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PREPARATION AND EVALUATION OF DIACEREIN LOADED MICROSPHERE WITH SODIUM ALGINATE AND ETHYL CELLULOSE BY DOUBLE EMULSION SOLVENT EVAPORATION METHOD

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ABSTRACT

The present study was conducted to prepare microsphere of poorly water soluble drug Diacerein by double emulsion method (W/O/W) where sodium alginate was used as drug release retarding polymer in microsphere formulation and ethyl cellulose was used as coating polymer. Diacerein was found to be compatible with matrix polymer and co-polymer by conducting the various physicochemical and instrumental analyses. Three different Diacerein loaded microspheric formulations were prepared using variable concentrations of Sodium alginate and Ethyl cellulose as it was found that entrapment efficiency and particle size of the microspheres were increased on increasing polymer concentration of formulations and on increasing polymer concentration the drug release of all the formulations were gradually decreased.Though not being categorized as anonsteroidal anti-inflammatory drug, Diacerein has significant role in pain therapy. It can be concluded that preparing Diacerein microsphere using sodium alginate and ethyl cellulose polymers can be a new addition in the field of pain management as sustained release therapy.

KEYWORDS: **Microsphere, Diacerein, Sodium alginate, Ethyl cellulose**

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INTRODUCTION

Sustained release, prolonged action, controlled release, depot release are the terms used to identify drug delivery system that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose of drug. Now a days conventional dosage forms of drugs are rapidly being replaced by the new and the novel drug delivery systems. Amongst these the controlled release/sustained release dosage forms have become extremely popular in modern therapeutics. A sustained release dosage form will provide a therapeutic concentration of the drug in the blood that is maintained throughout the dosing interval with a reduction in a peak concentration ratio[1-2]. For most drugs, conventional method of drug administration is effective, but some drugs are unstable or toxic and have shorter half-life, narrow therapeutic ranges. Some drugs also posses' solubility problems. Diacerein is a drug which has solubility problem. Diacerein or diacetylrhein is a slow acting anthraquinone derivative used to treat osteoarthritis. Diacerein acts by directly blocking the action of interlekin-1-beta, a protein involved in the inflammation and destruction of cartilage that play a role in the development of symptoms of degenerative disease like osteoarthritis. Numerous studies have been carried out to evaluate symptomatic efficacy,safety and mechanism of action of Diacerein in osteoarthriris[3-10].The aim of the study was to prepare microsphere of poorly water soluble drug Diacerein by double emulsion method (W/O/W) where sodium alginate was used as matrix polymer and ethyl cellulose was used as coating polymer. Drug polymer compatibility was established in microsphere by FTIR study and XRD analysis.

MATERIALS & METHODS

Materials

Drugs and Chemicals

Diacerein,also known as Diacetylrhein, is a slow acting medicine used to treat joint diseases.It works by inhibiting interleukin-1-beta.It is a unique drug to improve the pain and slowing the progress of osteoarthriris in hip and knee.For that reason we have selected the drug to improve its efficacy by preparing its microspheres which will also reduce the toxicity.Diacerein was purchased from Vardev Intermediates Pvt. Ltd (Mumbai, India). Sodium alginate was purchased from Star chemicals.All other commercial reagents like ethyl cellulose, sodium dihydrogen phosphate, potassium hydrogen phosphate, dichloromethane, Dimethyl formamide etc.used in this study were of analytical grade (from Merck Ltd. And sigma Aldrich).

Methods

Physical observation

The color and powder forms of the drug were observed and identified as per the Indian Pharmacopoeia (7th edition 2014) reported by WHO expert committee.

Determination of melting point of Drug

The melting point of Diacerein was determined by open capillary method using melting point apparatus VEEGO, (Model No.VMP-DS).

Development of Calibration Curve of Diacerein

Calibration curve of Diacerein was prepared in phosphate buffer pH 6.8. 10 mg of Diacerein was accurately weighed and transferred to 100 ml volumetric flask.. The drug was dissolved in 2 ml DMF and the volume was made up to 100 ml using phosphate buffer of pH 6.8 to obtain a stock solution of 100 µg/ml (stock solution I). One ml of this stock solution was again diluted with phosphate buffer (pH 6.8) up to 10 ml to obtain a solution of 10 µg/ml(stock solution II). From stock solution II aliquots of 2, 4, 6, 8, 10 ml were transferred to a series of 10 ml volumetric flasks. The volume was made up with phosphate buffer of pH 6.8 fluid to give 2, 4, 6 & 8, 10 µg/ml of concentration. The absorbances of these solutions were measured at 260 nm against blank.

