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Formulation and evaluation of Carisoprodol microsphere for sustained drug delivery

Authors & Affiliation:

Shantaram Gajanan Khanage[1*] and Vaibhav Ramrao Ptil[2]

1.Department of Pharmaceutical chemistry, N. M. S. M.' S Abasaheb Kakade College of B. Pharmacy, Bodhegaon, Tq-Shevgaon, Dist.-Ahmednagar, Maharashtra, India-414503.

2. Department of Quality assurance technique and PG studies, M. E. S.'S College of Pharmacy, Sonai, Tq-Newasa, Dist.-Ahmednagar, Maharashtra, India-414105.

Corresponding Author:

Shantaram Gajanan Khanage

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Abstract:

Sustained release microspheres of Carisoprodol were prepared by emulsification-solvent evaporation method using ethyl cellulose as release retarding agent. The influence of process parameters such as solvent mixture, composition, concentration of the emulsifying agent and speed of stirring has been examined. Microspheres were characterized for the particle size distribution, wall thickness by scanning electron microscopy (SEM), percent drug content, entrapment efficiency and *in-vitro* dissolution studies. Fourier transform infrared (FT-IR) and differential scanning calorimetric (DSC) studies showed that there was no significant interaction between the drug and polymer. The maximum yield of the microspheres was found to be 72.5 % and the encapsulation efficiency was found to be 90.28%. The prepared microspheres were white, free flowing and spherical in shape. The *in-vitro* release studies were carried out in phosphate buffer at pH 6.8. *In-vitro* release studies showed that Carisoprodol microspheres of 1:1 ratios showed better sustained effect over a period of 7h.

Introduction:

The goal in designing sustained or controlled delivery systems is to reduce frequency of dosing or to increase the effectiveness of the drug by localization at the site of action, reducing the dose required, providing uniform drug delivery. [1,2] Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticles. [3,4] Microparticulate drug delivery systems are interesting and promising carriers for developing an oral controlled release system due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability and sustained drug release characteristics. [5,6] Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm. [7,8] They are made of polymeric, waxy or other protective material, that is biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. Microspheres are small and have a large surface to volume ratio. [9-13] At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often indicating their activity. [14-16] This drug Carisoprodol has low half life of 2 h and which is rapidly eliminated from the body. So Carisoprodol is lacking to maintain its concentration at the site of action, so frequent dosing is required conventionally. Carisoprodol is slightly soluble in water and freely soluble in alcohol, chloroform and acetone. [17]

Carisoprodol is chemically N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate. The mechanism of action of Carisoprodol in relieving discomfort associated with acute painful musculoskeletal conditions has not been clearly identified. In animal studies, muscle relaxation induced by Carisoprodol is associated with altered interneuronal activity in the spinal cord and in the descending reticular formation of the brain. So, in the present work sustained release oral microsphere was prepared, which release the drug slowly over an extended period will be able to maintain therapeutic concentration at the site of action. So, frequency of dosing is reduced, as well as better control of plasma drug levels is possible with sustained release oral delivery system with less side effects, better efficacy and safety.

Material and Methods:

The pure sample of Carisoprodol was obtained from Watson (Actavis) Pharmaceutical Pvt. Ltd, Goa, India. The AR grade Ethyl cellulose and Polyvinyl alcohol were procured from Loba chemie Pvt Ltd, Mumbai. The EP grade Dichloromethane was collected from Ozone international, Mumbai. The AR grade Ethanol, Methanol and Furfuraldehyde were obtained from Changshu chemical, China and Oxford diagnostics, Mumbai. The absorbance of the solutions was measured on UV-Visible double beam spectrophotometer (V-630) JASCO Corporation, Japan. The particle size of microsphere was determined by binocular image analyser microscope (Motic BA-210) China.

Preparation of Carisoprodol microsphere:

The microsphere containing Carisoprodol was prepared by emulsification- solvent evaporation method using an internal phase that consisted of ethyl cellulose dissolved in 8 mL dichloromethane : ethanol (1:1) the mixture was then poured into aqueous solution of polyvinyl Alcohol which served as the external phase and it was stirred at 700 rpm for 3 h under constant stirring as shown in the Table 1. The dispersed drug and polymer solution was immediately transformed into fine droplets, which subsequently solidified into rigid microspheres due to the solvent evaporation. The microspheres were collected by vacuum filtration and washed repeatedly and dried at room temperature over a night to get free flowing microsphere.

Table 1: Optimum value for microsphere formulation.

Sr.No	Specification	Optimum value
1	Drug: polymer ratio	1:1,1:2,1:3
2	Amount of drug (mg)	100
3	Polyvinyl alcohol	0.5%
4	Inner phase solvent	Ethanol and Dichloromethane
5	Amount of Inner phase solvent	8 mL
6	Amount of water in outer phase	100 mL
7	Temperature of inner phase (℃)	37
8	Stirrer type	4 blade
9	Stirring rate (rpm)	700 rpm
10	Stirring time (h)	3 h

Characterization:

The prepared microspheres were characterized by Fourier Transformed Infrared Spectroscopic analysis. The FT-IR spectral measurements were taken at ambient temperature using Shimadzu Model 8033 (USA) using a KBr pellet method by applying 6000 kg/m2 pressure to study the polymer-drug interactions. The SEM analysis was carried out by using a JEOL 5400, Japan, operates at 5 Kv to determine the size, shape and surface morphology of the prepared microspheres. DSC was performed in order to assess the thermo tropic properties and thermal behavior of the drug by covering a temperature range of 40℃ to 300℃ under nitrogen atmosphere of flow rate 100 mL/min and DSC thermogram (Mettler-Toledo DSC) for pure drug was obtained.

