Research Article

Available Online at: www.ijphr.com

International Journal of Pharmaceuticals and Health care Research

ISSN: - 2306 – 6091

DEVELOPMENT AND EVALUATION OF MICROSPHERES CONTAINING ACECLOFENAC BY UTILIZING NATURAL POLYMERS

*Krishnamoorthy B, Basu S K

Division of Pharmaceutics, Department of Pharmaceutical Technology,
Jadavpur University, Kolkata- 700 032, India.

Abstract

In this present work an attempt was made to develop microsphere systems for sustained release of aceclofenac by employing ionotropic gelation method by utilising sodium alginate, chitosan and carrageenan. The prepared microspheres were characterized for their sizes, shapes and surface morphology by means of scanning electron microscopy (SEM), the physical state of the drug in formulations by X-ray powder diffraction analysis (X-RD), drug polymers interaction was studied by FTIR, entrapment efficiency, in vitro release and release kinetics studies were carried out as well. FTIR studies revealed that the absence of any interaction within or between drug- polymers and upto 99.18% ± 1.04 of entrapment efficiency was observed. The invitro study demonstrates that it is possible to control the release rate of aceclofenac over a wide time scale. The microspheres coated with carrageenan showed better results than the non-coated one and were the best with regards to the effectiveness for prolonged release of the drug.

Keywords: Aceclofenac, Microspheres, Carrageenan, Prolonged release.

Introduction

Microspheres made from natural products (e.g., polysaccharides), in the form of microparticules or microcapsules, have been proposed as advantageous delivery carriers for the controlled release of active compounds.[1-2] The micro-particulate carrier can be designed for use in various routes of administration as well as for the controlled and targeted release of drugs. This customized performance of the carrier leads to an enhanced efficacy of delivery, reduced toxicity, and improved patient compliance. Emulsification /internal gelation have been suggested as an alternative to extrusion/external gelation in the encapsulation of several compounds including sensitive biological such as anti inflammatory drug. An emulsification/internal gelation method is proposed for producing small diameter alginate microparticules in large quantity. The production procedure of chitosan microparticle based on external gelation, i.e., the mixture of chitosan and drug is extruded drop-wise through a needle into a gelling bath containing divalent cations to form gelated chitosan microparticules.

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line drugs in the relief of mild to moderate pain, acute and chronic inflammatory disorders such as rheumatoid

Author for Correspondence:
Krishnamoorthy B,
Division of Pharmaceutics,
Department of Pharmaceutical Technology,
Jadavpur University, Kolkata- 700 032, India.
Email: bkrishmoothy2004@gmail.com

arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac, phenyl acetic acid derivative related to diclofenac, is one of the widely used NSAIDs [3, 4] hence in the present study, it was attempted to use the non-steroidal anti-inflammatory drug Aceclofenac has been chosen as model drug. Therefore, the goal of this study was to prepare, optimize and fully characterize aceclofenac loaded chitosan microspheres.

Materials & Methods

Aceclofenac was received as a gift sample from Dr.Reddys Lab, Hyderabad; Chitosan powder was gifted from India Sea Foods, Cochin, Kerala; Sodium alginate procured from Fluka Chem, Buchs and Carrageenan from Sigma, USA; Lactic acid from Merck Limited, Mumbai; and all other chemicals and solvents were of analytical grade satisfying pharmacopoeial specifications.

Formulation of chitosan Microparticules

Method: 1 Chitosan coated alginate microspheres

1 to 4% (w/v) of sodium alginate was dissolved in double distilled water at a various concentrations (Table 1). Aceclofenac was slowly dispersed in the sodium alginate solution with constant stirring. The gelation medium was prepared by mixing equal proportion of $CaCl_2$ solution (0.5-2%w/v) with different concentrations of chitosan solution (0.5-2%) already prepared with 2.4% lactic acid and the pH of the medium was adjusted to 4.5 ± 0.1. The homogenous mixture of sodium alginate drug solution was added drop wise into the gelation medium using a 5 ml hypodermic syringe through a needle # 21 under constant stirring at room temperature. The microspheres thus formed were cured in the gelation medium for 4 hr, followed by washing with double distilled water and then allowed to dry at room temperature (25°C) in a dust free chamber till they attained constant weight.

Method: 2 Chitosan coated alginate/ carrageenan microspheres

The various concentrations of sodium alginate (1 to 4% (w/v)) were dissolved in double distilled water. This was mixed with carrageenan solution which was dissolved separately in various ratios (Table 2). Weighed amount of aceclofenac was dispersed slowly in the solution of sodium alginate and carrageenan with constant stirring. Then it was sonicated to remove air bubbles any that may have been formed during mixing. The gelation medium was prepared by mixing equal proportions of $CaCl_2$ solution (0.5-2%w/v) with different concentration of chitosan solution (0.5-2%) previously prepared with 2.4 % lactic acid and the pH of the medium was adjusted to 4.5 ± 0.1. The medium was mixed for 2h before use. The homogenous mixture of sodium alginate, carrageenan and drug was added drop wise into the gelation medium using a 5 ml hypodermic syringe through a needle # 21 under constant stirring at room temperature. The microspheres thus formed were cured in the gelation medium for 4 hr, followed by washing with double distilled water and then allowed to dry at room temperature (25°C) to attained constant weight. The prepared microspheres by both the methods were then stored in air tight container at room temperature for further evaluation.

IR Spectral Study

I.R spectroscopy can be used to investigate and predict any physiochemical interaction between different excipients. *I.R* spectra matching approach was used for detection of any possible chemical interaction between the drugs and polymer. A physical mixture of drug, polymer and other excipients were prepared and mixed with suitable quantity of potassium bromide. This mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It was scanned from 4000 to 400 cm-1 in a FTIR spectrophotometer (FTIR 8400 S, Shimadzu). The IR spectrum of the physical mixture was compared with those of pure drug and polymers and peak matching was done to detect any appearance or disappearance of peaks.

X-Ray Powder Diffractometry [X-RD] Analysis

The X-ray diffraction patterns of pure drug, polymer(s), physical mixture of drug & polymer(s), blank microspheres and drug loaded microspheres were recorded using Miniflex goniometer to investigate the physical state of the drug in the formulations. The instrument was operated at a scanning speed of 1°/ min, over a 2θ angle range of 10-70.

Particle size analysis and Surface morphology studies

Samples of the microspheres were analyzed for particle size by optical microscopy. The instrument was calibrated and one unit of the eyepiece micrometer was equal to 17.5µm. The sizes of

about 50 microspheres were determined for each sample. The shape and surface morphology of the prepared drug loaded microspheres for the drugs investigated were studied by Scanning Electron Microscopy (SEM) using Vega © Tescan, USA.