Preparation of Microsphere

Diacerein-loaded sodium alginate and ethyl cellulose microspheres were prepared by w/o/w multiple emulsion solvent evaporation method. First, an aqueous solution or suspension of sodium alginate with diacerein (internal aqueous phase) was emulsified in a solution of polymer(Ethyl cellulose) in organic solvent (Dichloromethane) containing 0.2ml span 80 using a Homogenizer (model RQ 127A, Remi Motors, Mumbai) at 5000 rpm for 5min. The resulting water in oil (w/o) emulsion was then transferred into 100ml water containing 0.2ml Tween 80 with continuous mechanical stirring (Type BL 433, RemiMotors,Mumbai) at 700rpm at room temperature for 3 hours to form w/o/w type multiple emulsion.Upon solvent evaporation, the polymer precipitated and core of the microspheres solidified. The microspheres were then filtered with muslin cloth, washed with cold double distilled water and dried at room temperature for 24hrs. The same procedure was adopted for the preparation of blank microspheres(without drug) and drug loaded microspheres (without alginate).Following are the different ratio of sodium alginate and ethyl cellulose used to prepare three different formulations.

Formulation	Amount of Drug (mg)	Amount of SodiumAlginate	Amount of Ethylcellulose	Drug polymerRatio.
F-1	30	30	360	1 : 1 : 12
F2	30	150	450	1 : 5 : 15
F3	30	90	720	1 : 3 : 24

Drug entrapment efficacy of the Microspheres

The entrapment efficacy is defined as the ratio of the amount of the drug encapsulated in the microsphere to that of total drug in the microsphere.Entrapment efficacy was calculated by using the formula:The drug entrapment efficiency (%) = (experimental drug content)

/ (theoretical drug content) x 100. For diacerein loaded microsphere, a weighed quantity (30mg) of loaded microspheres were crushed into powder and added to 30ml phosphate buffer (pH 6.8). The resulting mixture was kept under magnetic stirrer for 5 hr. The solution was then filtered through whatman filter paper. One ml of this stock solution was diluted using phosphate buffer(pH-6.9) and analysed spectrophtometically (Spectromax M5) for Diacerein content at 260 nm. The EE and content were determined in three separately prepared microspheres and were expressed as the mean+ standard deviation[11].

Scanning Electron Microscope Analysis

Scanning Electron Microscope(SEM) studies were carried out by using JEOL MAKE(UK), MODEL-JSM6360.It was used to characterize the shape and surface topography of the microspheres. Microspheres were mounted on conducting stubs and vacuum coated with gold palladium film using a sputter coater(Edward S-150,UK). Images were taken using 17 kV electron beam intensity in a scanning electron microscope to examine the surface morphology of the microspheres.

Fourier Transform Infrared Spectroscopy (FT-IR) Study

Drug-polymer interactions were studied by fourier transmission infrared spectroscopy. FTIR spectroscopy was done by IR (Model no. Prestige-21), Shimadzu, Japan. Samples were prepared in KBr discs (2mg sample in 200mg KBr). FTIR study was performed on Diacerein, Ethyl Cellulose, Sodium Alginate and Microspheres. The scanning region was from 400 to 4000 cm^{-1}.

X-Ray Diffraction Analysis

X-ray diffractometry of Sodium alginate, ethyl cellulose,Diacerein and Diacerein loaded microsphere were done by using MODEL- ULTIMA-III, RIGAKU MAKE (JAPAN), Cu target slit 10 mm.The samples were mounted on to the diffractrometer and ciliated the X-rays on to the powdered sample to get the diffraction peak of certain intensities and recorded scan speed and scan axis were 1.000 deg/min and 2θ/θ respectively.

In-Vitro drug release study

In-vitro dissolution studies were performed for all the formulations by using USP type II tablet dissolution tester (paddle type)at 37°C and 50 r.p.min phosphate buffer pH 6.8 (500 ml) dissolution medium. An accurate amount of microsphere (60 mg) were added to dissolution medium and at preset interval 5 ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. Aliquots following suitable dilution were analyzed spectrophotometrically at 260 nm. This study was performed for the period of 12 hrs[12].