Results and Discussion:

Quantitative analysis of Carisoprodol

Determination of calibration curve of Carisoprodol in phosphate buffer pH 6.8

Carisoprodol (10 mg) was accurately weighed and dissolved in sufficient amount of phosphate buffer pH 6.8 and the volume was made up to 10 mL with the phosphate buffer. By using the above stock solution with appropriate dilutions of the 2, 4, 6, 8 and 10 µg/mL concentration were prepared (Table 2). They were analyzed by UV Visible spectrophotometer by measuring the absorbance at 217.2 nm. A Linearity was obtained while absorbance values were plotted against concentrations (R2>0.0998) as shown in Figure 1.

Table 2: Absorbance and conc. of Carisoprodol in phosphate buffer pH 6.8.

Sr. No	Concentration(ug/mL)	Absorbance at 217.2 nm
1	2	0.1754
2	4	0.3561
3	6	0.5662
4	8	0.7458
5	10	0.9442

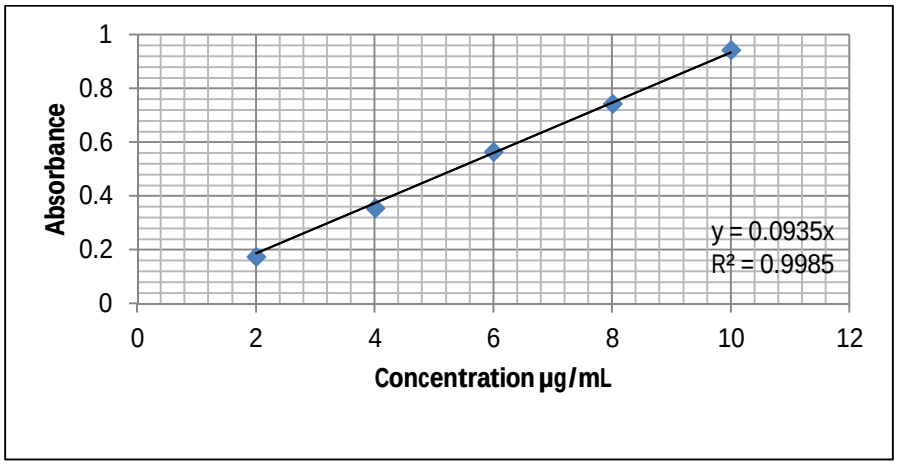


Figure 1: Calibration curve of Carisoprodol in phosphate buffer pH 6.8 (at 217.2 nm).

Evaluation of Carisoprodol Microspheres:

Determination of production yield of microsphere formulations

The production yield of all batches was observed in the range of 51% to 72.5 %. It was found that increasing the drug: polymer ratio increased the production yield (Table 3). When drug: polymer ratio was 1:1 (F1), the production yield was very low i.e. 51%, while in drug: polymer ratio 1:3 (F8), it was 72.5 %. The reason for increased production yield at high drug: polymer ratios could be due to the reduced diffusion rate of dichloromethane from concentrated solutions into the aqueous phase. This provides more time for the droplet formation and may improve the yield of microsphere as indicated in Figure 2.

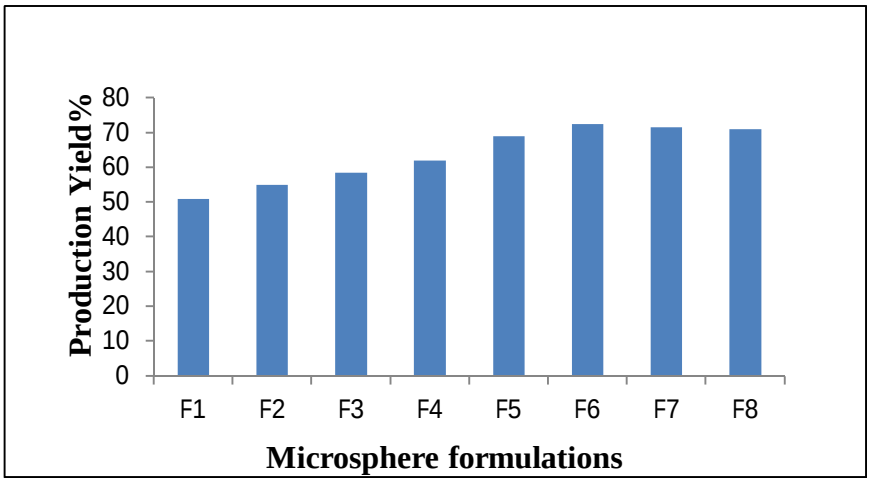


Figure 2: Graphical representation of production yield of microsphere formulations.

Table 3: Production yield of microsphere formulations.

Sr. No	Batch code	Speed (rpm)	Drug: Polymer Ratio	Theoretical Yield (mg)	Practical Yield (mg)	Production Yield (%)
1	F1	200	1:1	200	102	51
2	F2	300	1:1	200	110	55
3	F3	400	1:1	200	117	58.5
4	F4	500	1:1	200	125	62
5	F5	600	1:1	200	130	69
6	F6	700	1:1	200	142	71
7	F7	700	1:2	300	215	71.66
8	F8	700	1:3	400	290	72.5

Determination of actual drug content and encapsulation efficiency:

The result of percentage entrapment efficiency indicated maximum entrapment. The entrapment efficiency was in the range of 60.70-90.28% as shown in table 12. It was found that entrapment efficiency was greatly affected by drug: polymer ratio as well as by stirring speed. The highest (90.28 %) entrapment efficiency was achieved with polymer-drug ratio (1:1) and further increase in polymer-drug ratio from 1:1 to 1:2 and 1:3 decrease in encapsulation efficiency of Carisoprodol (Table 4). As the concentration of polymer increased the viscosity of the polymer solution increased, resulting in the formation of larger polymer/solvent droplets. The larger particles takes much time for hardening, allowing time for drug diffusion out of the particles, which tends to decrease encapsulation efficiency.

Table 4: Actual drug content and encapsulation efficiency of microsphere.

