International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

DEVELOPMENT OF SURFACTANT FREE NANOPARTICLES BY A SINGLE EMULSION HIGH PRESSURE HOMOGENIZATION TECHNIQUE AND EFFECT OF FORMULATION PARAMETERS ON THE DRUG ENTRAPMENT AND RELEASE

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ABSTRACT

The aim of this study was to prepare surfactant free Diclofenac sodium loaded ethyl cellulose nanoparticles by O/W single emulsion solvent evaporation high pressure homogenization technique.In the preparation of O/W single emulsion sodium alginate used as an emulsifying/stabilizing agent. Microparticles of nine different batches at different condition were prepared after that optimized batch was subjected to the high pressure Homogenizer for 3 cycles at 300 bar for the preparation of nanoparticles. The size of the nanoparticles was obtained in the range of 147.7nm to 256.6 nm .The maximum drugs loading of nanoparticles found about 90% and it showed about 66% of drug release in 12 hours. Physicochemical properties of these nanoparticles such as particle size, FESEM, X-RD and FTIR analysis were investigated. No drug-polymer interaction was observed in FTIR. FE-SEM images show that the particles are spherical and nanosized.

Keyword: Diclofenac sodium, High pressure Homogenizer, Polymer, O/W emulsion.

INTRODUCTION

Polyvinyl alcohol (PVA), Span series or Tween series, poly (ethylene oxide) (PEO), and poloxamer (PEO-poly (propylene oxide) block copolymer), etc. are used as surfactant/stabilizing agent For the synthesis of micro/nano particles in most of the cases[1-4]. It was known that PVA existed on the surface of PLGA micro/nanoparticles change the biodegradability, biodistribution, as well as the drug release behavior of micro/nano particles.[5-8]. Almost of these surfactants are non-digestible, non-biodegradable, and not always biocompatible. Also, these surfactants can affect to human body sometimes shows an allergy-like reactions [9]. Thus, need washing from the micro/nano particles [10]. It is easy to wash from the microparticles but in the case of nanoparticles it becomes difficult.

There are lots of natural polymers like sodium alginate, gelatin, pectin which can be used asstabilizing agent [11] alternative to PVA and others, in the synthesis of micro/nano particles. Sodium alginate is a naturally occurring biocompatible and biodegradable polymer, obtained from brown seaweed, *LaminariaHyperborean, Ascophyllumnodosum, and Macrocystispyrifera*[12,13]. The solvent evaporation method has been used to prepare biocompatible and biodegradable/non-biodegradable polymer micro/nano particles like PLGA, PLA, ethylcellulose, acrylate polymers etc..[14,15,11]. In O/W single emulsion solvent evaporation method, the polymers are dissolved in a suitable water miscible organic solvent, and active pharmaceutical ingredient is dissolved in this polymeric solution. This is emulsified in aqueous continuous phase containing surfactant/stabilizing/emulsifying agent [16, 17]. In the preparation of micro/nano particles several parameters have been identified which influence the property of the micro/nano particles, including drug solubility, types of solvent, rate of diffusion of the solvent, temperature, polymer composition, the nature of the polymers, viscosity and some cases pH of the external phase[18]. The effectiveness of the

O/W single emulsion solvent evaporation method to produce high entrapped API, microsphere and thus, this process is most successful with the drug either insoluble or poorly soluble in the external phase which comprises water in the case of O/W emulsion.
In the present work, we have prepared microparticles using O/W single emulsion solvent evaporation technique, and sodium alginate as an emulsifying/stabilizing agent in the external phase. The optimized microparticle batch was further subjected to high pressure Homogenizer for the preparation of nanoparticles. The effect the process parameters, the effect of pH of external solution and viscosity on drug loading were investigated. The effect of the drug to polymer concentration on drug loading as well as drug release was also investigated

MATERIALS AND METHODS

Materials: Diclofenac Sodium was obtained as a gift sample from Natco Pharma Pvt. Ltd; Hyderabad, India. Ethyl cellulose (20cp) purchased from LobaChem Pvt. Ltd. India. Sodium alginate was purchased from HIMEDIA Laboratories Pvt. Ltd., Mumbai, India. Dichloromethane (DCM) and Methanol was purchased from Merck Specialities Pvt. Ltd, Mumbai and RFCL Limited New Delhi, India respectively. All other chemicals were used of analytical grade.

Preparation of nanoparticles: Diclofenac sodium loaded ethyl cellulose nanoparticles were formulated by single emulsion (O/W) solvent evaporation and high pressure homogenization technique. Firstly the microparticles with Different drug to polymer ratio. 1:1, 1:2, 1:3 were prepared. For this purpose the drug and polymer was dissolved in a mixture of methanol and DCM each of 15 ml. and added drop wise into the aqueous sodium alginate solution (100 ml) under stirring. Remove the organic solvent and characterized for drug loading & drug release. The highest drug loaded and the desired drug release batch was subjected to high pressure Homogenizer(GEA Niro Soavi model Panda Plus) for three cycles at 300 bar for the preparation of nanoparticles. After that stirred for 3 h under lab stirrer and lyophilized (Labogene, SCANVAC Cool Safe) the naoparticles for 72 h.