RESULT AND DISCUSSION

Physical observation

Physical observation reveals that Diacerein is fine yellow crystalline powder.

Melting point determination

Melting point of the drug sample was found 217°c which matches the literature assuring the identity of the received sample.

Calibration curve of Diacerein

Calibration curve of Diacerein was prepared in phosphate buffer pH 6.8. The concentration was increased in a predetermined way. Regression equation was calculated and utilized for quantitative estimation of the drug. The correlation coefficient was found to be 0.9972.

Table I: Data for the preparation of calibration curve of Diacerein (mean ± SD, n=3)

Concentration (mcg/ml)	Absorbance (nm)
0	0.00
2	0.092
4	0.206
6	0.283
8	0.378

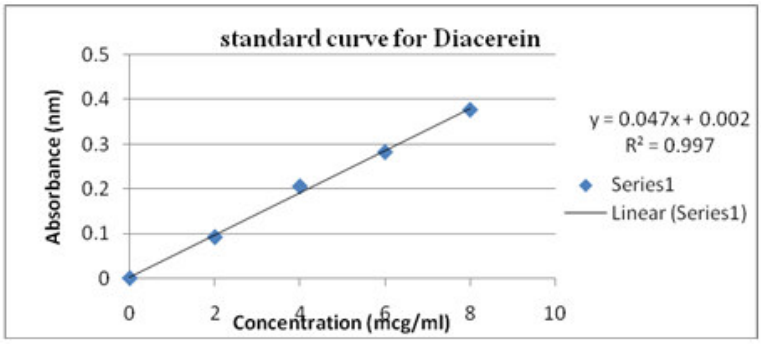

Figure I

Calibration curve of Diacerein in phosphate buffer 6.8 Characterization/Evaluation of Microsphere Percentage of Yield and Drug Entrapment Efficiency

The microspheres were prepared by double emulsion solvent evaporation (W/O/W) method with different drug to polymer ratio. So the influence of different polymer concentration on Diacerein loaded microsphere was

evaluated and yields were found from the range of 81-84%.It was observed that drug entrapment efficiency for various drug polymer ratio varied between 31-39% and increase in polymer concentration leads to formation of large microsphere, entrapping greater amount of drug. Entrapment increases with increase in drug to polymer ratio.

Table II:Percentage yield and drug entrapment efficiency measurement

Diacerein sodium alginate and ethyl cellulose ratio (F1, F2,F3 formulation code)	% yield Mean SD, n=3	Entrapment efficiency (%) Mean =SD, n=3
F1 (1:1:12)	84.52 = 2.9	30.82 + 2.1
F2 (1: 5:15)	82.86 = 3.6	32.18 + 2.4
F3 (1 : 5: 24	81.43 = 3.2	39.40 + 1.6

Scanning Electron Microscope

The microspheres were prepared by using different drug to polymer ratio. Microsphere was prepared by W/O/W method and the scanning electron microscopy (SEM) of drug loaded microsphere of sodium-alginate and ethyl-cellulose reveals that the microspheres posses spherical, non-aggregated and porous surface (figIIa,IIb&IIc). By SEM study it had been seen that the size of the optimized Diacerein loaded microspheres was 100 to 500 µm. The particle size of the microsphere increases with increase in polymer concentration. As the drug to polymer ratio increases the number and size of the pores decreases. Magnification of 120 times of formulation F1 shows that surface contains many pores and this may be one of the reason for its low drug entrapment as compared to formulation F2 and F3 because drug may be diffused out during hardening. The formation of pore may cause solvent to penetrate resulting in the swelling of the internal matrix release the drug either burst release or through diffusion, so this may be the reason for fast release of drug during dissolution. Formation of pore means diffusion mechanism to be responsible for sustained release.