Determination of Drug loading and Entrapment efficiency[5]

Fifty milligrams of drug loaded microspheres from chitosan coated alginate and chitosan coated alginate/carrageenan microspheres from each batch were dissolved in 100ml of Phosphate Buffer solution of pH 7.4 by shaking on a mechanical shaker for 24hrs. The solution was filtered through Whatmann filter paper. An aliquot with following suitable dilution was assayed spectro-photometrically (UV-Visible spectrophotometer, Shimadzu 1800, Japan) for Aceclofenac at 272nm.

Drug loading efficiency was determined by using the following relationship:

Drug Loading in % = W/W_t x 100 …..…… (Equation 1)

Where,

W = Drug content of the microspheres

W = Weight of the microspheres

Entrapment efficiency was determined by using the following relationship:

$$\text{Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100 \quad \text{(Equation 2)}$$

In-Vitro Drug Release Studies

Each sample (100mg) for release studies consisted of drug loaded microspheres was carried out using a USP XXI dissolution rate test apparatus, in 900 ml of 0.1N Hydrochloric acid for 2 h and followed in Phosphate buffer (pH 7.4) for 7 h at 37± 0.5°C. The apparatus was operated at a stirring speed of 75 rpm. 3 ml of the dissolution medium was sampled at predetermined time intervals and replenished with the same quantity of fresh dissolution medium in each occasion to keep the volume constant. The withdrawn samples were filtered through Whatmann No. 1 Qualitative filter paper and analyzed for drug content at 272 nm using Shimadzu 1800 UV spectrophotometer (Japan).

Drug release kinetics

To study the release kinetics, data obtained from in vitro drug release studies were fitted into various kinetic models: zero order (cumulative amount of drug released vs time), first order (log cumulative percentage of drug remaining vs time) and Higuchi's model (cumulative percentage of drug released vs square root of time).

Drug release obeying Zero order can be described as represented in equation 3:

$C = K_0 t$ (Equation 3)

Where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes.

Drug release obeying First order can be described as represented in equation 4:

$\text{Log } C = \text{Log } C_0 - kt/2.303$ (Equation 4)

Where C_0 is the initial concentration of drug, k is the first order constant, and t is the time.

Drug release obeying Higuchi model can be described as represented in equation 5:

$Q = Kt_{1/2}$ (Equation 5)

Where K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

The kinetic data obtained from the in vitro dissolution studies were analyzed to obtain correlation coefficients for the different kinetic equations.

Stability study[7]

Prepared microspheres were properly packed in aluminium foil and kept for stability studies at the following temperature and relative humidity (RH) for three months as per ICH guidelines:

- 25º C and 65% RH

- 40º C and 75% RH

The humidity was maintained using saturated solution of sodium chloride.

Results and discussion

From the data reported in Table 3, it can be seen that highest drug loading was observed in AF3 (24.50%). On the other hand, formulation AF7 showed the lowest drug loading of 19.15%. Increase in the drug loading was observed with increase in the concentration of calcium chloride (AFA8, AFA3). Increase in the sodium alginate (polymer) concentration from 2-3% w/v resulted in an increase in the percentage drug loading (AFA3, AFA7). Further increase in the concentration of the same to 4% w/v resulted in lower drug loading (AFA11). The addition of chitosan resulted in an increased drug loading when compared with microspheres prepared without the use of chitosan (AFA1, AFA2, AFA3).This is due to the formation of polyelectrolyte complex, which reduces the porosity at the surface of the microspheres, which results in the prevention of the drug diffusing out during and after gelation. Increase in the concentration of chitosan till 1% w/v shows increase in drug loading (AFA8); further increase in the concentration of chitosan leads to decrease in the drug loading. Entrapment efficiency was observed to be in the range of 85.96 to 99.18 % for different formulations of the prepared aceclofenac loaded chitosan alginate microspheres. In the case of aceclofenac loaded chitosan coated alginate & carrageenan microspheres the same was in range of 78.62% to 99.48%.

From the results of particle size determination of the prepared microspheres, it was observed that the mean particle size was in range of 813.66 µm to 891.66 µm for aceclofenac loaded chitosan alginate microspheres and 772 µm to 904.34 µm for aceclofenac loaded chitosan alginate & carrageenan microspheres. SEM photographs show that the prepared aceclofenac loaded chitosan alginate microspheres and aceclofenac loaded chitosan alginate & carrageenan microspheres were spherical in shape and rough surface as shown in Figure 1 & 2, it can be observed that the drug is dispersed in the polymeric matrix without having any core or coat, confirming that the system is a polymeric matrix system.

The FTIR of aceclofenac[8] (drug) shown intense bands at 1770cm^{-1}, 1718 cm^{-1} , 2937cm^{-1} 3193 cm^{-1} and 667 cm^{-1} corresponding to the functional groups C=O, OH, superimpose of OH, stretching NH stretching C-Cl as shown in Figure 3. In the FT-IR spectrum of aceclofenac loaded chitosan coated alginate microspheres and aceclofenac loaded chitosan coated alginate & carrageenan microspheres, the prominent bands of C=O stretching at 1772cm^{-1} and OH stretching was observed at 1716cm^{-1} respectively for aceclofenac loaded prepared microspheres Figures 3,4&5. This confirms the absence of any interaction between the drug and polymers during the preparation of the microspheres.

The X-ray diffraction patterns of aceclofenac loaded chitosan coated alginate microspheres and chitosan coated alginate & carrageenan microspheres are presented in figure 6 and 7 respectively. The X-ray diffraction pattern of aceclofenac shows many characteristic sharp peaks (Figure 6 d), which did not appear in the X-ray diffraction pattern (Figure 7c, 7d) of drug loaded microspheres. This indicates that the drug loaded inside the polymer matrix is not in the crystalline state for both chitosan coated alginate microspheres and chitosan coated alginate & carrageenan microspheres.

The effect of different experimental variables on the release of drug from aceclofenac loaded chitosan coated alginate Figure 8. Figure 8a shows the effect of sodium alginate concentration on drug release from aceclofenac loaded chitosan coated alginate microspheres. The microspheres prepared using a concentration of 4%w/v sodium alginate are able to prolong the drug release when compared to microspheres prepared with 2 and 3 % w/v sodium alginate. Increase in the concentration of calcium chloride resulted in delay in the drug release as shown in Figure 8c. Increase in the chitosan concentration leads to a prolongation in the drug release (Figure 8b), possibly due to positively charged amino groups of chitosan forming a membrane(coating) through ionic interaction with the carboxylic residues of alginate and addition of polycationic polymers to the gelation medium results in reduced permeability[9] . No major difference in the drug release profile was observed when alginate: drug ratio was altered. From the figure 8e depicted, it can be observed that

the curing time had a pronounced effect on the rate of drug release from the prepared microspheres. The increase in the curing time from 2 to 8 hours resulted in a delay in the release of the drug (Figure 8e) with increase in the curing time. This may be due to the penetration of calcium ions to the interior of the microspheres resulting in increased cross linking [10].