Sr. No	Batch Code	Speed (rpm)	Drug: Polymer Ratio	Theoretical Drug Content (%)	Actual Drug Content (%)	Encapsulation Efficiency (%)
1	F1	200	1:1	100	60.70	60.70
2	F2	300	1:1	100	63.82	63.82
3	F3	400	1:1	100	66.17	66.17
4	F4	500	1:1	100	67.16	67.16
5	F5	600	1:1	100	69.10	69.10
6	F6	700	1:1	100	90.28	90.28
7	F7	700	1:2	100	90.01	90.01
8	F8	700	1:3	100	88.98	88.98

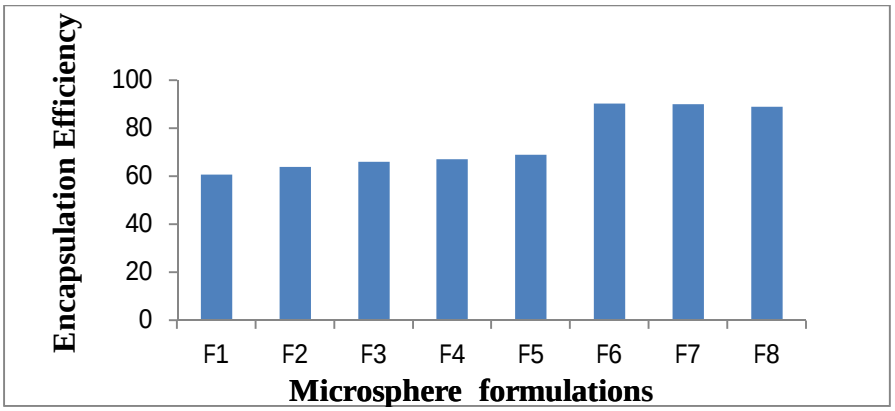


Figure 3: Graphical representation of entrapment efficiency of microsphere formulations.

Scanning electron microscopy:

The morphology of the optimized microsphere formulation (F6) prepared by emulsification-solvent evaporation method was investigated by SEM. It can be seen that at lower polymer concentrations (1:1) spherical and smooth surface microsphere was obtained. The SEM images of the microsphere are shown in Figure 4 to 8.

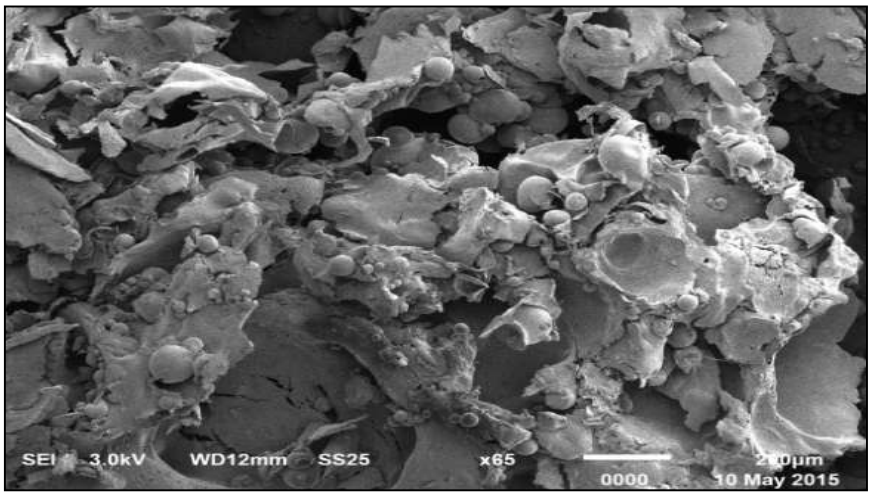


Figure 4: SEM image of optimized formulation (F6) at ×65.

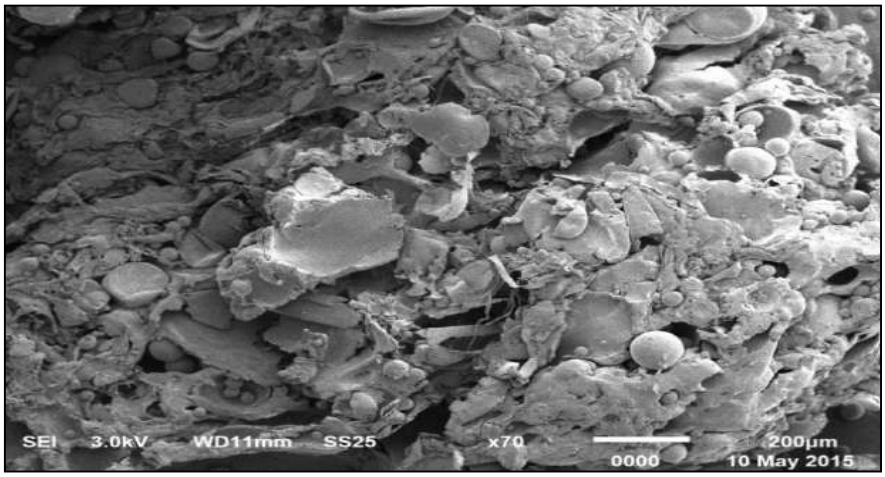


Figure 5: SEM image of optimized formulation (F6) at ×70.

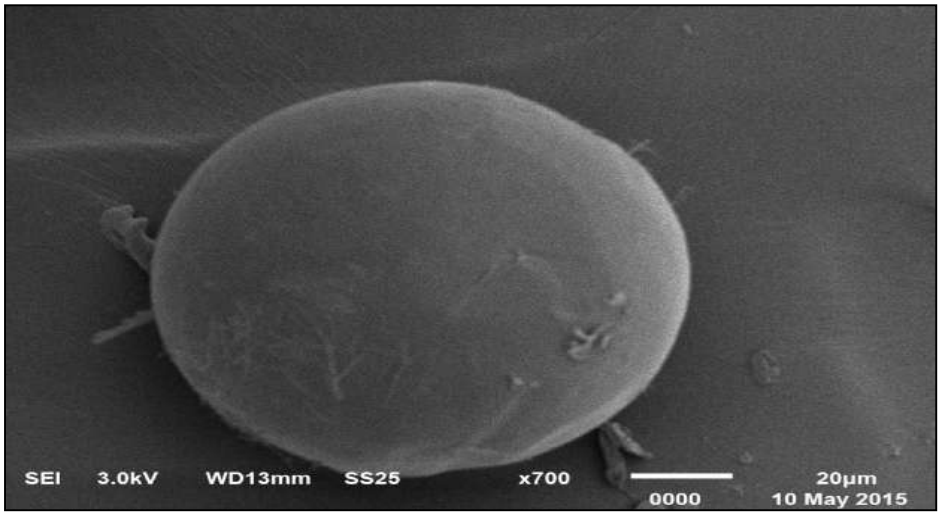


Figure 6: SEM image of optimized formulation (F6) at ×700.