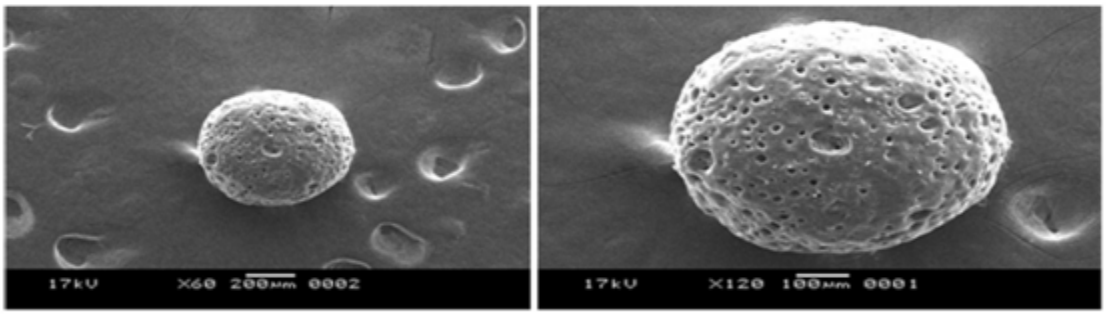

Figure II a
SEM photograph of Diacerein loaded microsphere (formulation F1)

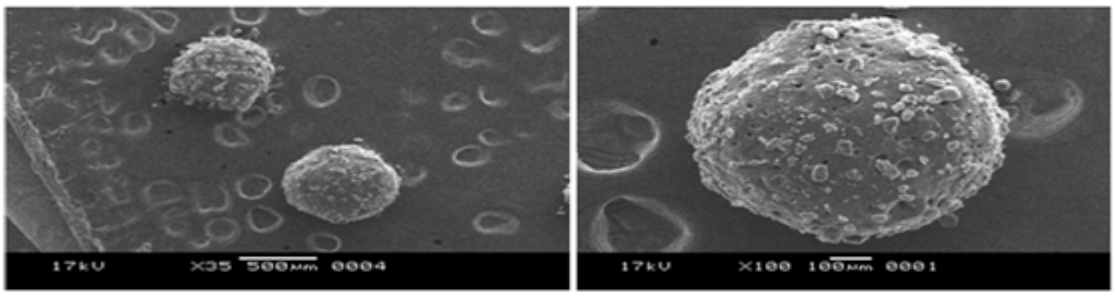

Figure II b
SEM photograph of Diacerein loaded microsphere (formulation F2)

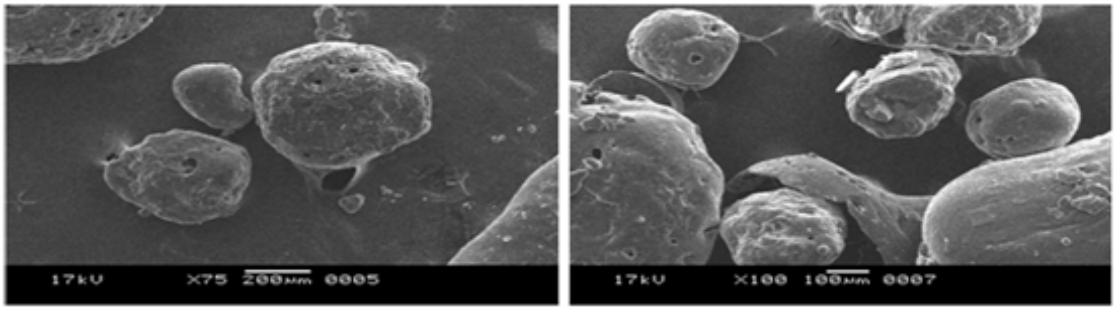

Figure II c
SEM photograph of Diacerein loaded microsphere (formulation F3)

FT-IR Study

The FTIR patterns of pure Diacerein and formulations are shown in (Figure III).The FT-IR spectra of pure Diacerein showed sharp peak at 1690cm^{-1} (which may be due to C=O stretching amide), 2937 cm^{-1} (C-H stretching aliphatic) and 1766 cm^{-1} (C=O stretch ester).The identical peaks also present in Diacerein loaded sodium alginate and ethyl-cellulose microsphere. All the peaks are also obtained in the microspheres, confirming the compatibility. This result suggests drug stability during encapsulation process.