The effect of different experiment variables on the release of drug from aceclofenac loaded chitosan coated alginate & carrageenan microspheres is represented in Figure 9. With increase in sodium alginate concentration, drug release is retarded from aceclofenac loaded microspheres. This is graphically represented in Figure 9b.

The effect of calcium chloride on aceclofenac release from prepared microspheres is presented in Figure 9c. The results indicate that the drug release was delayed with increase in calcium chloride concentration. No major difference in the drug release profile was observed when the curing time was altered (Figure 9e). Figure 11d reveals that increase in the carrageenan concentration results in an increase in retardation of drug release from prepared microspheres.

In order to characterize the in vitro release of aceclofenac from prepared aceclofenac loaded chitosan coated alginate microspheres and aceclofenac loaded chitosan coated alginate & carrageenan microspheres, the different drug release models were applied and studied by fitting the data obtained from in vitro drug release studies. Obtained values of correlation coefficient are given in Table 7 & 8. It was found that the in-vitro drug release of aceclofenac was best explained by first order, as the plots showed the highest linearity (r= 0.975), as compared with zero order and higuchi's plots. Linear regression analysis indicated a higher correlation coefficient for the first order model indicating that a linear relationship existed between the logarithm of the percent drug remaining to be released from the prepared microspheres and time. Hence, the release of aceclofenac from the aceclofenac loaded chitosan alginate microspheres and aceclofenac loaded chitosan alginate & carrageenan microspheres can be described by first-order kinetics model, followed by Higuchi's equation. This explains the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (or Higuchi's kinetics). This may be due to the fact that microspheres are matrix-type and swelling of microspheres took place during drug release experiments.

The data for all optimized batches kept under short term stability study showed that no appreciable changes in drug loading and cumulative release profile occurred. Hence formulation was found to be stable under the conditions used for the stability studies.

Conclusion

The study demonstrates that it is possible to control the release rate of aceclofenac over a wide time scale by using the 3: (1:1):2 alginate: (alginate: carrageenan): chitosan with 1:4 (alginate: aceclofenac). The beads coated with carrageenan showed better results than the non-coated beads, and the beads cross-linked with carrageenan were the best with regards to the effectiveness for prolonged release of the drug over 8 hours. It was also observed that the release of aceclofenac is slower in pH 1.2 and 7.4; therefore it can be utilized for the development of sustained release drug delivery system for aceclofenac based on information reported here in.

References

1. Elfstrand L, Eliasson AC, Wahlgren M. The effect of starch material, encapsulated protein and production conditions on the protein release from starch microspheres. Journal of Pharmaceutical Sciences, 98(10), 2009, 3802–3815.
2. Malafaya P, Stappers F, Reis R. Starch-based microspheres produced by emulsion crosslinking with a potential media dependent responsive behaviour to be used as drug delivery carriers. Journal of Materials Science Materials in Medicine, 17(4), 2006, 371–377.
3. Parfitt K. Analgesics, anti-inflammatory and antipyretics. In JEF. Reynolds (ed.), Martindale: The Complete Drug Reference. 32nd ed, Massachusetts, 1999, 1–12.
4. Kay AE, Alldred A. Rheumatoid arthritis and osteoarthritis. In R. Walker, and C. Edwards (eds.), Clinical Pharmacy and Therapeutics, 3rd ed, Churchill Livingstone, London, UK, 2003, 791–807.

5. Bhoyar PK, Morani DO, Biyani DM, Umekar MJ, Mahure JG, Amgaonka YM, Encapsulation of naproxen in lipid-based matrix microspheres: Characterization and release kinetics. Journal of young pharmacists. 3 (2), 2011, 105-111.
6. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pham. Acta Helv. 60, 1985, 110–111.
7. Kashappa Goud H, Desai TM, Pramod Kumar. Preparation and Evaluation of a Novel Buccal Adhesive System. AAPS PharmSciTech. 5, 2004, 35.
8. Mutalik S, Naha A, Usha AN. Preparation, in vitro, Preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac. Arch. Pharm. Res. 30, 2007, 222–234.
9. Ritger PL, Peppas N, A Simple equation for description of solute release. II Fickian and anomalous release from swellable devices. Journal of Controlled Release. 5, 1987, 37-42.
10. King GA, Dangulis AJ, Faulkner P, Goosen MFA. Alginate-polylysine microcapsules of controlled membrane molecular weight cut-off *for* mammalian cell culture engineering. Biotechnol Prog. 3(4), 1987, 231-240.

Table No. 01: Formulation design for Chitosan Coated Alginate Beads

Formulation code	Sodium alginate (in % w/v)	$CaCl_2$ (in % w/v)	Chitosan (in % w/v)	Drug: Alginate ratio	Gelation time (in h)
AFA1	3	2	-	1:4	4
AFA2	3	2	0.5	1:4	4
AFA3	3	2	1	1:4	4
AFA4	3	2	1	1:3	4
AFA5	3	2	1	1:2	4
AFA6	3	2	1	1:4	2
AFA7	2	2	1	1:4	4
AFA8	3	1	1	1:4	4
AFA9	3	2	2	1:4	4
AFA10	3	2	1	1:4	8
AFA11	4	2	1	1:4	4
AFA12	3	-	1	1:4	4

Table No. 02: Formulation design for chitosan coated alginate & carrageenan beads

Formulation code	Sodium alginate (in % w/v)	Sodium alginate : carrageenan ratio	$CaCl_2$ (in %w/v)	Chitosan (in % w/v)	Drug : Alginate ratio	Gelation time (in h)
AFAC1	3	3:1	2	0.5	1:4	4
AFAC2	3	3:1	2	1	1:4	4
AFAC3	3	3:1	2	1	1:3	4
AFAC4	3	3:1	2	1	1:2	4
AFAC5	3	3:1	2	1	1:4	2
AFAC6	2	2:1	2	1	1:4	4
AFAC7	3	3:1	1	1	1:4	4
AFAC8	3	3:1	2	2	1:4	8
AFAC9	3	3:2	2	2	1:4	4
AFAC10	3	1:1	2	2	1:4	4
AFAC11	3	3:1	-	2	1:4	4

