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Preparation and Evaluation of Diclofenac Gelatin Microspheres Using Coacervation Technique

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ABSTRACT

To improve bioavailability, physical stability and more target specificity with predetermined drug release profile, it is necessary to prepare a unique dosage form like microspheres. In this research we prepared 0.25%, 0.50% and 0.75% w/v Diclofenac Gelatin microspheres using phase separation coacervation technique. The Diclofenac was estimated using UV-Visible spectroscopically at 276nm. The drug entrapment efficacy of 0.75% w/v Diclofenac Gelatin was found to be maximum i.e., 94.45 ± 0.7%. From the *in-vitro* drug release studies it was confirmed that 0.75% w/v Diclofenac Gelatin microspheres has reported 68.6 ± 1.4% drug entrapment efficacy; which indicates prolong release profiling of the formulation. From the particles size analysis, it was confirmed that all the three batches were shown particles size in micro size range. From the TEM analysis it was confirmed that the prepared 0.75% w/v Diclofenac Gelatin microspheres were spherical with no aggregation and narrow distribution. From the motic microscopical images it was confirmed that the drug was properly encapsulated within the prepared microspheres. The outcome of the research indicates that, 0.75% Diclofenac Gelatin microspheres was physically more stable and capable of improving bioavailability of the Diclofenac sodium after oral administration.

Keywords: Diclofenac Gelatin microspheres, Transmission Electron Microscopy, Motic microscopy, Particle size, Drug entrapment efficacy, I*n-vitro* drug release studies

INTRODUCTION

Proper drug delivery in human body has a significant impact onoverall bioavailability of the formulation [1, 2]. In this direction, Multiarticular drug delivery system playing a significant role to improve target specificity, stability and predetermined controlled drug release [3]. Recently carrier technology is vividly used in pharmaceutical sciences viz. microspheres, nanoparticles, liposomes. Among all the carrier's microspheres are more stable and versatile to carry many hydrophobic drugs in it [4]. The unique characteristics of microspheres are basically flowability in the range of 1-1000 µm comprising of synthetic polymers and proteins [5]. These types of unique dosage forms have predominance in patient compliance with reduced toxicity. In microspheres the drug molecules are entrapped in the encapsulated matrix of hydrophilic substances [6]. Materials such as polymeric wax, protective materials are in use. The mechanism of drug release for microspheres is dissolution of diffusion [7]. In maximum time, the formulations are in matrix or in encapsulated form. There are many methods by which microspheres can be prepared i.e., coacervation, phase separation, interfacial

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polymerization [8]. The coating materials are very indispensable in adjusting thickness of microspheres or microcapsules (1-20 µm) [9]. In this experiment we considered gelation; a natural polymer, as our polymerization agent to prepare microcapsules or microspheres by coacervation method [10]. During preparation, we consider Diclofenac sodium as our model drug. Diclofenac is a non-steroidal anti-inflammatory drug which is in use for the treatment of inflammatory disease Viz. gout, joint stiffness, arthritis, and swelling. The biggest advantage of Diclofenac microspheres is it could reduce cytotoxicity and organ toxicity because of lower drug encapsulation (1mg/10mL) within microspheres with controlled released pattern.

MATERIALS

Diclofenac sodium API was a gift sample from Cipla pharmaceutical Ltd., India, Gelatin was purchased from Sigma Aldrich India, Castor oil was purchased from Sisco Research Laboratories Pvt Ltd (SRL), India, and Formaldehyde was purchased from Sisco Research Laboratories Pvt Ltd (SRL), India. Rest all the chemicals are from laboratory & analytical grads.

METHODS

Preparation of Diclofenac Gelatin Microspheres

The accurately weighted Gelatin was dissolved in 60 ml of water to prepared 0.25%, 0.50% and 0.75% w/v solution. Further, adequate amount of drug was incorporated in three different Gelatin solution. The solution of drug was stirred for 10 minutes for 300 rpm. For better emulsification process 2 drops of castor oil was added in the three different Gelatin solutions during the time of stirring, unless and until castor oil was not added, infusibility would not be good for the suspension, hence possible phase separation could may take place. The stirring was continued for 45 minutes for proper infusibility to produce a clear solution.