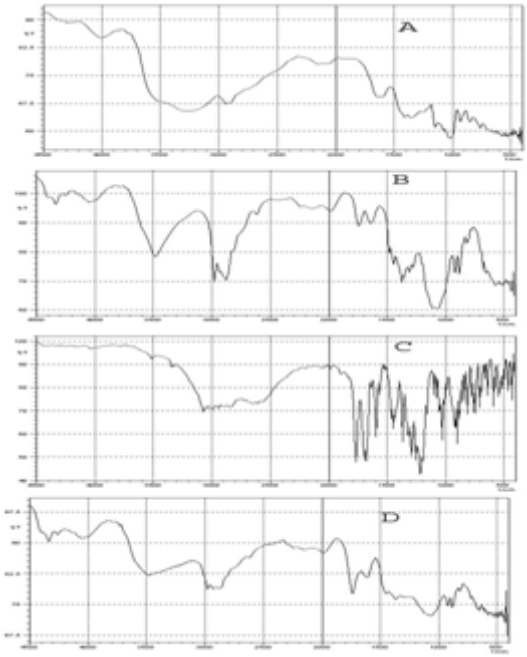

Figure III
FT-IR Spectrum of (a) sodium-alginate, (b) ethyl-cellulose, (c) pure drug Diacerein, (d) combination of sodium-alginate, ethyl-cellulose, and drug Diacerein

X-RAY Diffraction Spectroscopy (XRD)

X-Ray diffraction Spectroscopy was done to establish the stability and compatibility of the drugs with other excipients when formulated into microspheres. Since each diffraction pattern is characteristic to a specific crystalline lattice for a given compound, the purity and stability of the drug in the dosage form can be established through it. The XRD is done at 5-70^{o} 2θ. The diffraction pattern of the pure drug was compared with that of the formulations and establishes their characteristics.The X-Ray diffraction pattern shows that ethyl cellulose in combination with sodium alginate and ethyl-cellulose alone does not show any diffraction pattern so it most probably exists in amorphous form. But the formulation shows a little characteristic diffraction pattern of the drugs. Slight shift may take place due to the reduction of purity of the drugs during formulation into microspheres. The method which is followed during the preparation may also affect the diffraction pattern.