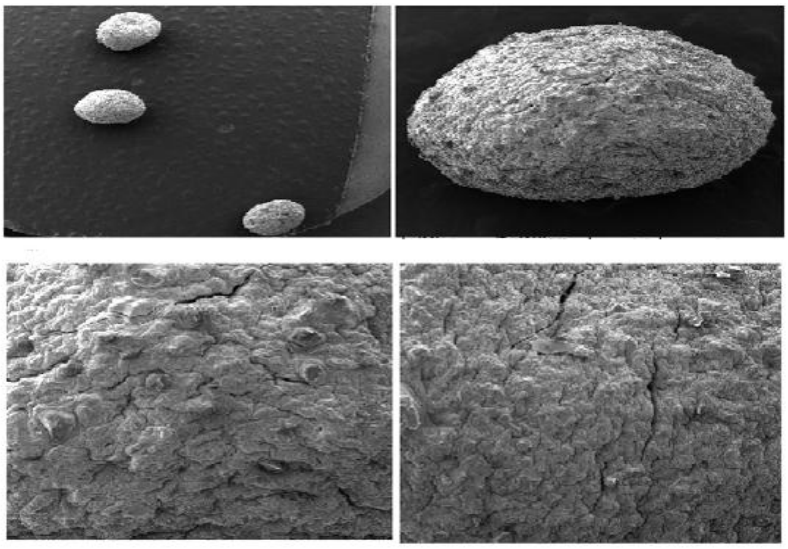


Fig. No. 01: Scanning electron micrographs of drug loaded chitosan coated alginate beads

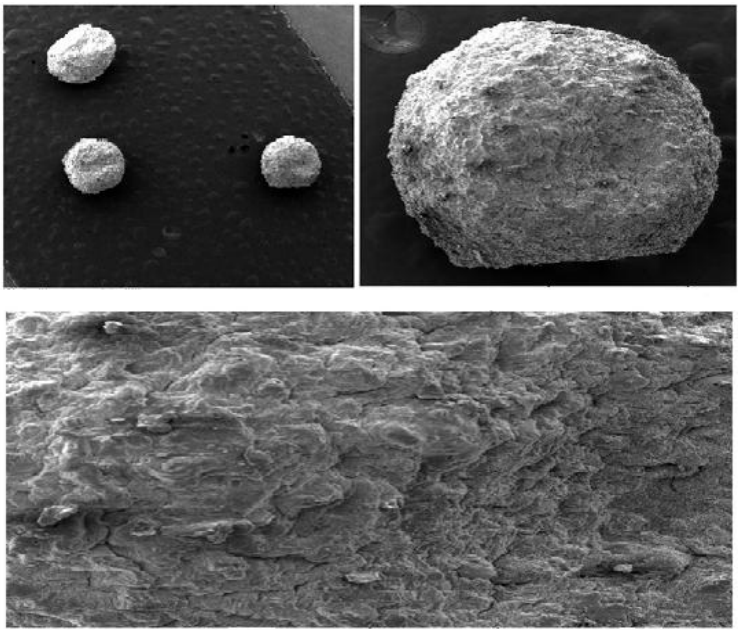


Fig. No. 02: Scanning electron micrographs of drug loaded chitosan coated alginate & carrageenan beads

Table No. 03: Morphological characters of aceclofenac loaded chitosan coated alginate beads

Formulation code	Particle size (µm)	Drug loading (in %)	Entrapment efficiency (in %)
AFA1	816.66 ± 3.05	21.42 ± 0.76	86.74 ± 1.34
AFA2	827.66 ± 15.04	24.16 ± 0.76	97.74 ± 1.51
AFA3	845.00 ± 5.00	24.50 ± 0.92	98.26 ± 1.60
AFA4	854.66 ± 2.51	24.26 ± 0.73	99.18 ± 1.04
AFA5	857.33 ± 7.09	23.30 ± 0.93	97.19 ± 3.45
AFA6	861.33 ± 6.11	22.71 ± 1.54	89.30 ± 2.28
AFA7	853.33 ± 10.40	19.15 ± 2.42	96.38 ± 3.26
AFA8	821.33 ± 2.30	20.69 ± 2.19	96.75 ± 1.86
AFA9	875.33 ± 4.50	20.78 ± 3.07	85.96 ± 1.72
AFA10	847.66 ± 5.50	19.42 ± 0.61	97.89 ± 1.64
AFA11	891.66 ± 1.52	19.89 ± 2.49	93.76 ± 1.52
AFA12	813.66 ± 3.21	23.90 ± 3.11	98.79 ± 0.21

(Mean ± SD, n=3)

Table No. 04: Morphological characters of aceclofenac loaded chitosan coated alginate and carrageenan beads

Formulation code	Particle size (µm)	Drug loading (in %)	Entrapment efficiency (in %)
AFAC1	862.27 ± 6.93	20.87 ± 2.91	94.50 ± 1.59
AFAC2	861.66 ± 12.58	18.40 ± 1.74	99.48 ± 0.52
AFAC3	881.66 ± 7.63	16.06 ± 0.39	85.19 ± 0.94
AFAC4	894.00 ± 12.12	22.88 ± 2.64	78.62 ± 0.978
AFAC5	902.93 ± 10.60	24.61 ± 0.45	83.50 ± 3.11
AFAC6	807.70 ± 11.46	12.03 ± 1.00	95.48 ± 4.47
AFAC7	818.87 ± 1.76	17.17 ± 2.68	92.01 ± 3.10
AFAC8	841.19 ± 23.08	19.07 ± 2.77	88.44 ± 8.00
AFAC9	772.96 ± 24.37	19.48 ± 0.22	93.38 ± 3.25
AFAC10	904.34 ± 6.27	19.97 ± 5.00	96.26 ± 3.41
AFAC11	807.75 ± 16.50	22.15 ± 1.60	98.88 ± 1.42

(Mean ± SD, n=3)