Evaluation Parameters

a. Standard curve of Diclofenac sodium

The dilution was made from stock solution of Diclofenac (100µg/ml) to get concentration of 0.5, 1, 2, 4, 6 and 8µg/ml respectively. Absorbance of diclofenac sodium was taken at 276 nm in Ultraviolet visible spectrophotometer. The calibration curve was plotted between absorbance verses concentration

b. Entrapment efficiency [%]

Entrapment efficiency [11] of three different formulations [0.25%, 0.50% and 0.75% w/v solution] containing same amount of drug was determined by centrifugation method. For this, 60 mL suspension was poured into centrifugation different tubes and centrifuged at 1000 rpm for 10 minutes. The clear fraction was further used for the determination of free drug by using UV/visible spectrometer at 276 nm examined under the UV visible spectroscopy. The entrapment efficiency was calculated using the following formula:

$$\textit{Entrapment efficiency \%} = (Ct-Cf)/Ct \times 100$$

Where, Ct is the concentration of total drug and Cf is the concentration of entrapped drug.

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c. Particle size analysis

For particle size measurement Zeta Sizer (Delsa C Particle Analyzer) was used [12]. Sample was filled in cuvette carefully and there should be no bubble formation in cuvette. Then the cuvette was inserted into the instrument by opening the lid of zeta sizer and sample was analysed.

d. Morphology and particle shape

Shape and morphology of the formulation were determined by microscope. Shape of microspheres is assumed to be spherical. The microspheres examined under the Motic Microscope [13]. The suspension of microspheres was stained using sulforhodamine B solution (sulforhodamine B solution previously prepared using 0.5% solutions of acetic acid and water)

e. Transition Electron Microscopy (TEM)

The dried thin film of formulation batches viz. 0.25%, 0.50% and 0.75% w/v solution of Diclofenac Gelatin microspheres were coated with gold using gold sputter coater in a high vacuum evaporator [Ion Sputter JFC-III00]. The gold coated of Diclofenac Gelatin microspheres were analysed in electron microscopy. To get the proper magnificent image of the nanoparticles, Transmission Electron Microscopy (TEM) was performed (Hitachi 7500, Japan) [2]. Before TEM analysis of Diclofenac Gelatin microspheres were carbon coated and placed in copper grid considering 1% Phosphotungstic acid as the native stain.

f. *In vitro* drug release study of Diclofenac Gelatin microspheres

For *in-vitro* drug release study the formulation batches viz. 0.25%, 0.50% and 0.75% w/v solution of Diclofenac Gelatin microspheres was incorporated in a dialysis beg (12000 Dalton). The dialysis bag was tight by both the end submerged in 500 ml conical flask containing 400 ml of phosphate buffer solution [7.4] with 0.8% tween 20 as a medium. The flask was kept at 37°C in shaker incubator at different period of time. 5ml of actuates amount was withdrawn and 5 ml buffer was incorporated to maintain sink condition. The amount of drug release from Diclofenac Gelatin microspheres was measured by UV-Visible spectroscopy at 276 nm.

RESULT AND DISCUSSION

Standard curve of Diclofenac sodium

The calibration curve was plotted between absorbance verses concentration and UV-Spectra were reported in Figure 1.