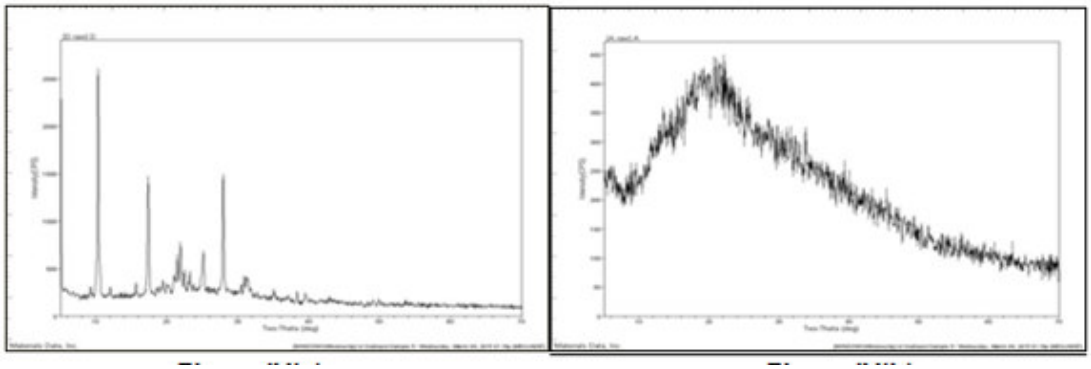

Figure IV(a)
X-RD Spectra of pure drug Diacerein

Figure IV(b)
X-RD Spectra of Sodium-alginate

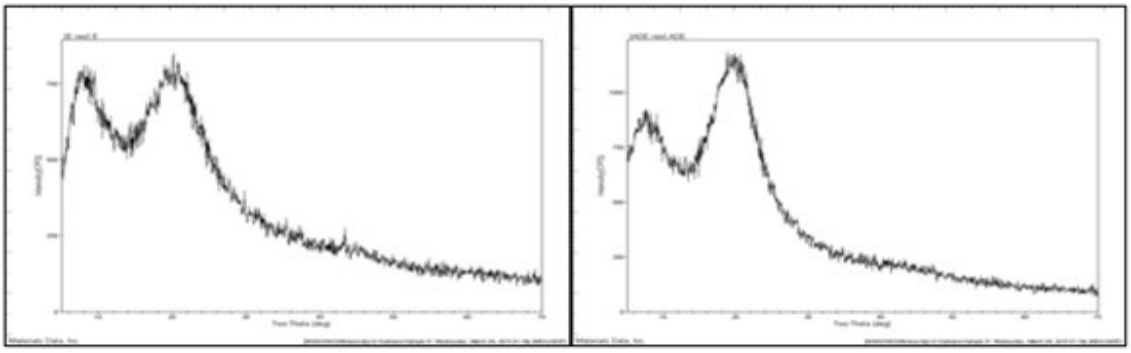

Figure IV(c)
X-RD Spectra of Ethyl cellulose

Figure IV(d)
XRD Spectra of combination of drug

In-Vitro Drug Release Behavior&Release kinetics

In-vitro dissolution studies were performed for all the formulations using USP type II tablet dissolution tester employing paddle type in phosphate buffer pH 6.8 dissolution medium for 11 hr. The samples withdrawn were analyzed by using UV spectrophotometer. For the different three formulations (F1, F2, F3) it has been observed that initial burst release after 30 min which was followed by a slow and continuous manner. It has been found that the drug release is very slow which decrease with the increase in drug to polymer ratio (shown in the following table and figures).A polymer's ability to retard the drug release rate is related to its viscosity. Higher alginate viscosity slowed down drug release rate in the buffer phase. The results showed that sodium alginate matrices can sustain drug release for 11 hr. The initial burst effect may be attributed as a desired effect to ensure initial therapeutic plasma Concentrations of drug.The drug release was decreased with increase in concentration of the polymer as increase in the polymer solution viscosity has produced microspheres with reduced porosity due to the thickening of the polymer wall. It is understood that higher polymer concentration results in a longer diffusional path length, so drug release is extended. The thick polymeric barrier slows the entry of surrounding dissolution medium in to the microspheres and hence less quantity of drug leaches out from the polymer matrices of the microspheres exhibiting extended release. Drug release profile of different formulation (F1, F2, and F3) of mixed polymeric matrix microsphere provides linear relationship for Higuchi &Korsmeyer-Peppas model. The effect of drug to polymer concentration on in-vitro release was studied. Figure V, VI, &VII show that the release of Diacerein from sodium-alginate and in combination of ethyl-cellulose microsphere illustrating the rate of drug release depend on the polymer concentration of the prepared devices, which indicates that the release rate decreases with increasing the amount of polymer. This can be explained by a decreased amount of drug present close to the surface and also by the fact that amount of uncoated drug decreases with higher concentration (Alex and Bodmeier 1990, Kristmundsdottir*et al.* 1996)[13-14] .In order to describe the kinetics of the release process of drugs from controlled/ sustained release preparation the data were with different kinetics models shown in table I. The first order equation[15] describes the release from systems when dissolution rate is depending on the concentration of the dissolving species. The Higuchi square root equation[16] describes the release from the systems where the solid drug is dispersed in an insoluble matrix and the rate of drug release is related to the rate of drug diffusion. The applicability of all this equations was tested in the present work. The release of drugs from sodium-alginate and ethyl-cellulose microsphere is found to be tri-phasic release profile, first initial burst release followed by diffusion for certain time then sustained release. In order to the model which will represent a best release kinetics model for the prepared formulations, the dissolution data was analyzed using the Peppas and Korsemeyer equation[17] which is expressed as $-M_t/M_\infty = k.t^n$ Where, M_t is the amount of drug release at time t and M_∞ is the amount release at time $t=\infty$, thus M_t/M_∞ is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusion exponent which can be used to characterize both mechanism for both solvent penetration and drug release. The different kinetic models for different formulation are shown in figure V, VI, &VII.

Table III(a): percent drug release for formulation F1

time(hr)	% release (Mean ± SD, n=3)	AUC
0	0	0
0.5	12.04	3.00906
1	13.87	6.47603
1.5	16.48	7.58738
2	17.00	8.36403
3	22.83	19.9037
5	28.91	51.7489
7	29.80	58.7231
9	31.33	61.1395
11	31.62	62.9546

Table III(b): percent drug release for formulation F2		
time(hr)	% release, (Mean ± SD, n=3)	AUC
0	0	0
0.5	7.82	2.069
1	9.83	4.52775
1.5	12.66	5.62409
2	13.68	6.58556
3	15.73	14.7096
5	18.39	34.1346
7	23.94	42.339
9	25.38	49.3249
11	26.47	51.8582

Table III(c): percent drug release for formulation F3		
time(hr)	% release, (Mean ± SD, n=3)	AUC
0	0	0
0.5	14.80	3.70138
1	15.60	7.60367
1.5	16.73	8.08676
2	18.21	8.73717
3	20.58	19.3958
5	21.89	42.4793
7	25.80	47.7011
9	27.32	53.1270
11	28.53	55.8447