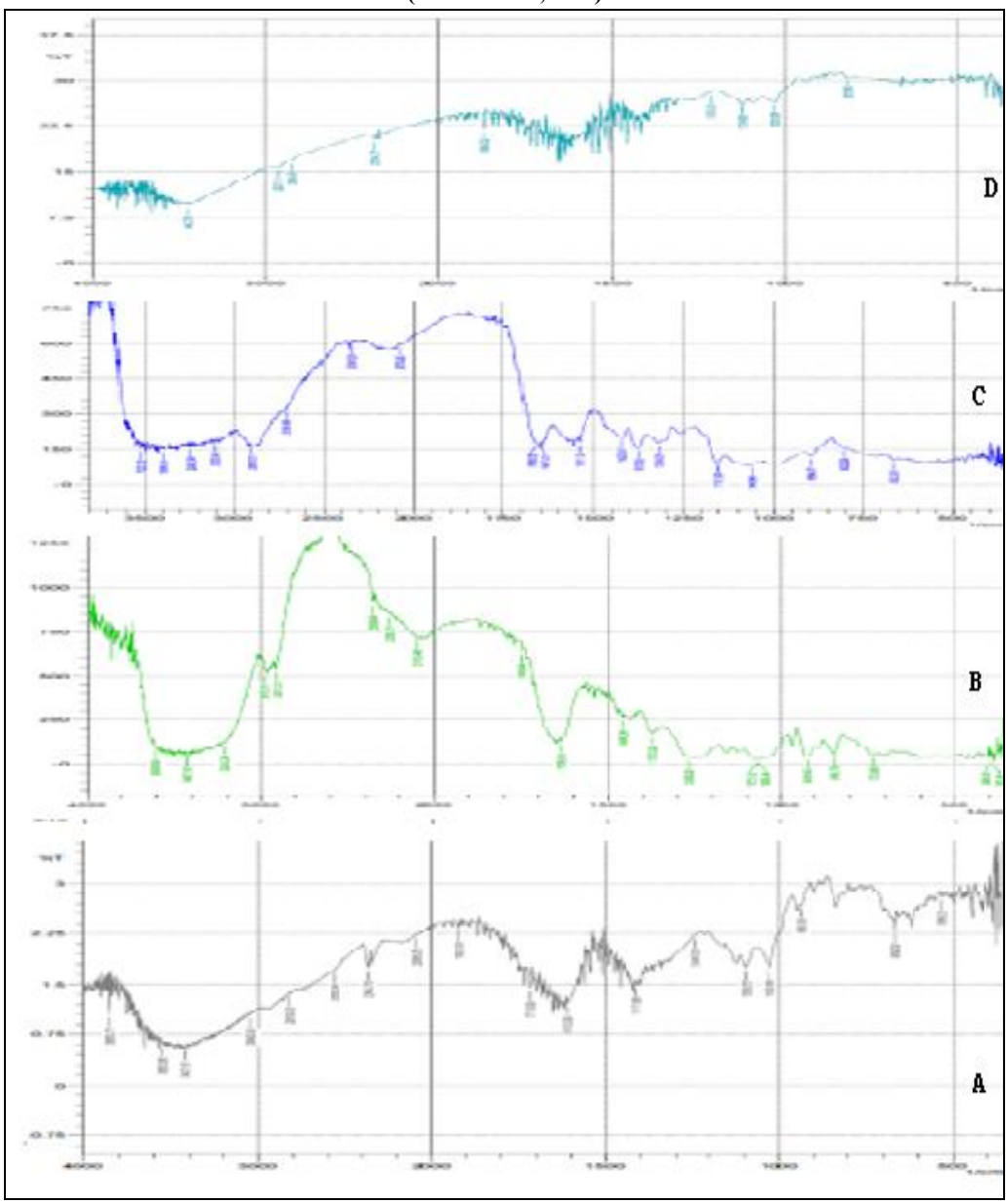


Fig. No. 03: FT-IR spectra of a) sodium alginate b) carrageenan c) chitosan d) unloaded chitosan coated alginate and carrageenan beads

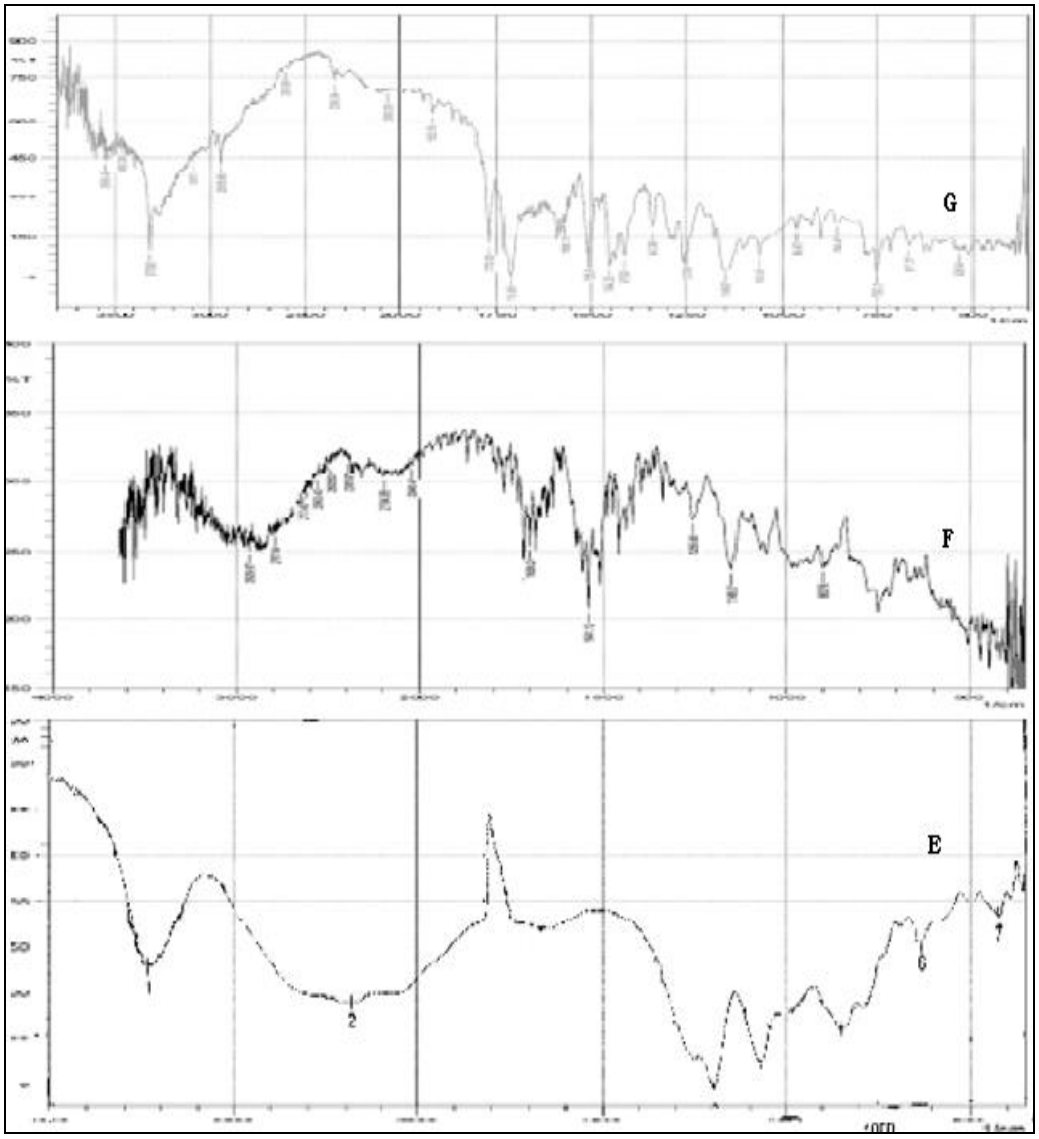


Fig. No. 04: FT-IR spectra of e) unloaded chitosan coated alginate beads f) physical mixture of aceclofenac, chitosan and alginate g) physical mixture of aceclofenac, alginate, chitosan and carrageenan