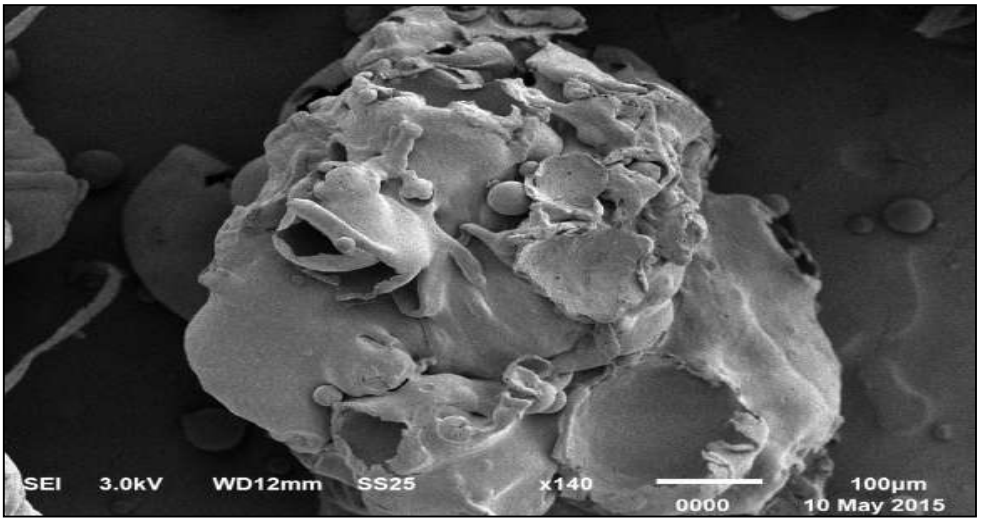


Figure 7: SEM image of optimized formulation (F6) at ×140.

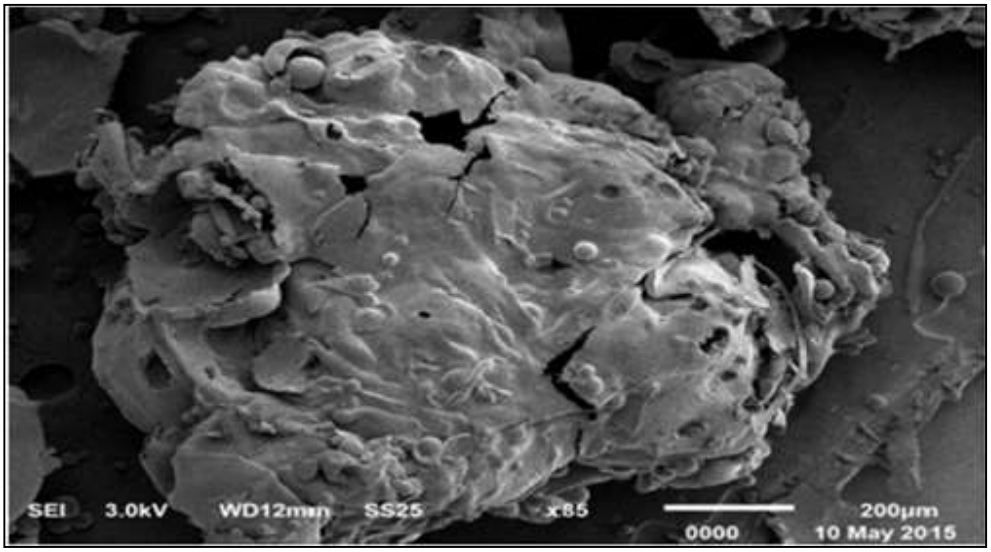


Figure 8: SEM image of optimized formulation (F6) at ×85.

The images of microsphere formulation F6 were also observed by binocular image analyser microscope (Motic) under 10x as shown in Figure 9 from binocular image it was revealed that all microsphere were spherical in shape.