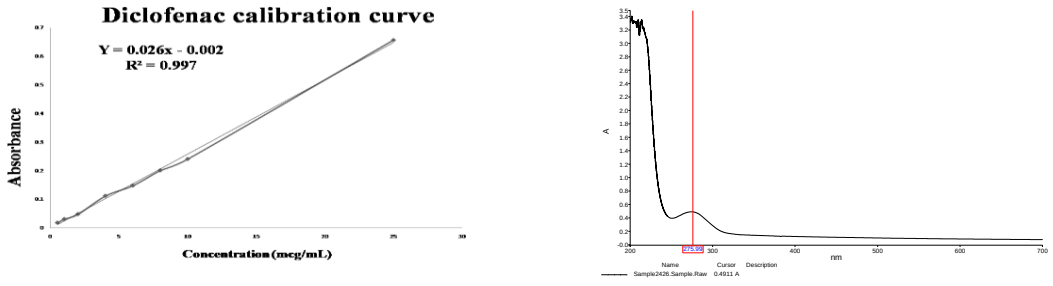

Figure 1: (A) Diclofenac calibration curve; (B) Standard curve at 275.77~ 276nm

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***In vitro* drug release study of Diclofenac Gelatin microspheres**

The release of diclofenac was found to be 93.63 ± 1.4 % after 120 min of studies. Where else 3 different combinations of 0.25%, 0.50% and 0.75% w/v solution of Diclofenac Gelatin microspheres produces 102.8 ± 1.2%, 91.6 ± 1.2% and 68.6 ± 1.4% of cumulative drug release after 300 min of *in-vitro* drug release studies. It was reported that 0.75% w/v solution of Diclofenac Gelatinwas found to have best control drug release profiling as after 300 minutes studies it was showing slow and controlled release profiling (Figure 2).

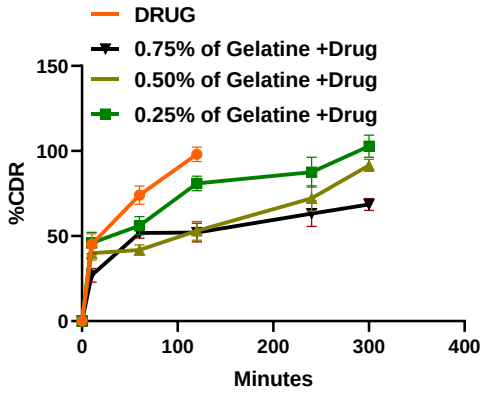

Figure 2: *In-vitro* drug release studies of Diclofenac Gelatin microspheres in three different concentrations.

Drug entrapment efficacy [%]

Drug entrapment efficacy of 0.25%, 0.50% and 0.75% w/v solution of Diclofenac Gelatin microspheres was measured by UV-Visible spectroscopically method and was found to be 64.56 ± 1.2%, 78.25 ± 2.2% & 94.45 ± 0.7% respectively; which indicating that increased concentration of Gelatin improves drug entrapment efficacy percentage.

Transition Electron Microscopy [TEM]

TEM studies of 0.75% w/v solution of Diclofenac Gelatin microspheres indicating that the prepared 0.75% w/v Diclofenac Gelatin microspheres were spherical with no aggregation and narrow distribution (Figure 3).

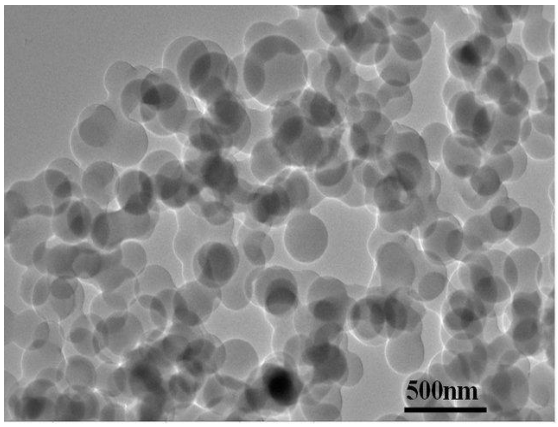

Figure 3: TEM analysis of Diclofenac Sodium microspheres containing of 0.75 % of Gelatin.