Table No. 07: Various kinetic data of aceclofenac -loaded chitosan coated alginate beads

Formulation code	Zero-Order [r] (Mt vs t)	First-Order [r] $\log(M_0-M_t)$ vs t	Higuchi-Matrix[r] (M vs $t^{0.5}$)
AFA1	0.534	0.944	0.978
AFA2	0.464	0.940	0.950
AFA3	0.555	0.953	0.970
AFA4	0.332	0.949	0.913
AFA5	0.349	0.975	0.923
AFA6	0.374	0.967	0.930
AFA7	0.261	0.969	0.899
AFA8	0.294	0.945	0.905
AFA9	0.646	0.957	0.982
AFA10	0.610	0.962	0.978
AFA11	0.457	0.967	0.947
AFA12	0.203	0.954	0.879

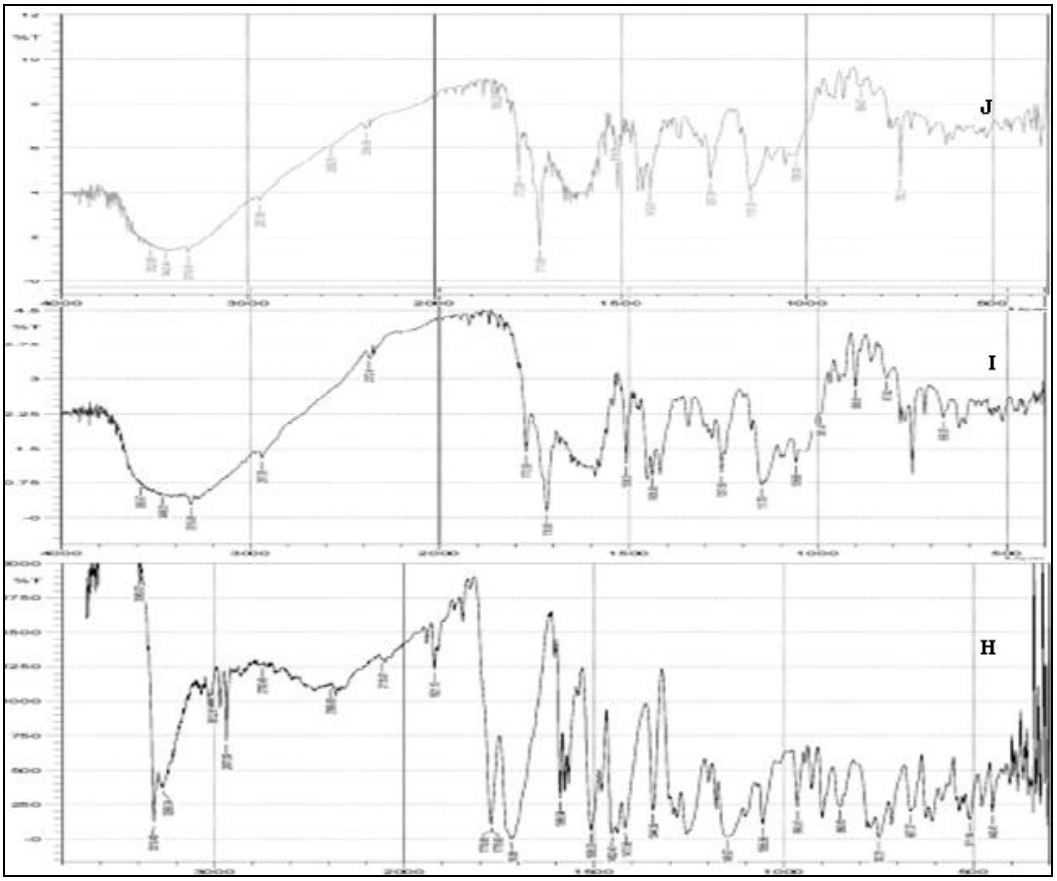


Fig. No. 05: FT-IR spectra of h) pure aceclofenac i) aceclofenac chitosan coated alginate and carrageenan beads j) aceclofenac chitosan coated alginate beads

Table No. 08: Various kinetic data of aceclofenac – loaded chitosan coated alginate and carrageenan beads

Formulation code	Zero-Order [r] (Mt vs t)	First-Order [r] $\log(M_0-M_t)$ vs t	Higuchi-Matrix[r] (M vs $t^{0.5}$)
AFAC1	0.534	0.944	0.9781
AFAC2	0.464	0.9407	0.9505
AFAC3	0.555	0.9537	0.9709
AFAC4	0.332	0.9493	0.9134
AFAC5	0.349	0.9755	0.9238
AFAC6	0.374	0.9676	0.9301
AFAC7	0.261	0.9698	0.8991
AFAC8	0.294	0.9453	0.9052
AFAC9	0.646	0.9573	0.9823
AFAC10	0.610	0.9629	0.9783
AFAC11	0.457	0.9674	0.9473
AFAC12	0.203	0.9548	0.8797