Viscosity study: Three stock solutions (0.2%, 0.6% and 1%) of sodium alginate were freshly prepared at room temperature in de-ionized water and pH was adjusted to 3.9. Viscosity measurement was carried out using cone and plate type Viscometer (Brookfield RSR/S plus). A single drop of specific concentration of aqueous sodium alginate was transferred to the plate of Viscometer and measured the coefficient of viscosity shown in table 1[19].

CHARACTERIZATION

Encapsulation efficiency determination: For determination of encapsulation efficiency the micro/nanoparticles having 20 mg equivalent of the drug were added into the 10 ml of DCM to dissolve the ethyl cellulose coat and subsequently added phosphate buffer (10 ml) of pH 6.8, stirred it to remove the DCM. The dispersion was filtered and analyzed under UV spectrophotometer at 276 nm (Hitachi U 2900) and drug content was determined using pre-estimated calibration curve. The percent drug encapsulation efficiency of micro/nanoparticles was calculated using the following equation:
% Encapsulation efficiency = [drug content /equivalent drug] ×100

In-vitro release study: *In- vitro* dissolution study was performed using eight stations fully calibrated dissolution test apparatus USP type II (TDT 06T, Electrolab India.),initially for 2 h in an acidic media (pH 1.2) and after 2 h media was replaced by fresh phosphate buffer (pH 6.8). The prepared nanoparticles equivalent to 50 mg of the drug were filled into the dialysis membrane (Himedia), to this, 10 ml of phosphate buffer having pH 6.8 was added. The release of drug from formulation was determined by using standard the samples were withdrawn at 30, 60, 90, 120, 180 min and then at 60 min interval up to 720 min. and Diclofenac sodium content was determined spectrophotometrically at 276 nm. The initial volume of dissolution fluid was maintained by adding the same volume of fresh dissolution fluid after each withdrawal to maintain the sink condition.

Fourier transform Infrared (FTIR) spectroscopy: The chemical structure of nanoparticles, pure Diclofenac sodium, and polymer was analyzed by FTIR (Schimadzu FTIR-8400) in transmission mode. The sample was prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was used from 4000 to 400 cm^{-1} [20].

X-ray diffraction: In order to confirm the crystalline or amorphous nature of pure drug, polymer and nanoparticles were subjected to X-ray diffractometer (Bruker, D-8 advance). The data collection was performed using Cu anode and a voltage of the monochromator at 40 kV. The diffraction pattern was determined in the area $3^0 < 2\Theta < 80^0$ using continuous scan.

Particle size determination: The average mean diameter of Diclofenac nanoparticles was determined by the Malvern particle size analyzer (Zetasizer Ver. 6.34) at room temperature. For measurement of particle size of nanoparticles were diluted with de-ionized water and the size of nanoparticles were evaluated.

Field emission Scanning electron microscope (SEM): [21] The surface morphology of nanoparticles was observed by Field emission scanning electron microscope (HITACHI, Model-S4800 type II). For the surface morphology, nanoparticles were mounted on metal stub using double sided adhesive tape and coated with gold for 80 second under vacuum, this operation was performed at 5.0-10 kV.

RESULT AND DISCUSSION

This study was carried out to investigate and feasibility of preparing Diclofenac sodium loaded ethyl cellulose nanoparticles by emulsion solvent evaporation technique with the different drug-polymer ratio [22,23]. In this study, the several parameters such as emulsifying/stabilizing agent concentration (viscosity of external aqueous phase) and pH of external medium on drug encapsulation efficiency and drug release on Diclofenac loaded nanoparticles were investigated. Spherical shape of particles can be seen in the Fig 1A and 1B.

Effect of pH on drug loading: As the pH changes from 6 to 3.9 the ionization property of Diclofenac changes[24]. At pH 6 Diclofenac sodium was in ionized form, which shows that its aqueous solubility at higher pH is larger, due to this, high amount of drug is loss in external aqueous phase at above pH 5., After, evaluation of results of all 9 batches (microparticles). It reflects that the encapsulation efficiency significantly increases with decrease in pH (Table 2), Whereas at pH 3.9 show higher encapsulation efficiency because of the less solubility of DS, due to which, no leaching of drug into external phase take place during Micro/nanoparticle preparation.

