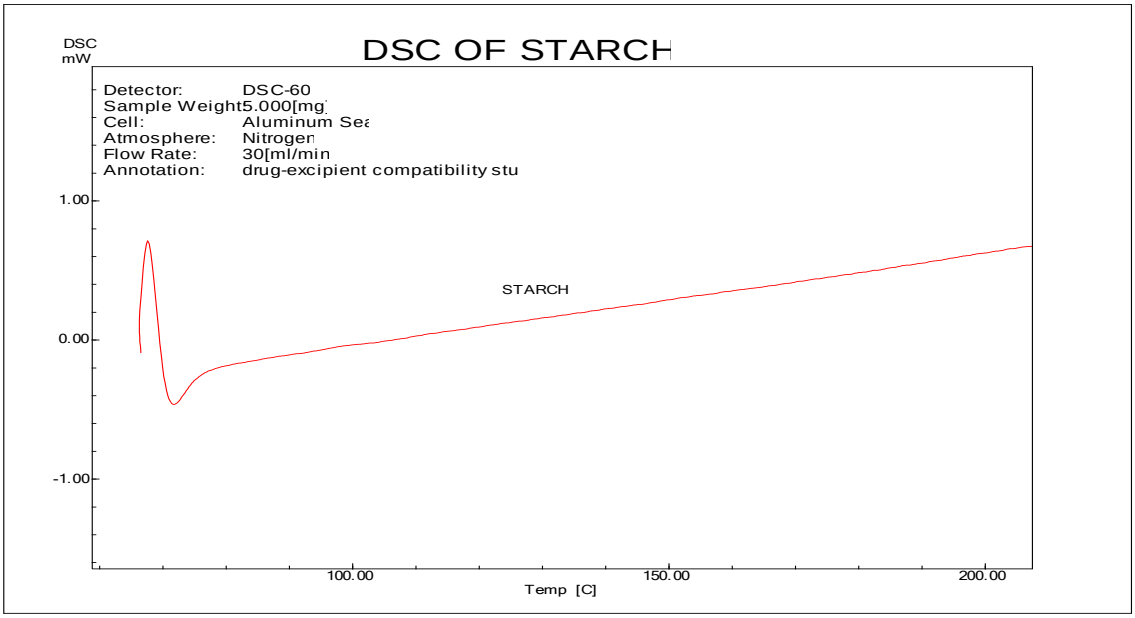


Fig-2B DSC Thermogramof Starch

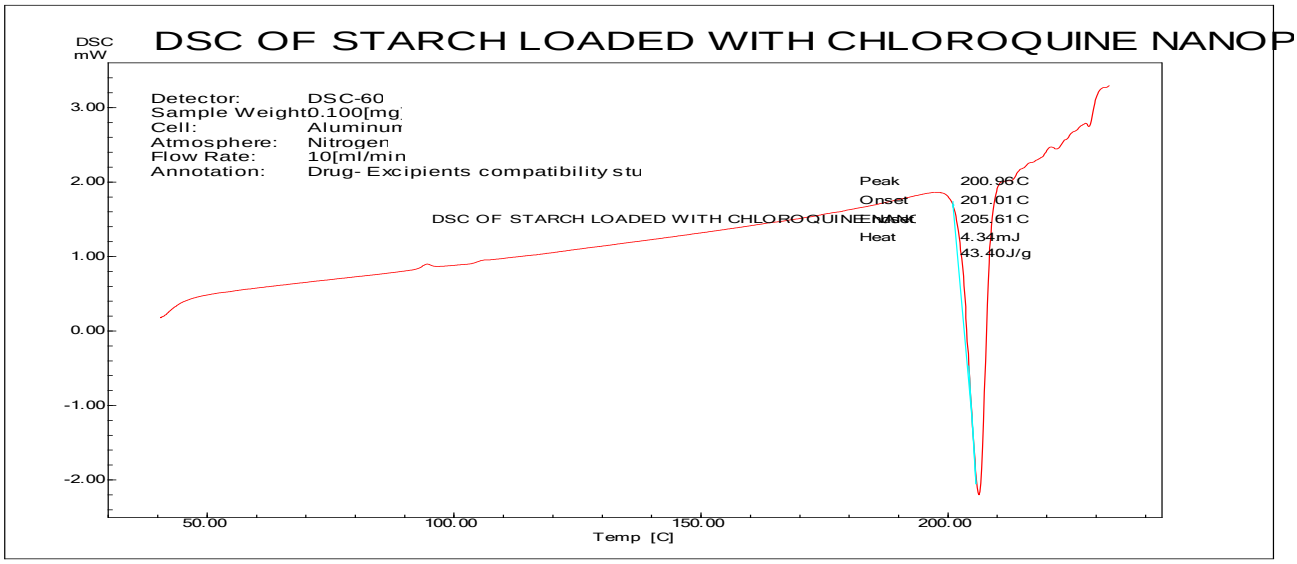


Fig-2C DSC Thermogramof chloroquine loaded starch nanoparticles

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 easily prepared under mild conditions, and can be incorporated in macromolecular bioactive compounds.

Among the various methods developed for preparation of nanoparticles, nanoprecipitation method 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). It is concluded that Chloroquine loaded starch nanoparticles exhibited excellent capacity for the association of chloroquine.

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