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ORIGINAL ARTICLE

Formulation and Evaluation of Diclofenac Loaded Silver Nanoparticles

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ABSTRACT
The preset study deals with formulation and development of silver nanoparticles based on polysaccharides as reducing and stabilizing agent. In this study chitosan stabilized silver nanoparticles were prepared by controlled heating technique and Diclofenac was conjugated with silver nanoparticles to treat the inflammation. The Diclofenac loaded silver nanoparticles were characterized by UV–Visible Spectrophotometer, FT-IR, Scanning electron microscopy and Zeta sizer. The absorption maximum of the silver nanoparticles was 276nm. SEM images ofDiclofenac Silver nanoparticles showed irregular particles in the range of 90nm to 140nm. Drug loading efficiency was carried out and percent drug loading was found to be 69.74% for formulation F1. An in vitro drug release study was carried out and percentage drug release was found to be 40.78% at the end of 12 hours for formulation F1. The study concluded that, silver nanoparticles formulation plays a dual role, to target the diseased site and to release the drug in a controlled manner and produces synergetic effect to the inflammatory sites.
Keywords: *Diclofenac, Chitosan, Silver nanoparticles, Inflammation.*

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INTRODUCTION

Nanoparticles are small colloidal particles that are made up of non-biodegradable and biodegradable polymers and their diameter is around 1nm to 1000nm. Nanoparticles possess large surface area and their surface to mass ratio is extremely high compared to other particles. The nanoparticles are able to bind, adsorb and carry other compounds such as drugs, probes and proteins due to large surface area. Nanoparticulate carrier system permits entrapment /encapsulation of therapeutics without modification, [1] as it requisite for polymer drug conjugates. Both metallic and polymeric nanoparticles are used to encapsulate drugs within the solid core. The use of metals can yield multifunctional nanoparticles whereby both therapeutic delivery and imaging are facilitated. [2]

Nano Silver may have different shapes, such as spheres, rods, cubes etc., at nano scale, the silver particles exhibit deviating physicochemical properties and biological activities compared with the regular metal. This is due to the higher surface area per mass, allowing a large amount of atoms to interact with their surroundings [3]. Due to the properties of silver at the nanoscale, nano-silver is now a day's an increasing number of consumer and medicinal products.

As the chitosan and silverhave multifunctional properties, the AgNPs reduced withchitosan may be a better therapeutic agent to treatinflammation. Silver nanoparticleshave anti-inflammatory and antibacterial property. With anti-inflammatory drug silver nanoparticles produces the synergistic effect to the inflammatory site [4]

Chitin is known biodegradable natural polymer based on polysaccharides,which is obtained from crustacean shell (e.g. crab, shrimp, and lobster), someinsect (e.g. true fly, Sulphur butterfly) and fungi like yeasts and plants.Chitosan is obtained from partial deacetylation of chitin. Chitosan is a

linearpolysaccharide comprising copolymers of randomly distributed -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine(acetylated unit). Chitosan a natural polysaccharide that has uniquepolycationic, chelating, film forming, reducing, stabilizing properties andvarious biological properties such as anti-inflammatory, anti-proliferative,anti-oxidant, antimicrobial and anticholesterolemic etc., Chitosan is anabundant, naturally occurring polymer with versatile functionalities [5].

MATERIAL AND METHODS

Materials:

Diclofenac, Sodium was obtained as gift sample from Yarrow chem. Products Mumbai. Chitosan 85% deacetylated was obtained as gift samplefrom Central Institute of Fisheries Technology, Kochi.Silver Nitrate (99%) , was purchased from Sigma Aldrich(Analytical Grade). Deionized water was used in thisexperiment. All the other chemicals were of analyticalgrade.

Synthesis of Silver Nanoparticles:

0.1 % chitosan solution was preheated at 80°C±2°C for 30 minute. Aqueous solution of 0.1M silver nitrate was added to above solution and kept on mechanical stirrer until solution get change to yellowish brown colour, it confirms that the formation of silver nanoparticle. Silver nanoparticles collect by centrifugation. Residue washed with isopropyl alcohol, dried at 45°C in hot air oven for 15 minutes [6].