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Size distribution studies

The Diclofenac Gelatin microspheres were characterized for size distribution using dynamic light scattering technology (Zeta Sizer). The sizes of the Diclofenac Gelatin microspherewere found to be 2679.5 nm for 0.25% w/vof Diclofenac Gelatin microspheres, 4466.7 nm for 0.50% w/v of Diclofenac Gelatin microspheres & 11512.0 nm for 0.75 % w/v of Diclofenac Gelatin microspheres. Hence, it was confirmed that increased amount of Gelatin concentration increases particle size (Figure 4).

Vesicle morphology study

The Diclofenac Gelatin microspheres were observed under a Motic microscope and the particles were observed spherical in shape and surrounded by the Gelatin layer. In some figures the drug encapsulation was clearly observed. The images taken from the microscope are shown in & graph plots of particle size are mentioned in (Figure 5).

CONCLUSION

Microspheres are novel drug carriers to design effective drug delivery systems for prolong and controlling the drug delivery. The main agenda of this research work was to improve drug release profiling and entrapment efficacy of the prepared formulations. The microspheres were prepared by using phase separation coacervation technique. Numerous researches was highlighted to target inflammatory mediators by conventional techniques, but microspheres approach of Diclofenac sodium is unique within. The prepared particles not only shown good morphological characteristics but also shown higher entrapment efficacy. From the entrapment efficacy and *in-vitro* drug release profiling, it was confirmed that 0.75% w/v of Diclofenac Gelatin microspheres shows good stability and morphology. This research demonstrated that Diclofenac Gelatin microspheres improves stability of the entrapped drugs, proper dose encapsulation & enable targeted delivery to a specific type of tissue.

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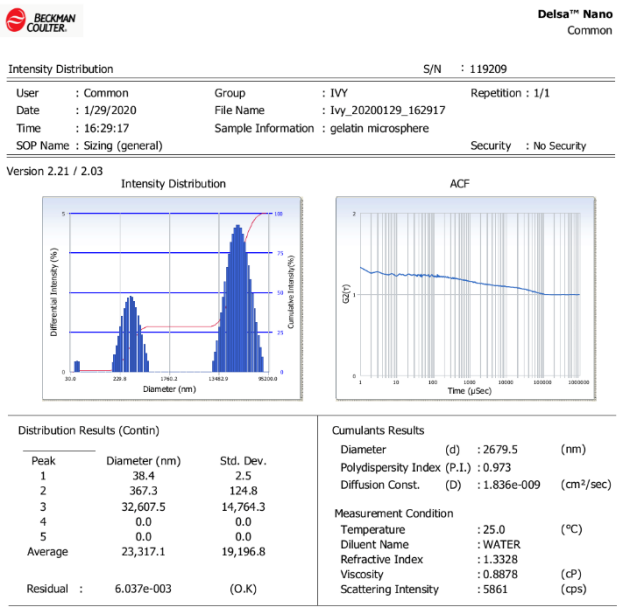
A



BECKMAN COULTER

Delsa™ Nano
Common

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SOP Name	: Sizing (general)			Security	: No Security

Version 2.21 / 2.03

Intensity Distribution

ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	38.4	2.5
2	367.3	124.8
3	32,607.5	14,764.3
4	0.0	0.0
5	0.0	0.0
Average	23,317.1	19,196.8

Residual : 6.037e-003 (O.K)