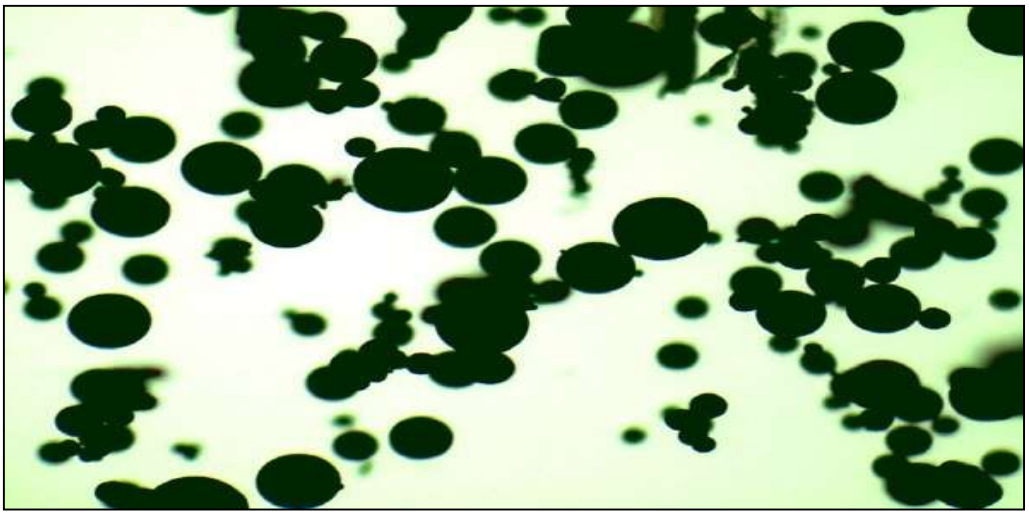
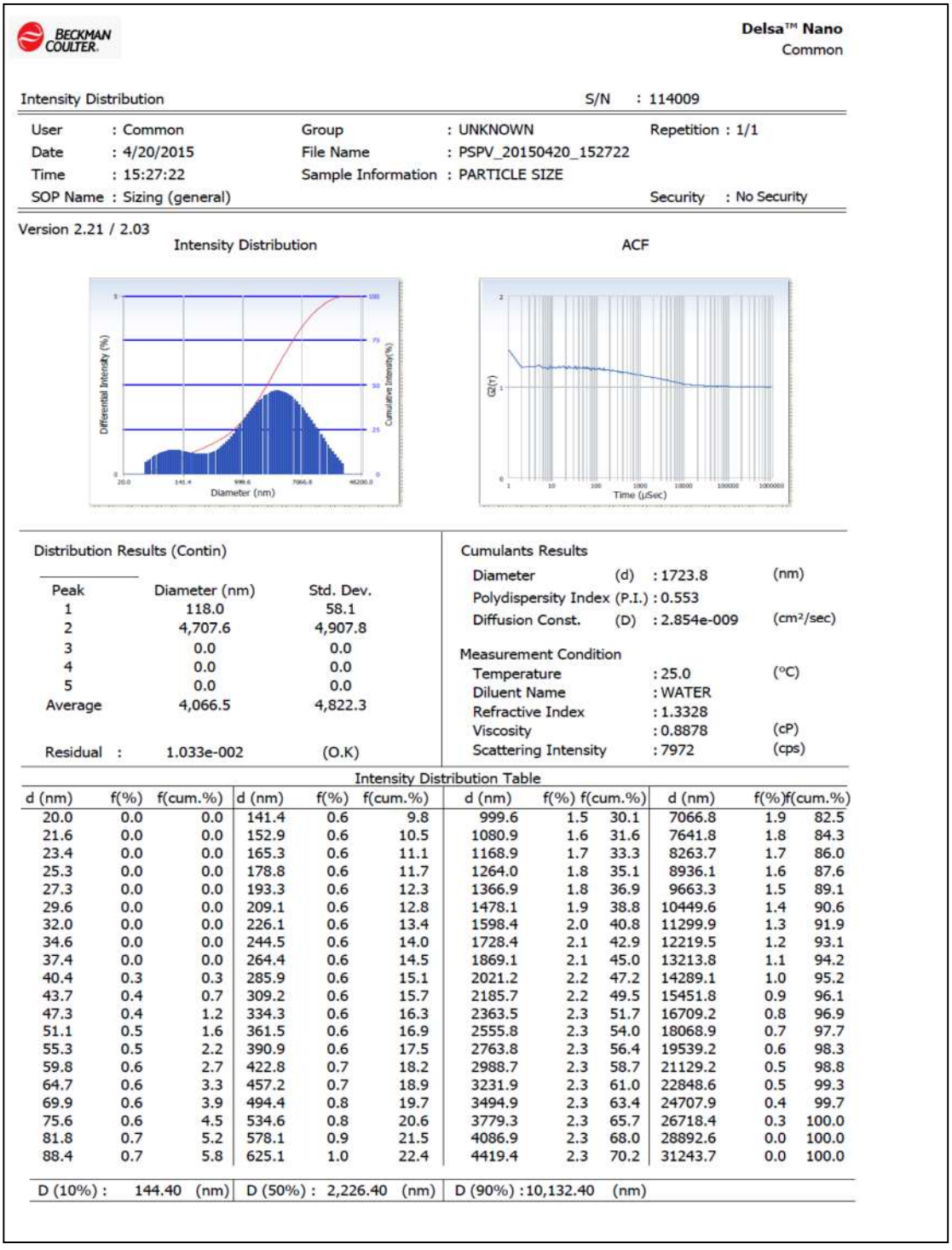


Figure 9: Photo microscopic images (10X) of microsphere formulations (F6).

Particle size analysis:

The particle size of Carisoprodol loaded microsphere was analyzed by binocular image analyzer microscope (Motic). The F6 batch possessed more, uniform, spherical particles in optical microscopy, so this batch was chosen for further analysis using photon correlation spectroscopy (Beckman Coulter, Delsa^{TM} Nano). The results of optimized microsphere formulation F6 batch revealed (Figure 10) that particle size is 10.13 µm.



BECKMAN COULTER

Delsa™ Nano
Common

Intensity Distribution S/N : 114009

User	: Common	Group	: UNKNOWN	Repetition	: 1/1
Date	: 4/20/2015	File Name	: PSPV_20150420_152722		
Time	: 15:27:22	Sample Information	: PARTICLE SIZE		
SOP Name	: Sizing (general)			Security	: No Security

Version 2.21 / 2.03

Intensity Distribution

ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	118.0	58.1
2	4,707.6	4,907.8
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	4,066.5	4,822.3

Residual : 1.033e-002 (O.K)