Effect of Viscosity (sodium alginate concentration) on drug loading: The effect of viscosity of sodium alginate in external phase was studied. From the figure 2 we can conclude that the viscosity of external phase increases from 1.868 Pas to 2.392 Pas the encapsulation efficiency also increases, in graph 'C' we studied the effect of different viscosity for the batches having polymer concentration 750 mg (i.e. 1:3 drug: polymer ratio). But drug release increases only up to a certain value (i.e. 2.092 Pas) after that at 2.392 Pas the graph indicates that the rate of drug release decreased. Batch no. 18 shows highest encapsulation efficiency (90.15 %) and rate of release of drug is low (52.36%) shown in Table no. 2

In-vitro Dissolution analysis: *In-vitro* release study of microparticles was carried out are shown in figure 3, which indicate that batch no 18 showed good release property, initially burst release was 37% and nearly 50% drug release was found in 12 h. This was desired result . This batch was further converted into nanopartilces and dissolution test were performed initially for 2 h in an acidic medium (pH 1.2) and after 2 h it was replaced by fresh phosphate buffer (pH 6.8). The prepared Diclofenac nanoparticles equivalent to 50 mg of the drug were filled into the dialysis membrane (Himedia), to this dialysis membrane 10 ml of phosphate buffer having pH 6.8 was added. The release of drug from formulation was determined by using dissolution test apparatus USP type II (TDT 06T, Electrolab India.). Diclofenac sodium content was determined spectrophotometrically at 276 nm after withdrawal of sample at 30, 60, 90, 120, 180 min and then at 60 min interval up to 720 min. The initial volume of dissolution fluid was maintained by adding the same volume of fresh dissolution fluid after each withdrawal to maintain the sink condition. *In-vitro* release pattern of diclofenac sodium nanoparticles is shown in figure 4, nanoparticles shows near about 70% of drug release in 12 hrs.

Fourier transform infrared analysis: Figure 5 showed the FT-IR spectra of diclofenac sodium, ethyl cellulose and nanopartilces. The functional groups responsible for the anti-inflammatory activity is-COO^- showed Stretching vibration at the region 1700-1680 cm^{-1}confirming -C=O and stretching vibration at 1250 cm^{-1}& bending vibration at 1404 cm^{-1}, 920cm^{-1} confirming C-O of COOH group respectively. It is obvious that the most characteristic stretching vibration at 3259 cm^{-1}& bending vibration at 1600-1500 cm-1region and stretching vibration at 1190 cm^{-1} confirming the presence of N-H and C-N respectively at the aromatic ring of Diclofenac sodium. The FTIR spectra of Diclofenac nanoparticles (Fig 2A) shows all characteristic bands of Diclofenac sodium, indicating that no interactions of drug and polymer and the successful loading of the drug into nanoparticles.

X-Ray Diffraction analysis: The nature of entrapped drug is also an important factor to take into consideration in the drug delivery system, there may be transitions from amorphous to crystalline structure occurred. These transitions may affect the drug

release. For this purpose, X-RD study done to show the physical nature of encapsulated material [25]. The graph depicted in figure 6 shows the X-RD pattern of a) Diclofenac sodium b) Ethyl cellulose and c) Diclofenac nanoparticles. From figure 6 B observed that ethyl cellulose is an amorphous polymer (57.7 % amorphous) where as Diclofenac sodium is crystalline in nature (93.4% crystalline). The figure 6C clearly demonstrated that the obtained nanoparticles are (38.1%) amorphous in nature, which indicates homogeneous distribution of drug into polymer.

Particles size analysis: Reduction of particle size diameter down to nanometer range has known to increase the dissolution rate [26]. The particle size of Diclofenac sodium Nanoparticles was investigated by the particle size analyzer and it was found that most of the particles are in the range of 147.7nm to 256.6 nm (Figure 7).

CONCLUSION

From this study we can conclude that the viscosity of external aqueous phase in O/W solvent evaporation method was affecting an encapsulation efficiency as well as drug release of nanoparticles. The increase in viscosity of an external phase shows increase in encapsulation efficiency but In the case of drug release, an increase in the viscosity upto a certain level increases the drug release rate but after that decrease the rate of release. pH of the external phase linearly affects the encapsulation efficiency as the pH of an external phase decreases from 6 to 3.9 encapsulation efficiency increases.