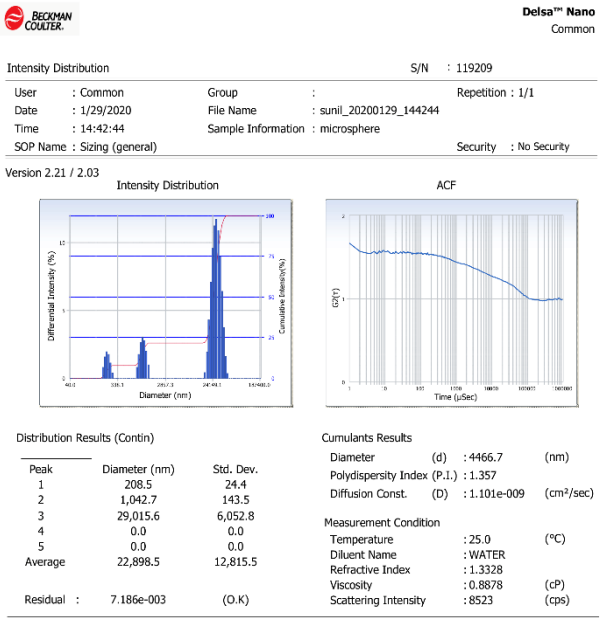
Cumulants Results

Diameter	(d)	: 2679.5	(nm)
Polydispersity Index	(P.I.)	: 0.973	
Diffusion Const.	(D)	: 1.836e-009	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 5861	(cps)

B



BECKMAN COULTER

Delsa™ Nano
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Version 2.21 / 2.03

Intensity Distribution

ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	208.5	24.4
2	1,042.7	143.5
3	29,015.6	6,052.8
4	0.0	0.0
5	0.0	0.0
Average	22,898.5	12,815.5

Residual : 7.186e-003 (O.K)

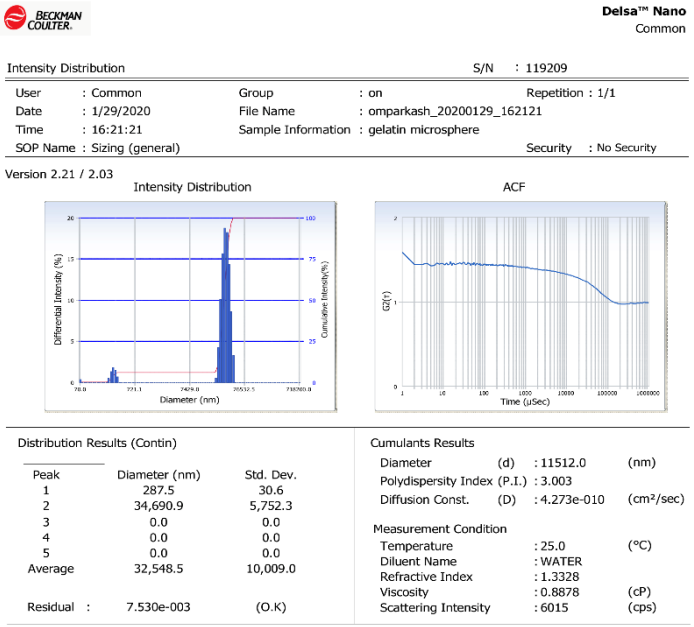
Cumulants Results

Diameter	(d)	: 4466.7	(nm)
Polydispersity Index	(P.I.)	: 1.357	
Diffusion Const.	(D)	: 1.101e-009	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 8523	(cps)

C



BECKMAN COULTER

Delsa™ Nano
Common

Intensity Distribution S/N : 119209

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Time	: 16:21:21	Sample Information	: gelatin microsphere		
SOP Name	: Sizing (general)			Security	: No Security

Version 2.21 / 2.03

Intensity Distribution

ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	287.5	30.6
2	34,690.9	5,752.3
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	32,548.5	10,009.0

Residual : 7.530e-003 (O.K)

Cumulants Results

Diameter	(d)	: 11512.0	(nm)
Polydispersity Index	(P.I.)	: 3.003	
Diffusion Const.	(D)	: 4.273e-010	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 6015	(cps)

Figure 4: Particle size analysis using Dynamic Light Scattering; (A) Particle size of Diclofenac Sodium microspheres containing of 0.25 % of Gelatin (B) Particle size of Diclofenac Sodium microspheres containing of 0.50 % of Gelatin (C) Particle size of Diclofenac sodium