CHARACTERIZATION:

Fourier Transformation Infrared Spectroscopy:

FTIR spectrum of Diclofenac and Diclofenac loaded silver nanoparticles were recorded using Nicolet Fourier transformation Infrared spectroscopy (FT-IR) combined to PC (with spectrum 2000 analysis software) in the range of 4000 cm-1 to 400 cm-1.The particles was placed in the light path and the spectra were analyzed. [7]

Drug loading Efficiency:

Surface adsorption of Diclofenac sodium on the silver nanoparticles was determined by measuring the amount of free drug present in the supernant solution [8].

Drug loading efficiency of Nanoparticles is calculated by the following formula,

$$\text{Loading efficiency} = \frac{\text{Total amount of Drug} - \text{Free drug} \times 100}{\text{Total amount of drug}}$$

Particle size analysis:

Particles size of optimized nanoparticles was measured by Malvern Zetasizer (version 6.32). The Nano-suspension of AgNPs was diluted to 10 fold with water and transferred to sample holder to get the actual particle size.[9]

Scanning electron microscopy:

The optimized nanoparticles were photographed using scanning electron microscope. The sample is smeared on a small piece of adhesive carbon tape. This is fixed on a brass stub. Then sample was subjected to gold coating using sputtering unit (model-JFC1600) for 10 sec at 10 mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390 LA) and secondary images are recorded. [10]

In-vitro drug release study:

The Franz diffusion cell consists of two parts; the upper part that is donor compartment with semi permeable membrane; the bottom part contains the receptor solution, the water jacket for temperature control, and the sampling port. The effective permeation area of the diffusion cell and receptor and cell volume was 1cm2 and 20ml, respectively. The temperature was maintain at 37±2°C. the receptor compartment contained 20ml of phosphate buffer IP PH 7.2 stirred by magnetic stirrer. The permeability studies were carried out semi permeable membrane. Sample 5ml were withdraw and replace with the same volume of fresh receptor solution, to the sampling port of the diffusion cell predetermine time intervals till 6hrs.the absorbance of withdrawn samples were measured at 276 nm for Diclofenac sodium [7]. Find out the %Cumulative Drug release of each formulation.

RESULTS AND DISCUSSION

FTIR Spectroscopy:

FTIR spectroscopy was carried out to study the compatibility of pure drug Diclofenac sodium with the Chitosan.

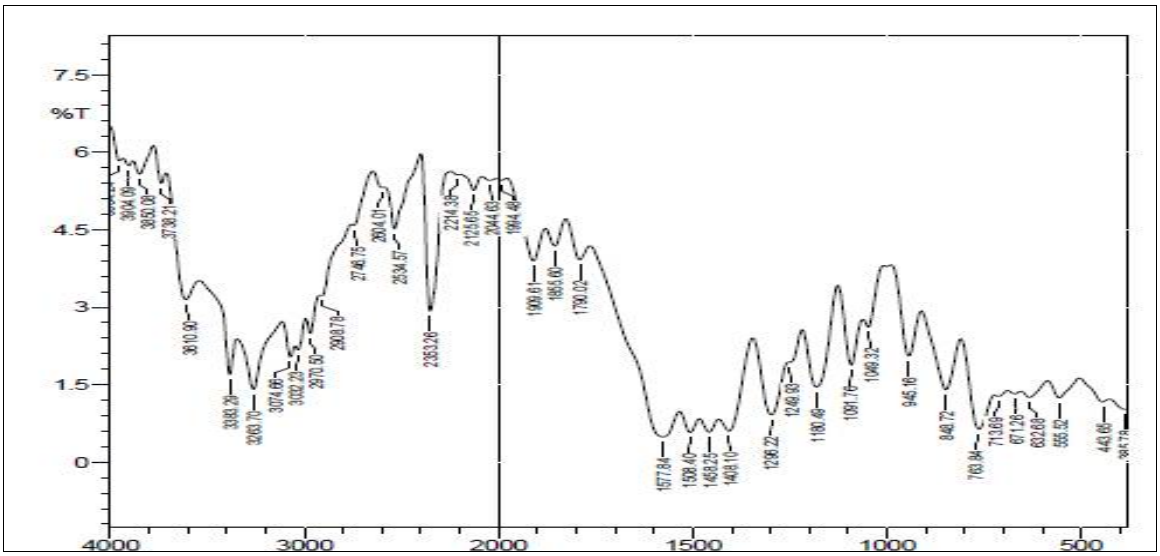


Figure no: 01 FTIR spectrum of Diclofenac sodium

Table no: 01IR Interpretation of Diclofenac sodium

Wave Number (cm^{-1})	Interpretation
3383.29	-NH
2353.26	C=C
1296.22	C-O stretching of carboxylic acid
1790.02	C=O stretching of carboxylic acid
1180.49	C-N