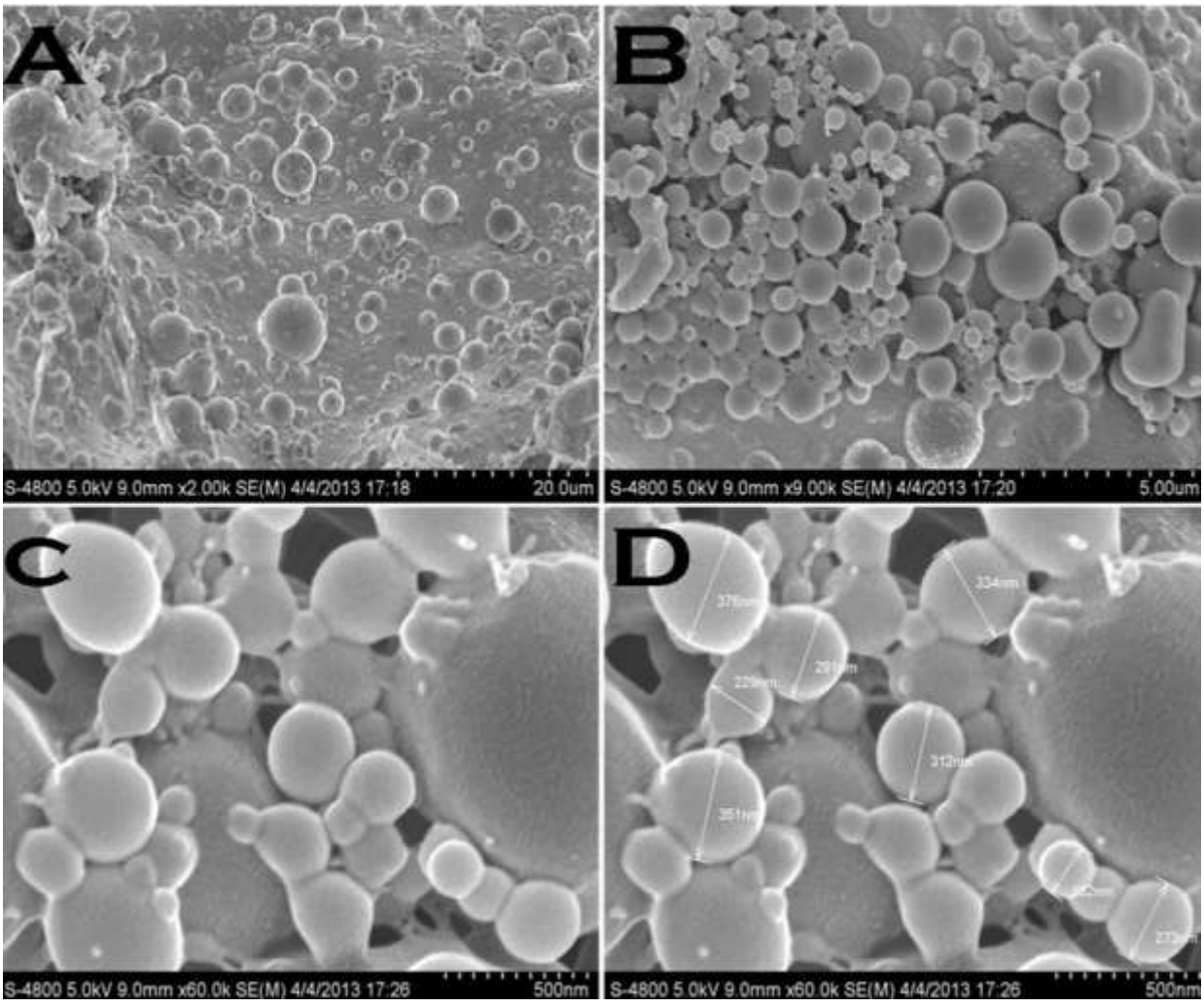

Figure I: FE-SEM image of Diclofenac nanoparticles.