Cumulants Results

Diameter	(d)	: 1723.8	(nm)
Polydispersity Index	(P.I.)	: 0.553	
Diffusion Const.	(D)	: 2.854e-009	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 7972	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
20.0	0.0	0.0	141.4	0.6	9.8	999.6	1.5	30.1	7066.8	1.9	82.5
21.6	0.0	0.0	152.9	0.6	10.5	1080.9	1.6	31.6	7641.8	1.8	84.3
23.4	0.0	0.0	165.3	0.6	11.1	1168.9	1.7	33.3	8263.7	1.7	86.0
25.3	0.0	0.0	178.8	0.6	11.7	1264.0	1.8	35.1	8936.1	1.6	87.6
27.3	0.0	0.0	193.3	0.6	12.3	1366.9	1.8	36.9	9663.3	1.5	89.1
29.6	0.0	0.0	209.1	0.6	12.8	1478.1	1.9	38.8	10449.6	1.4	90.6
32.0	0.0	0.0	226.1	0.6	13.4	1598.4	2.0	40.8	11299.9	1.3	91.9
34.6	0.0	0.0	244.5	0.6	14.0	1728.4	2.1	42.9	12219.5	1.2	93.1
37.4	0.0	0.0	264.4	0.6	14.5	1869.1	2.1	45.0	13213.8	1.1	94.2
40.4	0.3	0.3	285.9	0.6	15.1	2021.2	2.2	47.2	14289.1	1.0	95.2
43.7	0.4	0.7	309.2	0.6	15.7	2185.7	2.2	49.5	15451.8	0.9	96.1
47.3	0.4	1.2	334.3	0.6	16.3	2363.5	2.3	51.7	16709.2	0.8	96.9
51.1	0.5	1.6	361.5	0.6	16.9	2555.8	2.3	54.0	18068.9	0.7	97.7
55.3	0.5	2.2	390.9	0.6	17.5	2763.8	2.3	56.4	19539.2	0.6	98.3
59.8	0.6	2.7	422.8	0.7	18.2	2988.7	2.3	58.7	21129.2	0.5	98.8
64.7	0.6	3.3	457.2	0.7	18.9	3231.9	2.3	61.0	22848.6	0.5	99.3
69.9	0.6	3.9	494.4	0.8	19.7	3494.9	2.3	63.4	24707.9	0.4	99.7
75.6	0.6	4.5	534.6	0.8	20.6	3779.3	2.3	65.7	26718.4	0.3	100.0
81.8	0.7	5.2	578.1	0.9	21.5	4086.9	2.3	68.0	28892.6	0.0	100.0
88.4	0.7	5.8	625.1	1.0	22.4	4419.4	2.3	70.2	31243.7	0.0	100.0

D (10%) : 144.40 (nm) D (50%) : 2,226.40 (nm) D (90%) : 10,132.40 (nm)

Figure 10: Particle size analysis of optimized microsphere formulation F6.

Drug - Excipients Interaction study

Drug-excipients interaction study by FT-IR spectrum

FT-IR data of Carisoprodol, ethyl cellulose and microsphere formulation F6 were shown in Table 5.

Table 5: FT-IR data of Carisoprodol, ethyl cellulose and optimized microsphere formulation F6.

Functional group	Carisoprodol	Ethyl cellulose	F6
N-H	3213 cm^{-1}	-	3213cm^{-1}
CH_3 stretching	2964cm^{-1}	2974cm^{-1}	2974cm^{-1}
C=O	1676cm^{-1}	-	1676 cm^{-1}
C-O	1300 cm^{-1}	1300 cm^{-1}	1300 cm^{-1}

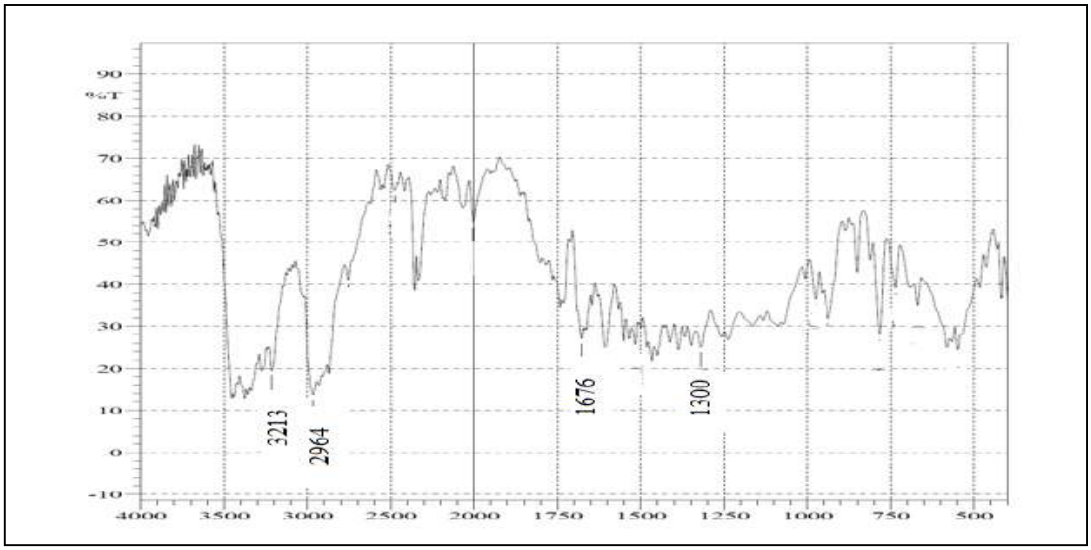


Figure 11: FT-IR spectrum of Carisoprodol.