Table IV: Correlation coefficient (R^2) for drug to polymer ratios 1: 13, 1: 20, 1: 27 (prepared by w/o/w method) after fitting of dissolution data to the different kinetic models

Formulation Code	Drug-polymer ratio	Kinetic Model				
		Zero-order	First order	Higuchi	Korsmeyer-Peppas	
		R^2	R^2	R^2	R^2	N
F1	1:1:12	0.7716	0.8764	0.9433	0.9670	0.3519
F2	1:5:15	0.8633	0.9597	0.9826	0.9885	0.3936
F3	1:3:24	0.6980	0.9708	0.9889	0.9666	0.2289

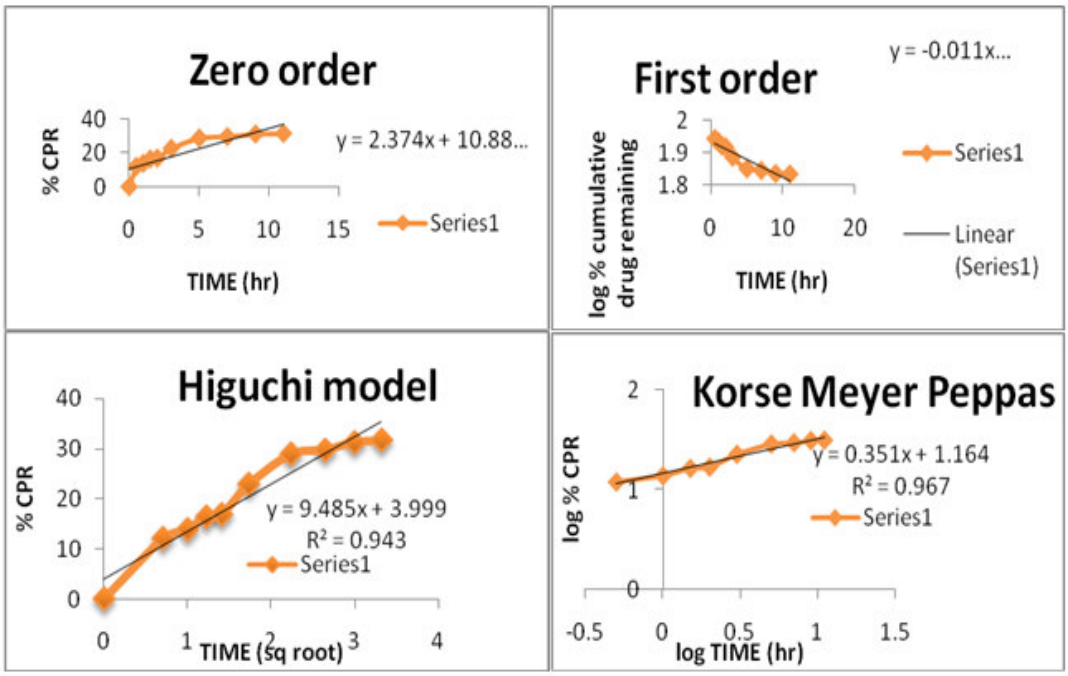

Figure V

Different kinetic model for formulation F1 (1:1:12) Prepared by w/o/w emulsion solvent evaporation method