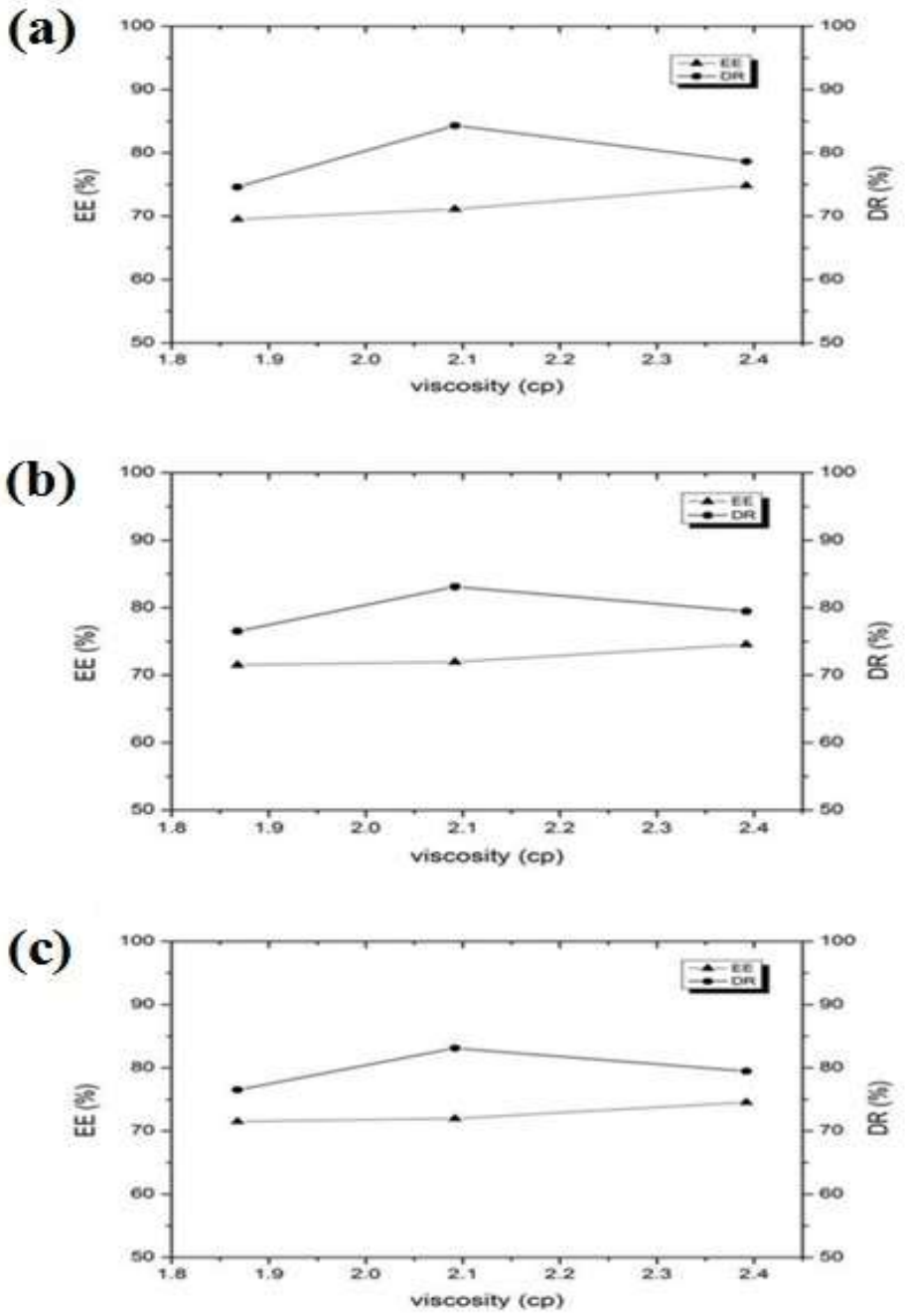

Figure II: Effect of viscosity on percent encapsulation efficiency and drug release a) effect of viscosity of batches having polymer concentration 250 mg (i.e. 1:1 drug : polymer ratio) b) effect of viscosity of batches having polymer concentration 500 mg (i.e. 1:2 drug : polymer ratio) c) effect of viscosity of batches having polymer concentration 750 mg (i.e. 1:3 drug : polymer ratio)

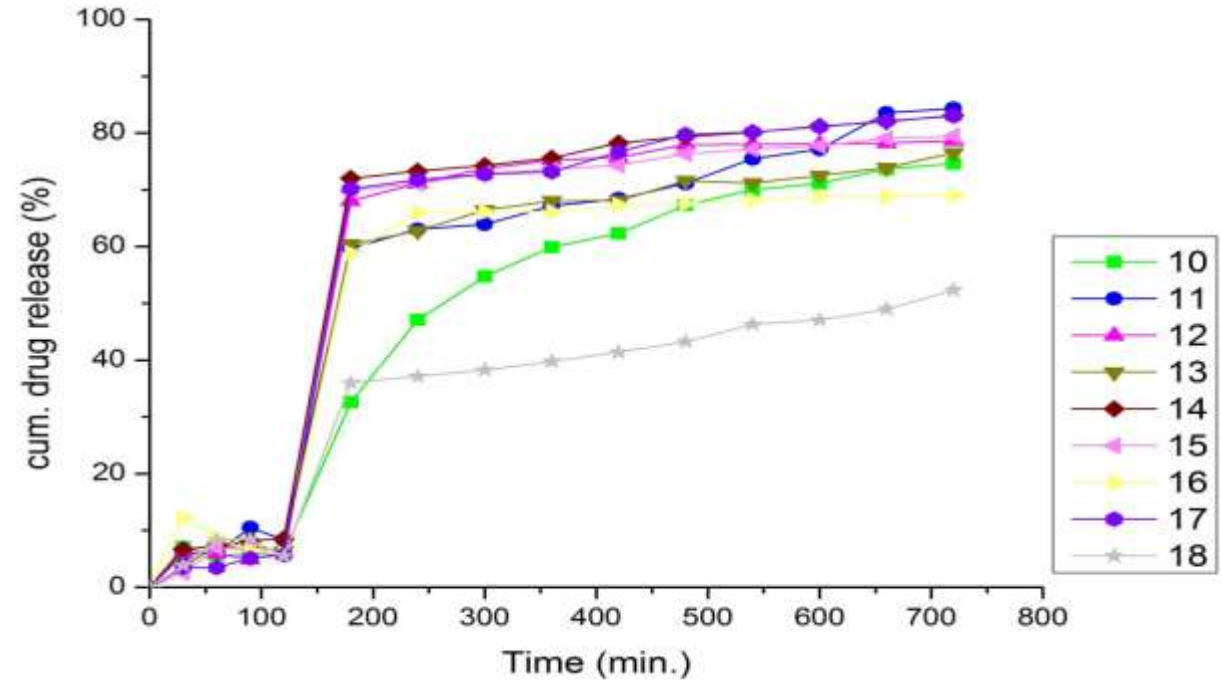

Figure III: *In-Vitro* dissolution of 9 batches of microspheres showing sustained release.