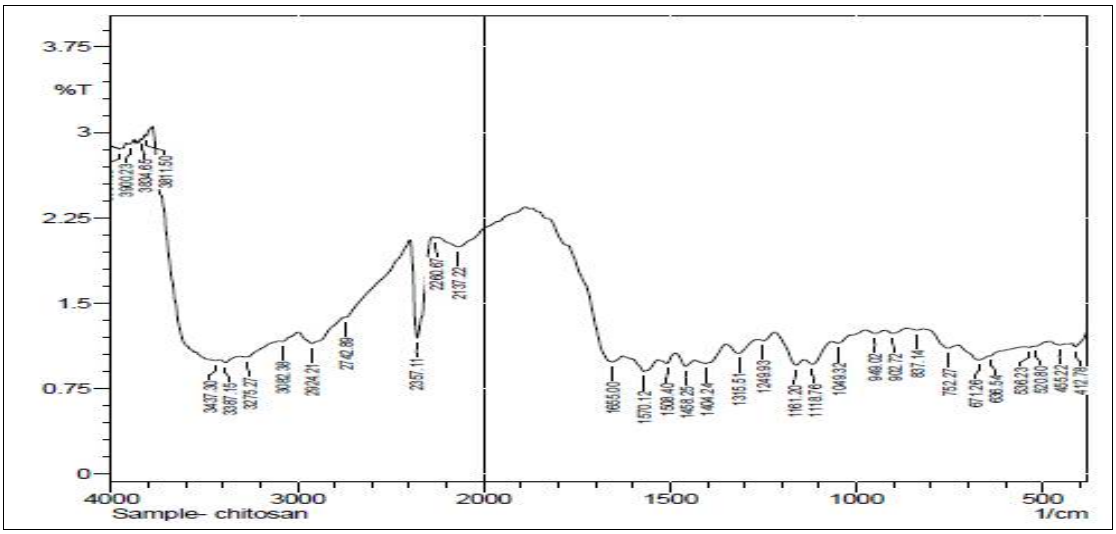


Figure no:02 FTIR spectrum of Chitosan

Table no: 02 IR Interpretation of Chitosan

Wave Number (cm^{-1})	Interpretation
2924.21	-CH
1161.20	C-O-C
3387.76	-OH
1118.76	C-C
3437.30	-NH