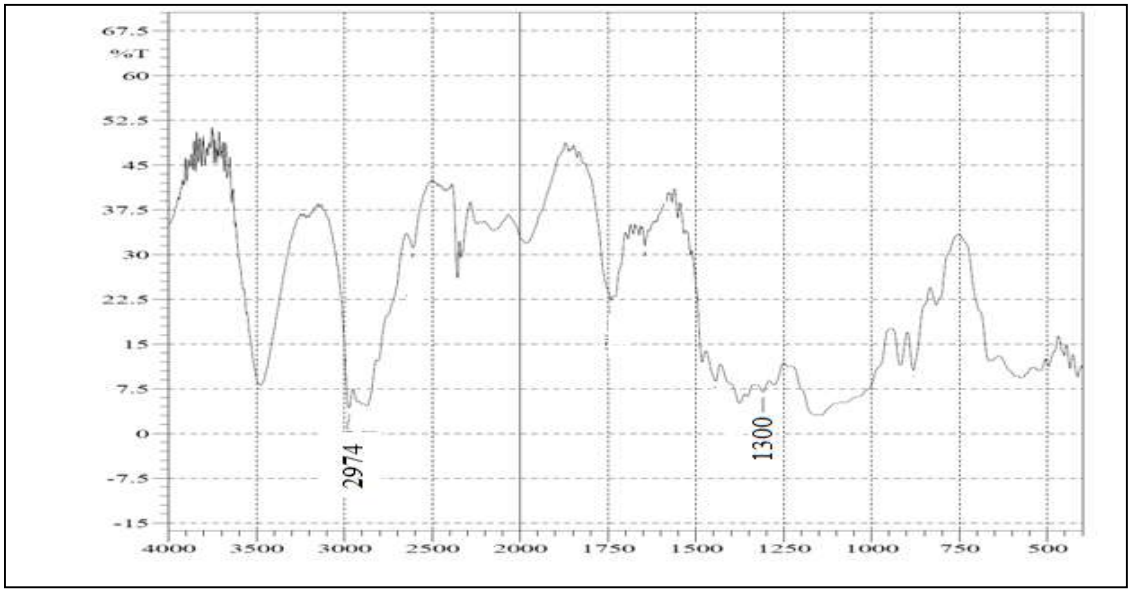


Figure 12: FT-IR spectrum of Ethyl cellulose.

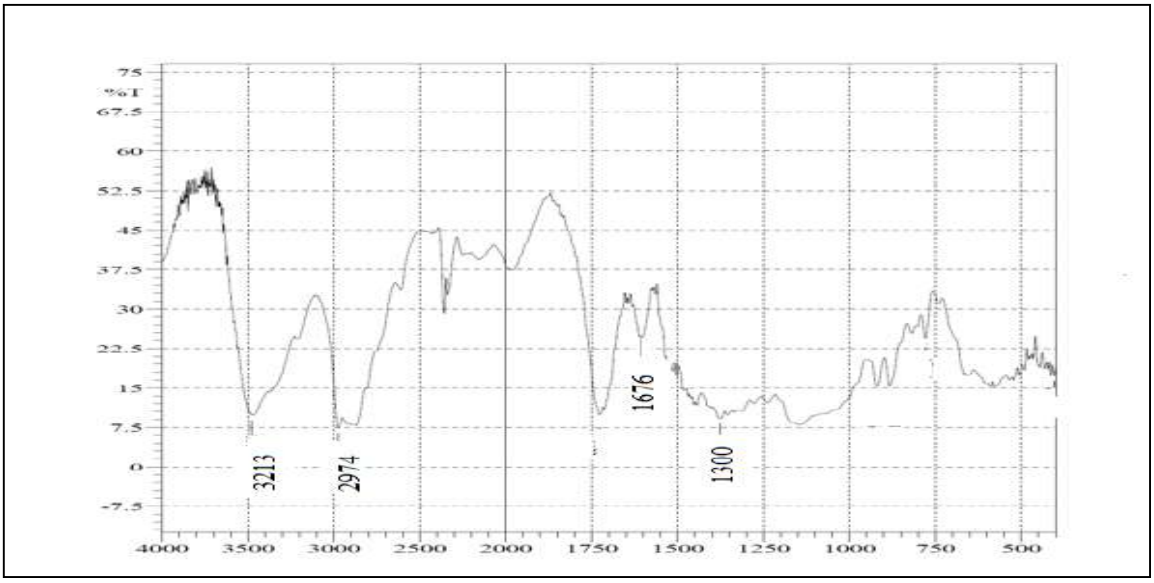


Figure 13: FT-IR spectrum of optimized microsphere formulation F6.

All the characteristic peaks of Carisoprodol were observed in the FT-IR spectrum of microsphere formulations, spectra shows that Carisoprodol was stable in microsphere formulations. From the FT-IR studies revealed that there was no appearance of new peaks and disappearance of existing parks, which indicated that there is no interaction between the Carisoprodol and ethyl cellulose used.

Drug-excipients interaction study by DSC thermogram

In order to confirm the physical state of Carisoprodol microsphere, The DSC thermogram of Carisoprodol, physical mixture of drug and polymer and Carisoprodol loaded sustained release microsphere were carried out and shown in figure 31 to 33. The DSC thermogram of Carisoprodol exhibited a sharp endothermic peak at 92.78^{0}C which is same to the melting point of drug (Carisoprodol) while the thermogram of representative, physical mixture exhibited a sharp endothermic peak at 92.78 ^{0}C which was same to the DSC thermogram of drug (Carisoprodol). Thus, from this study, it was clear that the drug was compatible with the excipients. Absence of endothermic peak at 92.78^{0}C the DSC of the optimized microsphere formulation (F6). (Figure 16) suggest that the Carisoprodol existed in amorphous or disordered crystalline phase as a molecular dispersion in a polymeric matrix.

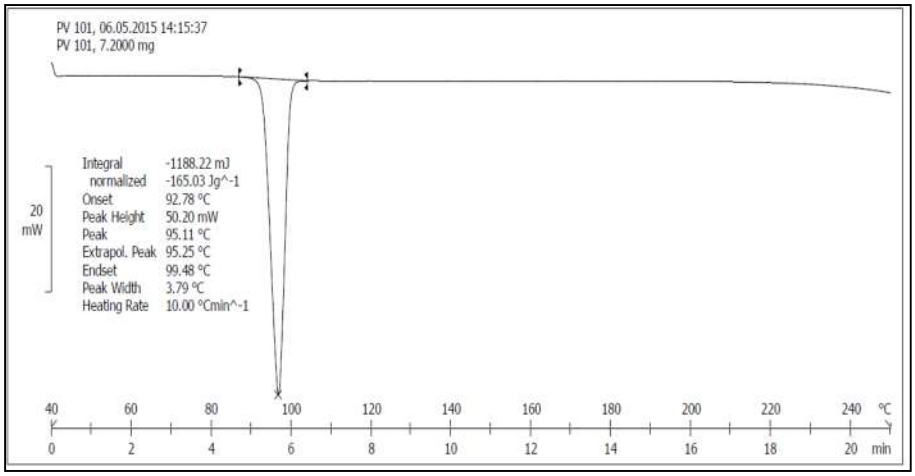


Figure 14: DSC Thermogram of Carisoprodol.