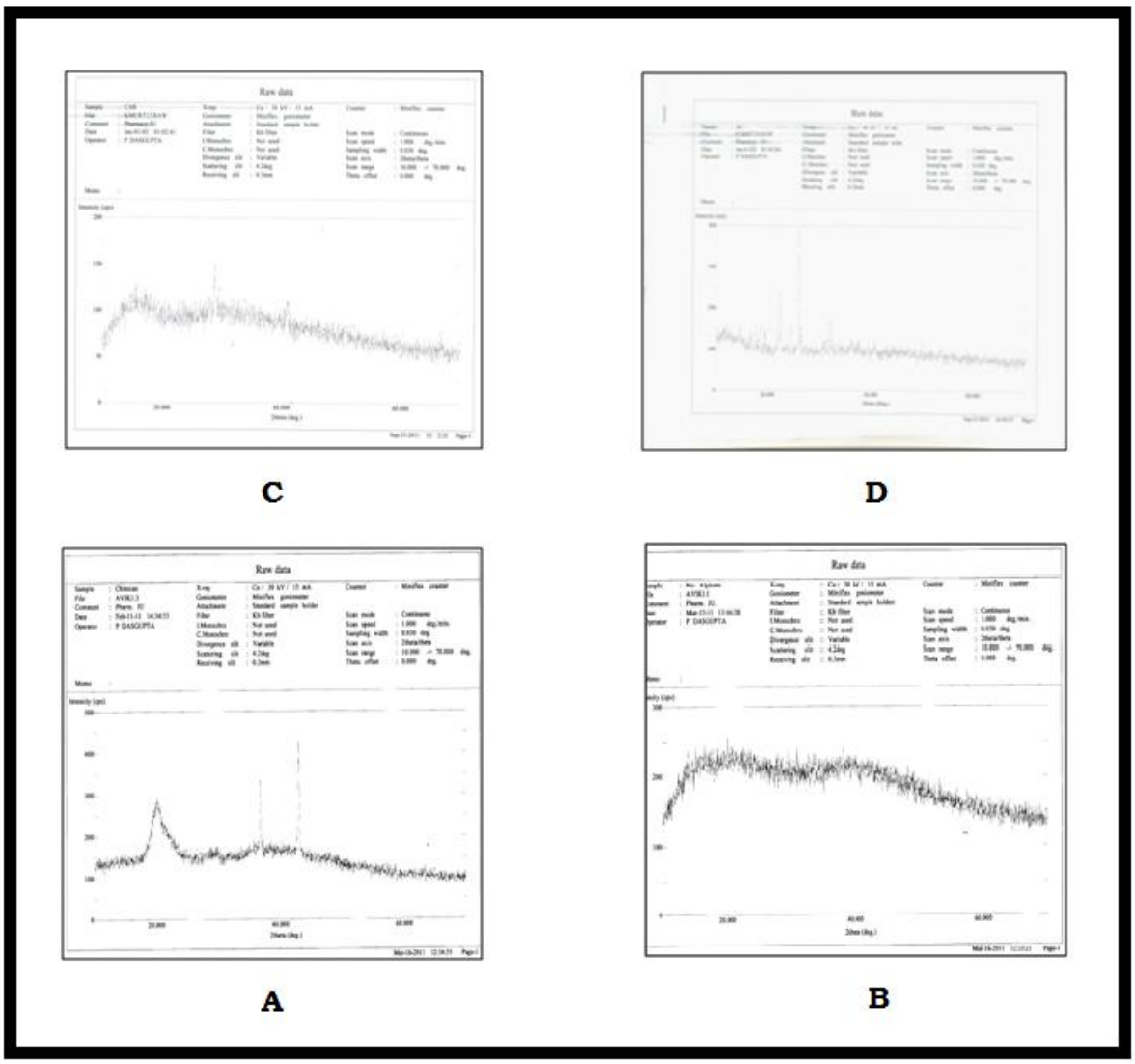


Fig. No. 06: X-ray diffractogram of a) sodium alginate; b) chitosan; c) carrageenan; d) pure aceclofenac

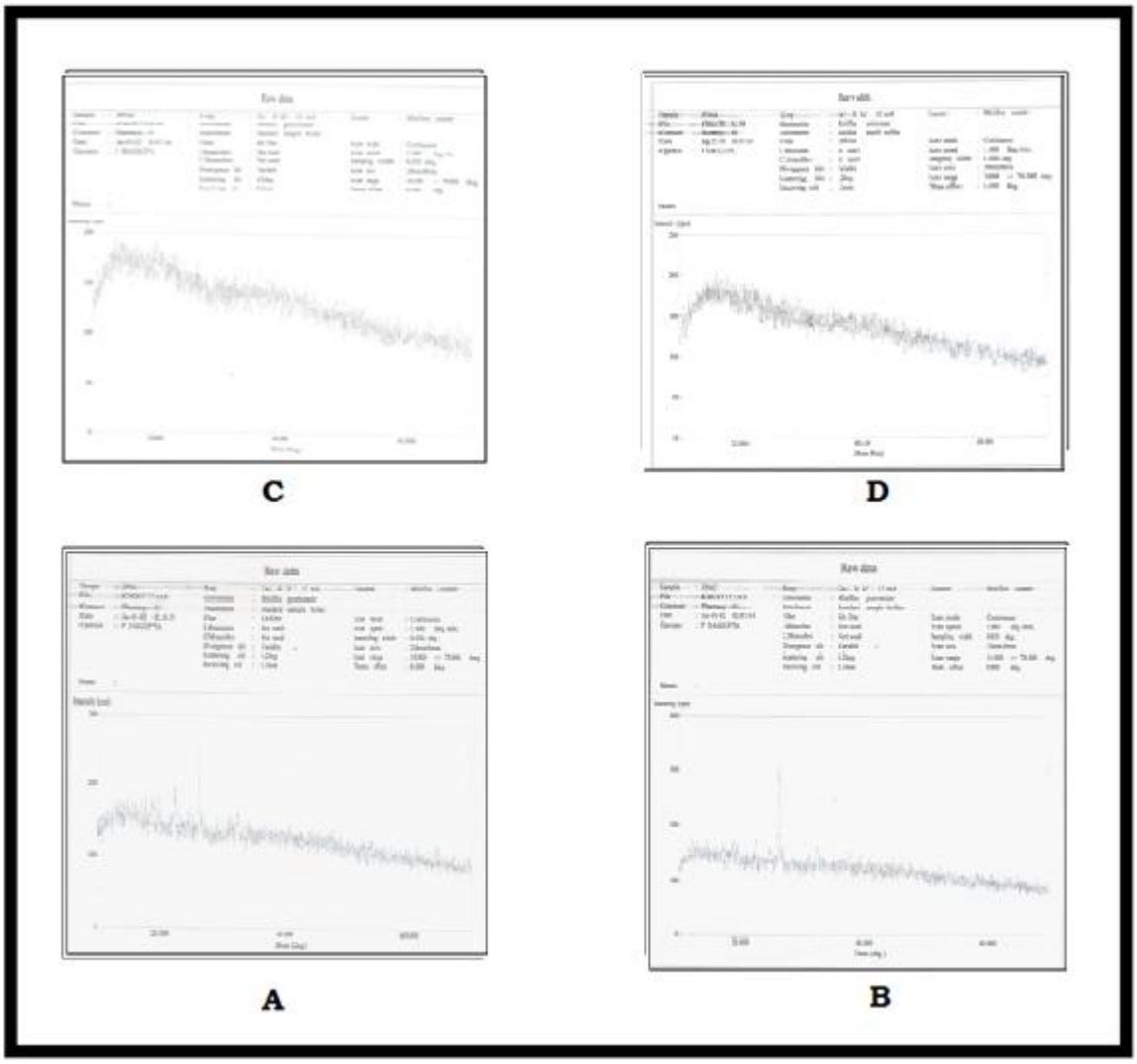
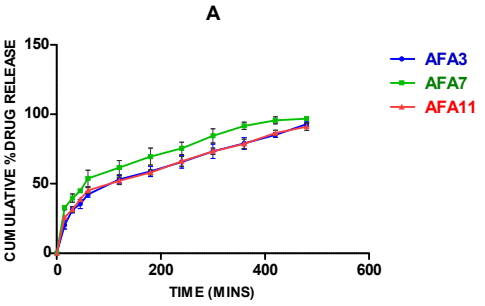
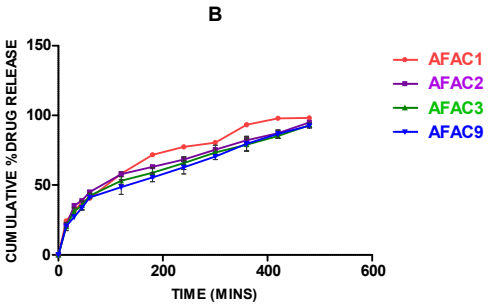