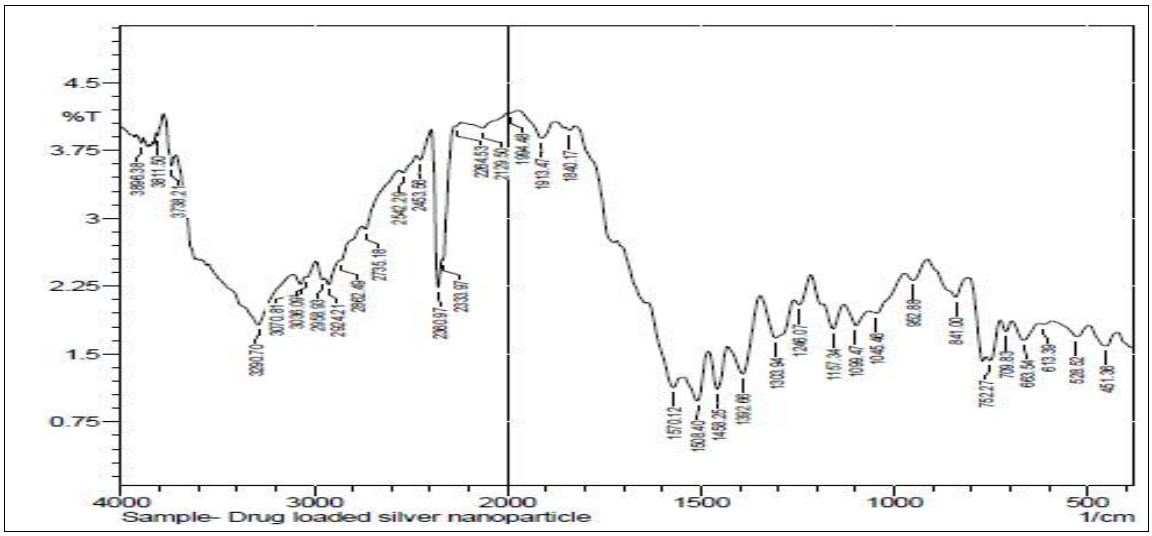


Figure no: 03 FTIR spectrum of Diclofenac loaded silver nanoparticle

Table no: 03 IR Interpretation of Diclofenac loaded silver nanoparticle

Wave Number (cm^{-1})	Interpretation
2360.97	C=C
1246.07	C-O stretching of carboxylic acid
1570.12	C=O stretching of carboxylic acid
1157.34	C-N
3263.70	-OH

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the silver nanoparticles and other Excipients.

Drug loading efficiency:

On formation silver nanoparticles which are confirmed by change in colour (yellowish brown). Formulations are filtered and collect the filtrate to determine the free drug content present in supernant liquid. 1 ml of filtrate is subjected to UV spectrophotometer to check the absorbance and calculate % drug loading efficiency.

Table No: 04 Drug loading efficiency.

Sr no.	Batches code	Practically drug loaded (mg)	Percent drug loaded (%,w/w)
1	F1	34.87	69.74
2	F2	35.62	71
3	F3	35.17	70.35
4	F4	32.85	65
5	F5	38.38	76
6	F6	38.93	77.87
7	F7	42.94	85
8	F8	34.93	69.87
9	F9	35.10	70.20

Surface adsorption of Diclofenac sodium on the silver nanoparticles was determined by measuring the amount of free drug present in the supernant solution. Actual drug loading was found in the range of 65 to 85 %. The highest drug loading was found in batch F7.

Particle size analysis:

Particles size of optimized nanoparticles was measured by Malvern Zeta sizer (version 6.32). The nano-suspension of AgNPs was diluted to 10 fold with water and transferred to sample holder to get the actual particle size. Particle size of prepared silver nanoparticles was found in the range of 90.49 to 140.05 nm. From the results obtained batch F1 showed lowest particle size, hence it is selected as optimized formulation.

Table no: 05 Particle sizes of optimized silver nanoparticles.

Sr no.	Batches code	Particle size (nm)
1	F1	90.49
2	F2	94.93
3	F3	114.18
4	F4	118.24
5	F5	121.08
6	F6	126.37
7	F7	128.09
8	F8	132.33
9	F9	140.05

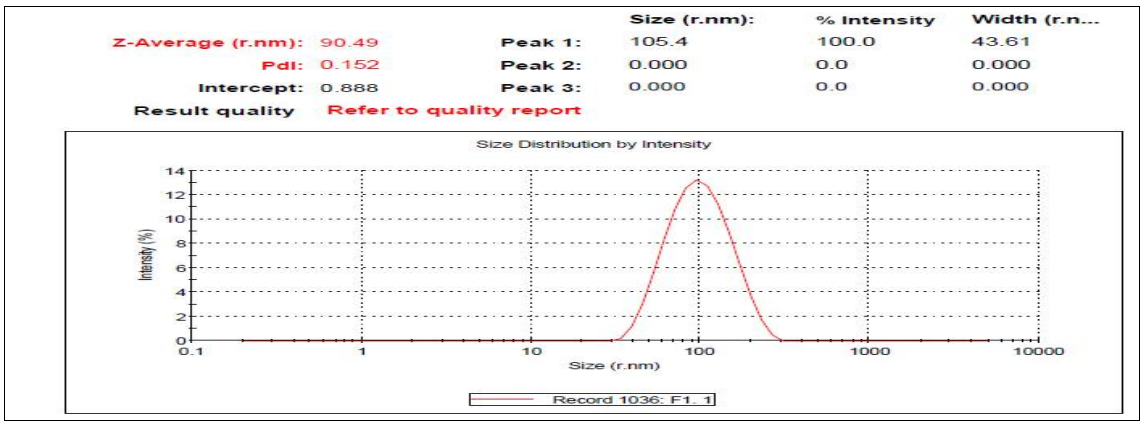


Figure no: 04 Particle size of batch F1.