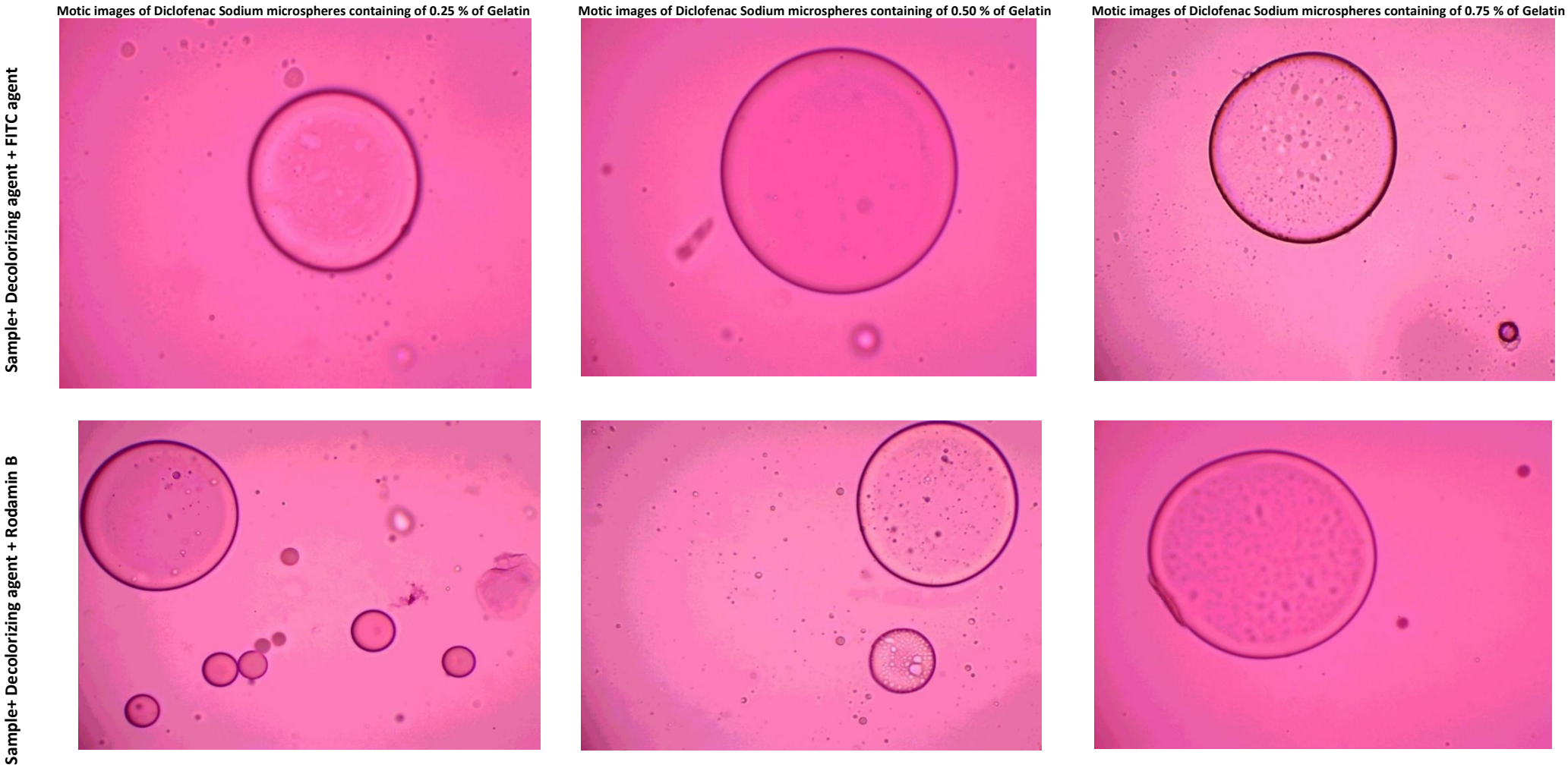

Figure 5:Morphological studies of Diclofenac Sodium microspheres containing of 0.25 %,0.50%& 0.75% W/V of Gelatin