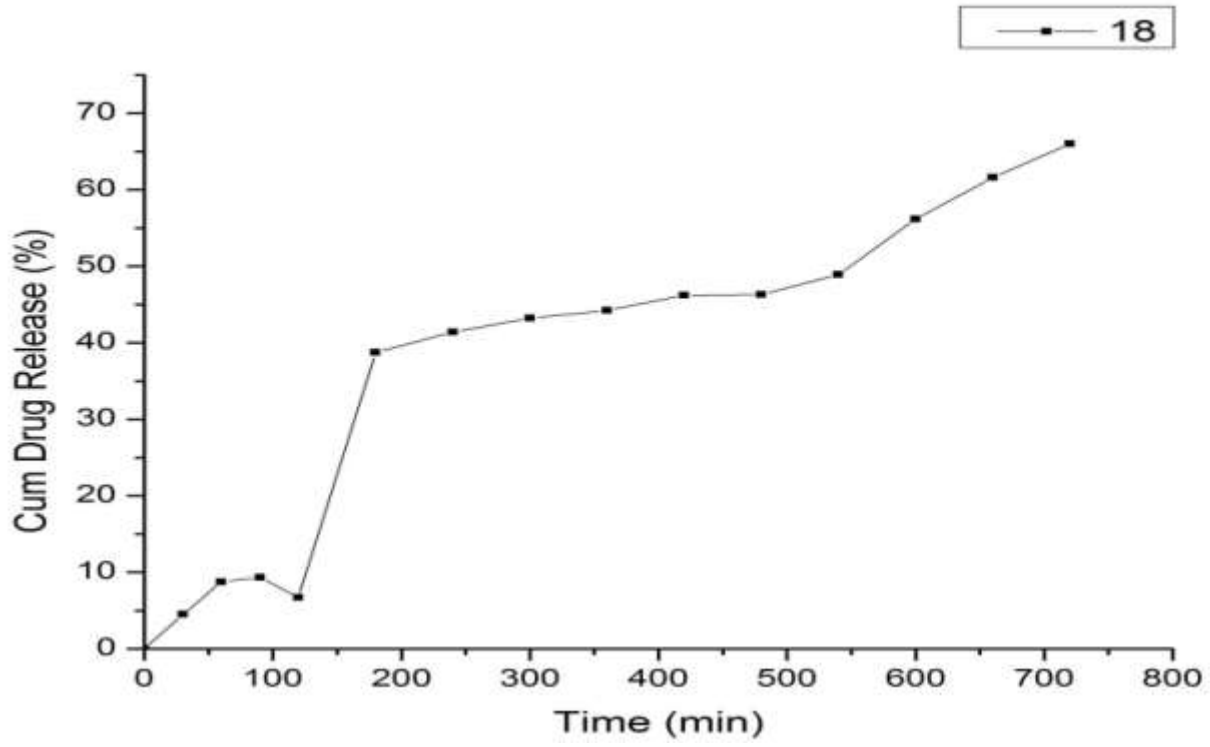

Figure IV: *In-Vitro* dissolution of optimized nanoparticles of Diclofenac.