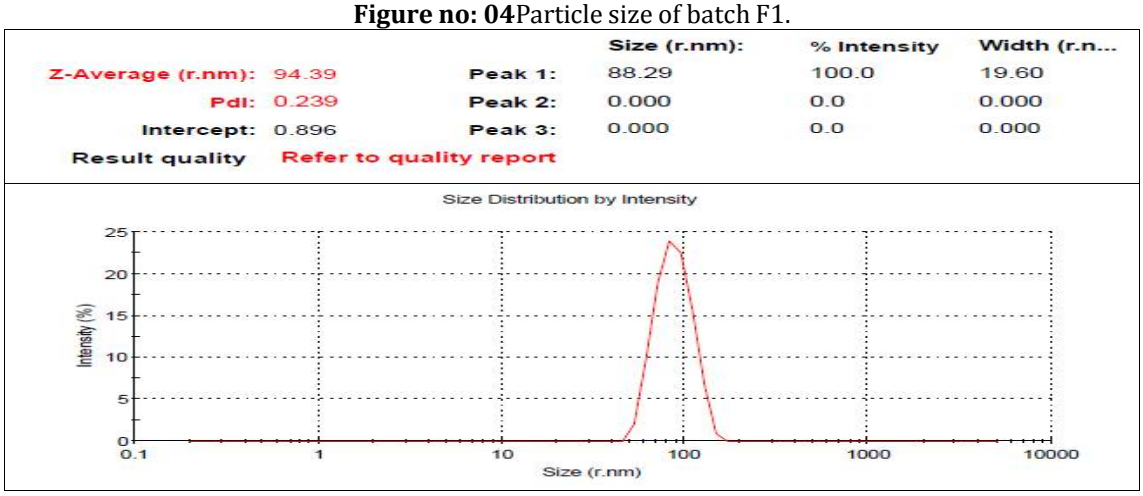
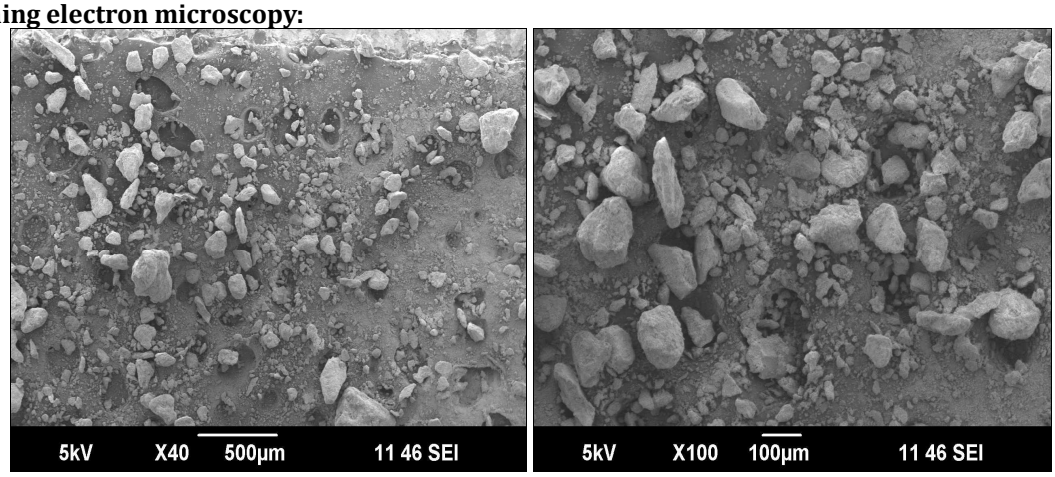


Figure No: 05 Particle size of batch F2.

Scanning electron microscopy:



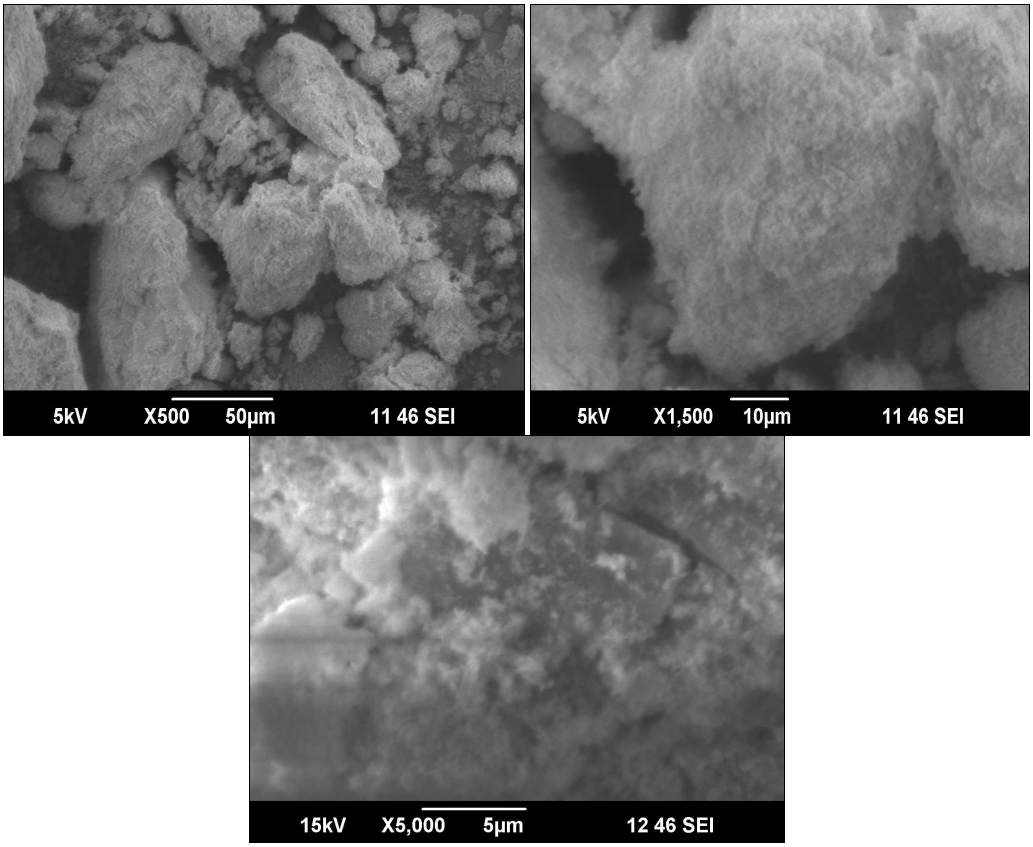


Figure No: 06SEM analysis of diclofenac loaded silver nanoparticle at different magnification.

***In-vitro* drug release study:**

Table no: 6 *In-Vitro*% Drug Release of Batches, F1-F9.

Time in Hrs	Cumulative % drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.298	2.727	1.941	4.298	4.084	1.227	1.941	2.084	0.513
4	7.635	8.135	5.446	9.156	11.124	5.839	6.803	12.538	11.874
6	13.679	14.293	12.782	16.075	21.342	13.257	14.064	16.800	18.785
8	19.248	19.844	18.023	20.697	22.144	17.734	20.126	22.997	23.611
10	25.445	27.227	24.063	26.480	28.348	26.463	26.309	27.795	28.780
12	40.780	42.205	39.159	38.041	43.883	36.738	38.609	44.533	41.148

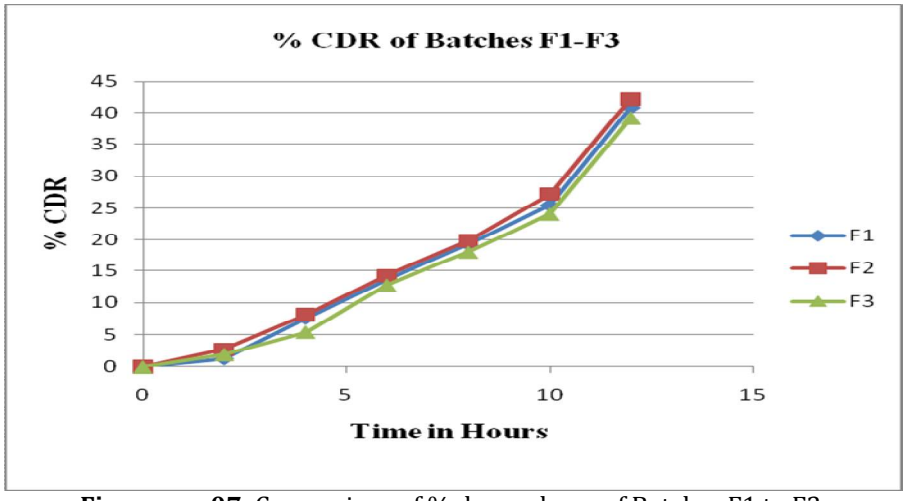


Figure no: 07: Comparison of % drug release of Batches F1 to F3.