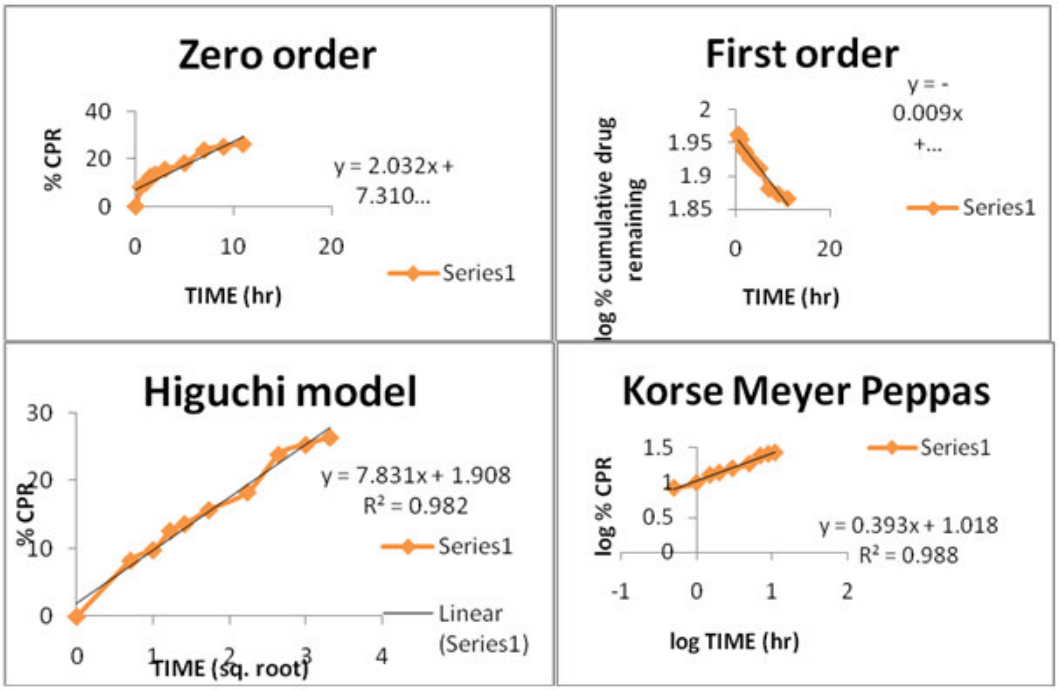

Figure VI
Different kinetic model for formulation F2 (1:5:15) Prepared by w/o/w emulsion solvent evaporation method

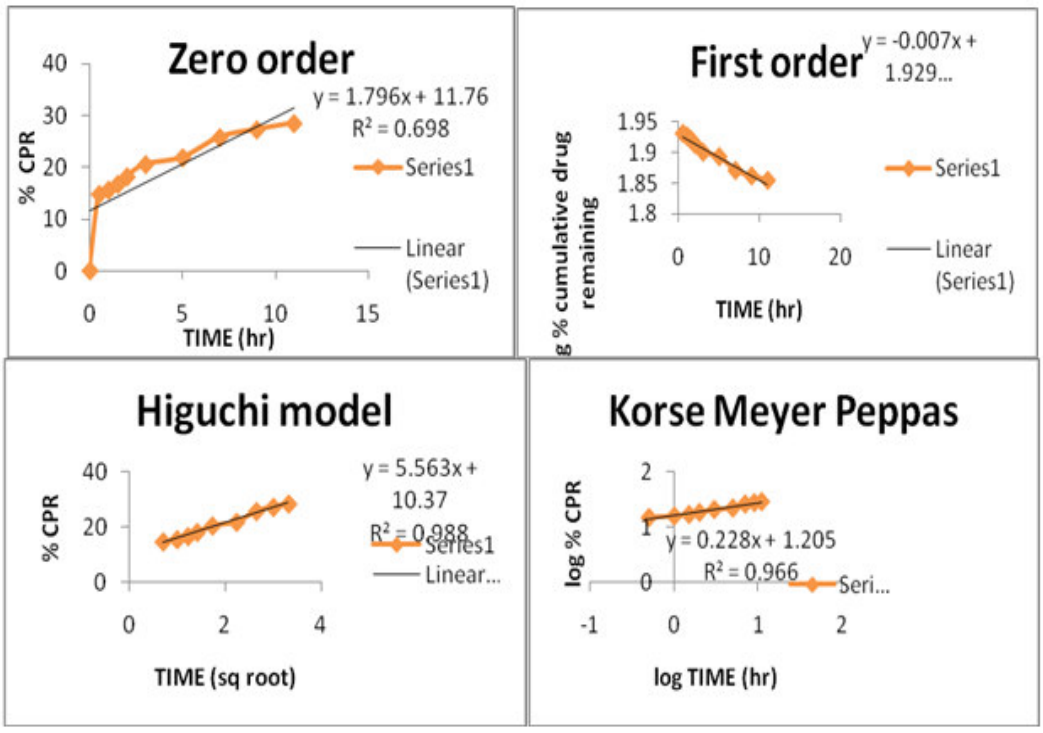

Figure VII
Different kinetic model for formulation F3 (1:3:24)Prepared by w/o/w emulsion solvent evaporation method

CONCLUSION

The study confirms that Diacerein loaded microspheres can be prepared using sodium alginate and ethyl cellulose polymers by solvent evaporation method for sustained release drug delivery. So it can be concluded that, this preparation can be used in near future for new drug delivery system against osteoarthritis.

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