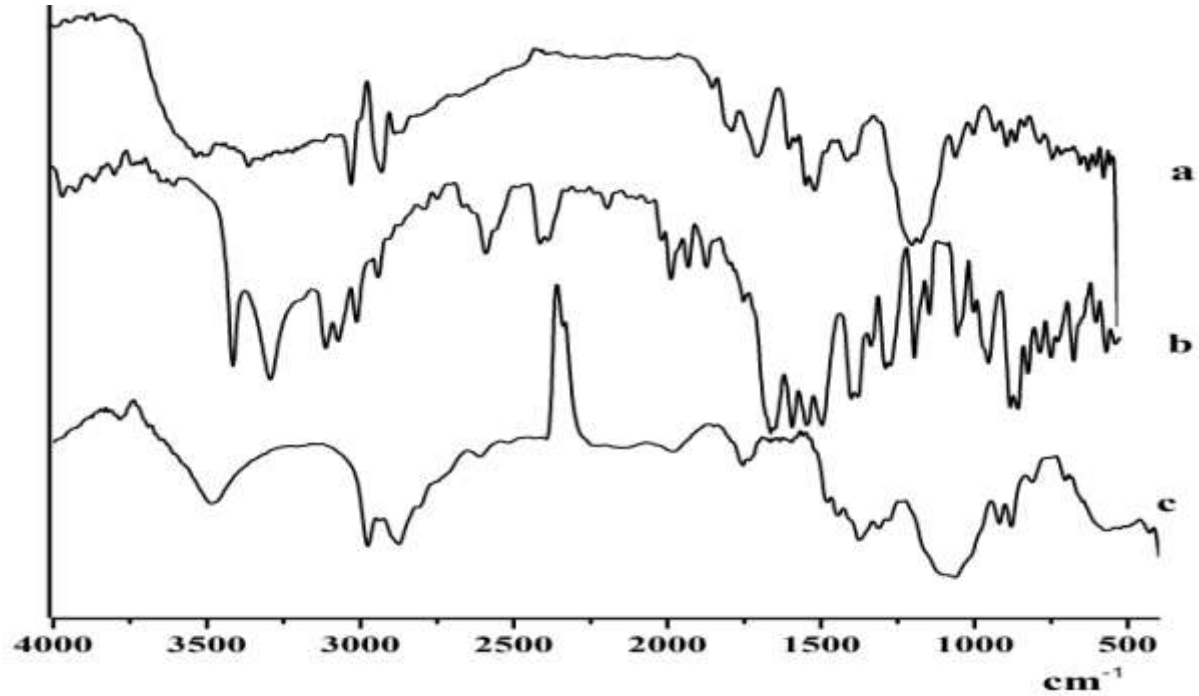

Figure V: Infrared spectroscopy of (a) Diclofenac nanoparticles , (b) Pure Diclofenac, (c) Ethyl cellulose.

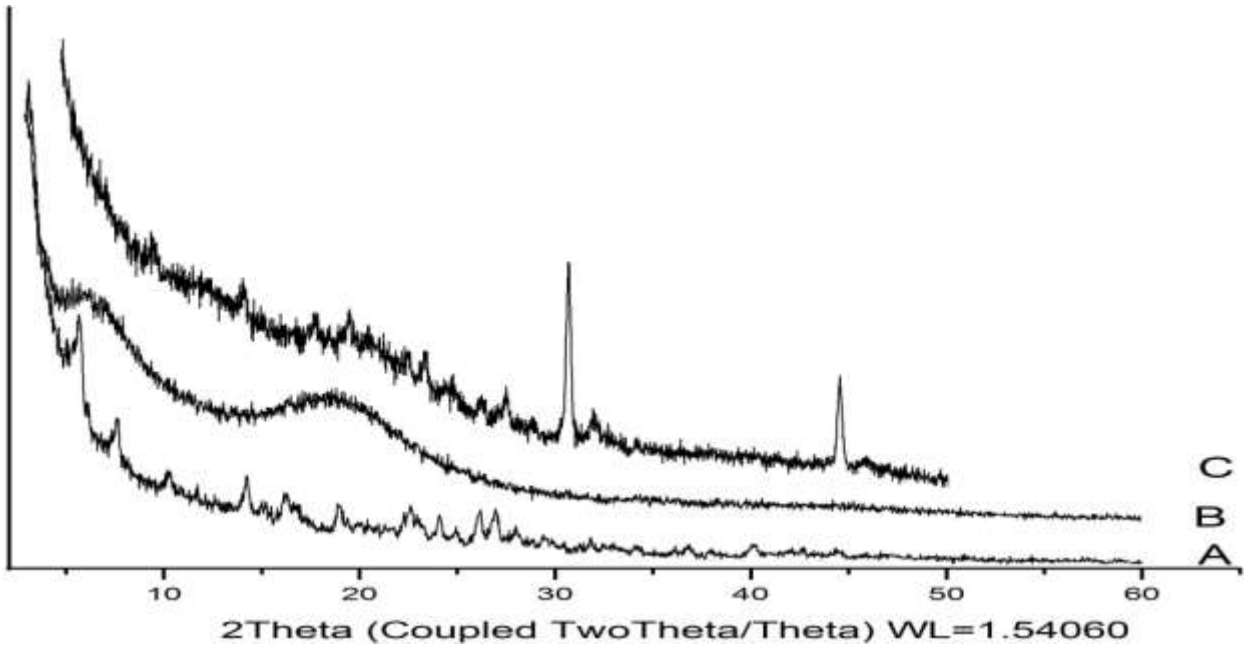

Figure VI: X-RD pattern of A) Diclofenac sodium B) Ethyl cellulose C) Diclofenac nanoparticles.