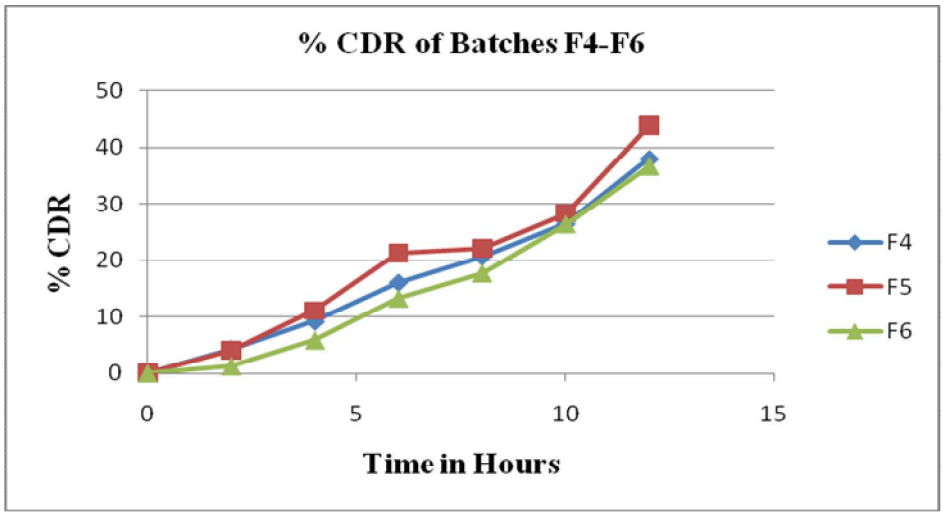


Figure no: 08 Comparison of % drug release of Batches F4 to F6.

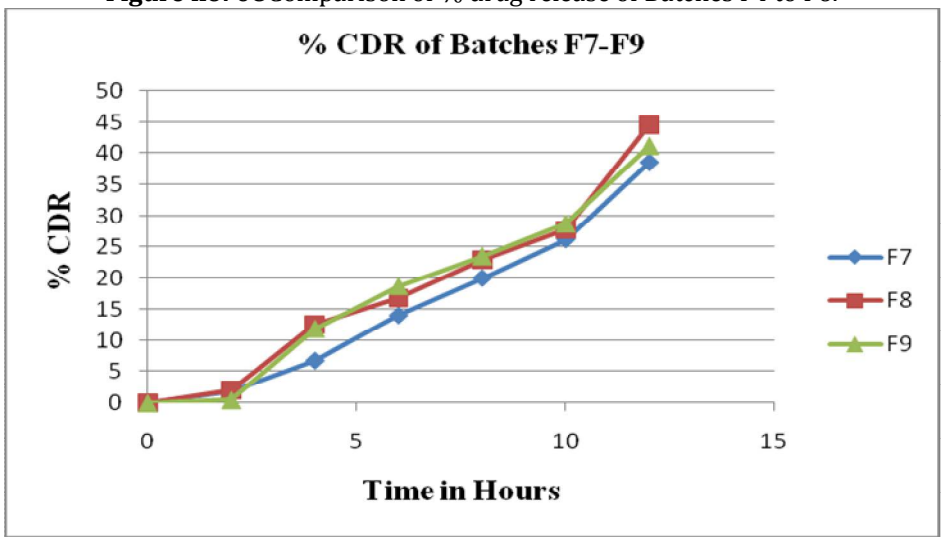


Figure no: 09: Comparison of % drug release of Batches F7 to F9.

In vitro drug release of diclofenac loaded silver nanoparticles showed release in pattern of sustained release, from all the batches drug release was found in the range of 36.73 to 44.53 %.Therefore F1 was selected as optimized batch.

CONCLUSION

The silver Nanoparticles were prepared using environmental benign natural polymer Chitosan (85% deacetylated). The optimum concentration of silver nitrate required to form silvernanoparticles was found to be 0.1M AgNO3. In controlled heating technique, the stable silver nanoparticles were formed at 80ºC±2ºC for 3 hours. As the chitosan and silver have multifunctional properties, the AgNPs reduced with chitosan may be a better therapeutic agent to treat inflammation/inflammatory sites. Silver nanoparticles have anti-inflammatory and antibacterial property. Chitosan is used as anti-inflammatory, antioxidant, reducing and stabilizing agent. The chitosan stabilized silver nanoparticles produce synergistic effect to treat inflammation.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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