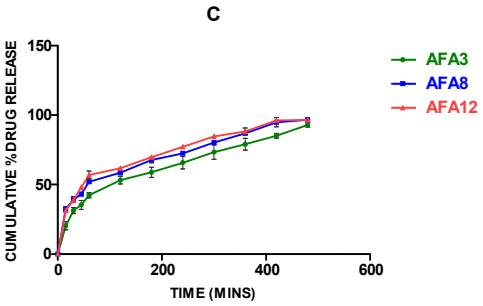
Fig. No. 07: X-ray diffractograms of a) physical of aceclofenac, sodium alginate, chitosan polymers; b) physical of aceclofenac, sodium alginate, chitosan & carrageenan polymers chitosan; c) aceclofenac loaded chitosan coated alginate beads; d) aceclofenac loaded chitosan coated alginate& carrageenan beads



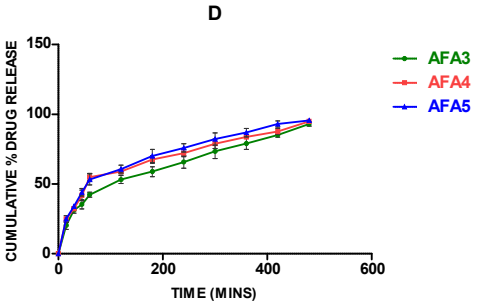
Effect of alginate concentration on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate beads



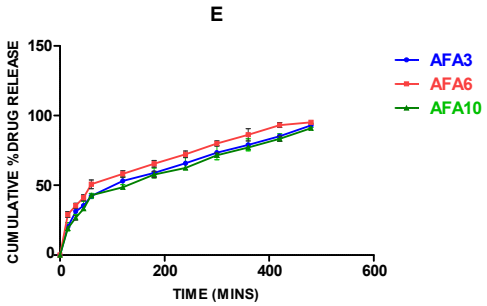
Effect of chitosan concentration on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate beads



Effect of $CaCl_2$ concentration on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate beads

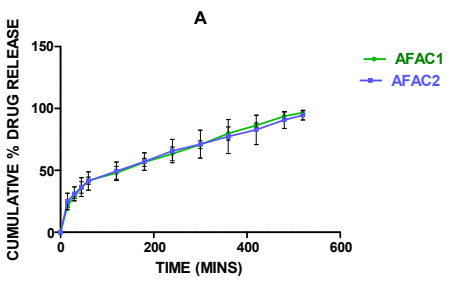


Effect of alginate, drug ratio on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate beads

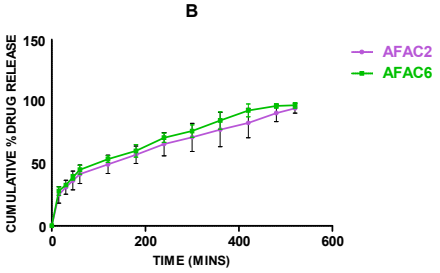


Effect of gelation time on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate beads

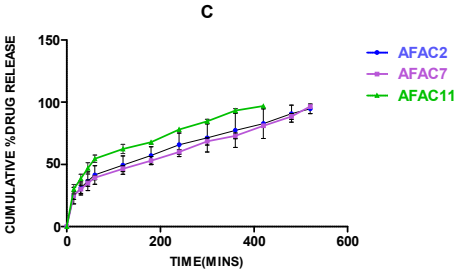
Fig. No. 08: Aceclofenac release profiles from chitosan coated alginate microspheres with different concentration and variable of (a) Alginate, (b) $CaCl_2$, (c) Chitosan, (d) Alginate-Drug ratio and (e) Alginate-Carrageenan ratio, (f)Curing time.



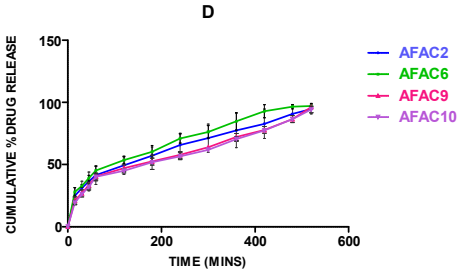
Effect of chitosan concentration on the cumulative percent release of drug from aceclofenacloaded chitosan coated alginate & carrageenan beads



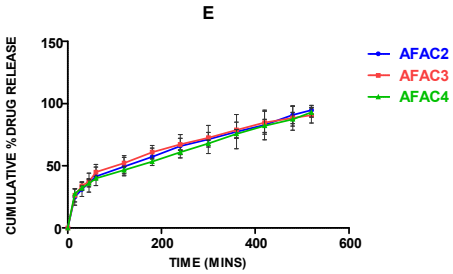
Effect of sodium alginate concentration on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate & carrageenan beads



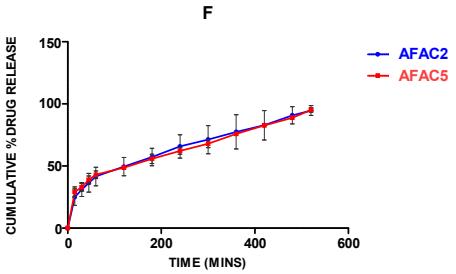
Effect of $CaCl_2$ concentration on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate& carrageenan beads



Effect of sodium alginate carrageenan ratio on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate & carrageenan beads



Effect of drug, sodium alginate ratio on the cumulative percent release of drug from aceclofenac loaded chitosan coated alginate & carrageenan beads



Effect of gelation time on the cumulative percent release of drug from aceclfenac loaded chitosan coated alginate & carrageenan beads

Fig. No. 09: Aceclofenac release profiles from chitosan coated alginate & carrageenan microspheres with different concentration and variable of (a) Alginate, (b) $CaCl_2$, (c) Chitosan, (d) Alginate-Drug ratio and (e) Alginate-Carrageenan ratio, (f)Curing time.