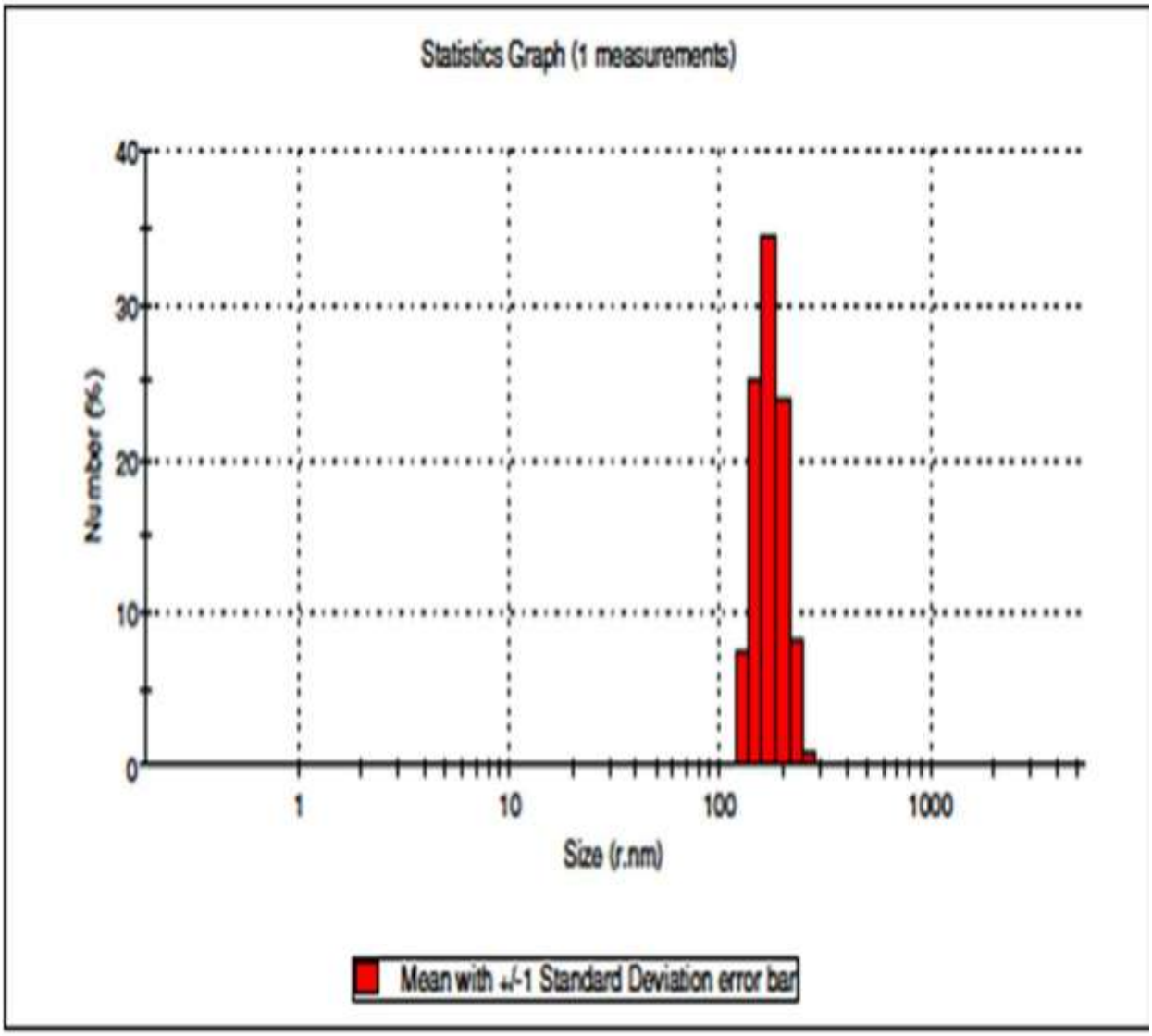

Figure VII: Particle size analysis of Diclofenac nanoparticles.

Table 1: Viscosity of aqueous sodium alginate solution at different concentration (0.2%, 0.6%, and 1%)

Concentration (Sodium alginate)	Coeff. Of viscosity (Pas)	Torque (pa)	Temp. (^{0}C)
0.2%	1.868	560.315	31.3
0.6%	2.092	627.602	31.3
1%	2.392	717.490	31.3

Table 2: Effect of PH adjustment and viscosity on Encapsulation efficiency and % Drug release of Diclofenacmicroparticles

Batch No.	Drug/Polymer Ratio	pH	Con. Of sodium alginate (%)	Encapsulation efficiency	% Drug Release
1.	1:1	5.86	0.2	37.75	-
2.	1:2	5.84	0.6	51.66	-
3.	1:3	5.90	1	48.93	-
4.	1:1	5.81	0.6	33.63	-
5.	1:2	5.93	1	36.81	-
6.	1:3	6.0	0.2	41.66	-
7.	1:1	5.76	1	46.51	-
8.	1:2	5.85	0.2	48.48	-
9.	1:3	5.99	0.6	50.30	-
10.	1:1	3.99	0.2	69.54	74.61
11.	1:2	3.97	0.6	71.12	84.32
12.	1:3	3.9	1	74.84	78.65
13.	1:1	3.96	0.6	71.51	76.52
14.	1:2	3.9	1	71.96	83.12
15.	1:3	3.92	0.2	74.54	79.47
16.	1:1	3.99	1	71.36	69.05
17.	1:2	3.91	0.2	79.84	83.01
18.	1:3	3.9	0.6	90.15	52.36

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