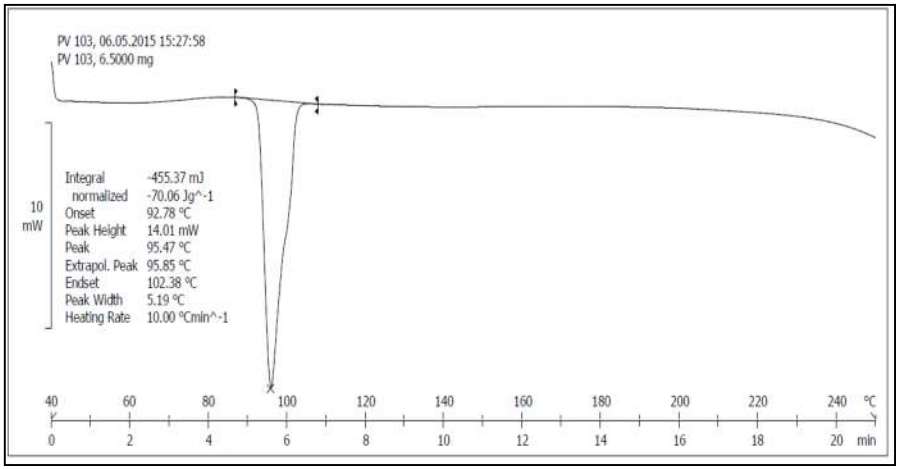


Figure 15: DSC thermogram of physical mixture of Carisoprodol and Ethyl cellulose.

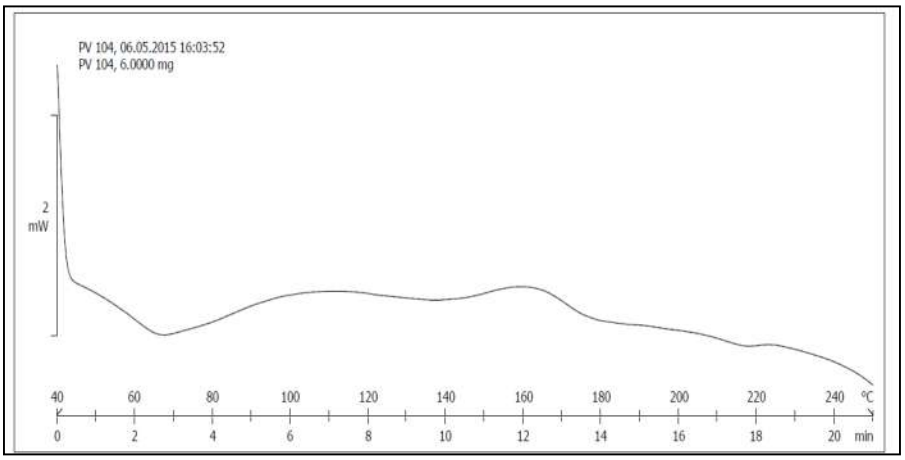


Figure 16: DSC thermogram of optimized microsphere formulation (F6).

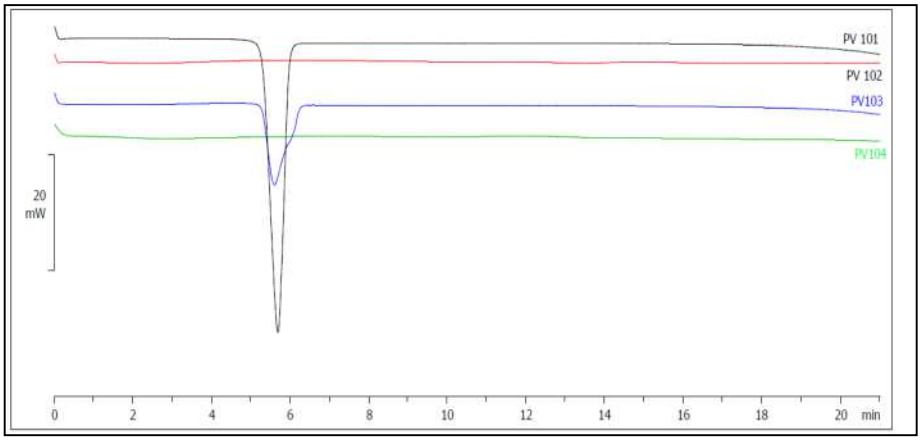


Figure 17: Overlay of DSC thermogram of drug (Carisoprodol), polymer (Ethyl cellulose), physical mixture (Carisoprodol- Ethyl Cellulose) and optimized microsphere formulation (F6).

The *in-vitro* dissolution study:

The dissolution study of Carisoprodol microsphere was carried out using the USP-type-II dissolution test apparatus in the solution at 37±0.5 ℃ with 100 rpm rotating speed. Samples of 5 mL were withdrawn at regular time interval of 30, 60, 120, 180, 240, 300, 360 and 420 min and filtered using 0.45 µm Whatman filter paper as indicated in Table 6. Drug content of the sample was analyzed using UV-spectrophotometer at 217.2 nm. All measurements were done in triplicate from three independent samples.

Table 6: % Drug release profile of F6-F8.

Time (min)	Formulations (%CDR)		
	F6±SD	F7±SD	F8±SD
0	0	0	0
30	5.52±0.005	4.91±0.001	4.40±0.005
60	18.75±0.000	5.54±0.000	17.80±0.02
120	31.37±0.005	29.82±0.000	28.39±0.005
180	45.71±0.005	44.50±0.000	43.73±0.005
240	52.30±0.005	50.44±0.005	49.88±0.005
300	56.65±0.000	55.66±0.000	53.25±0.005
360	60.37±0.000	59.10±0.005	54.17±0.005
420	65.51±0.005	61.36±0.034	55.68±0.005

SD= (Standard Deviation) n=3

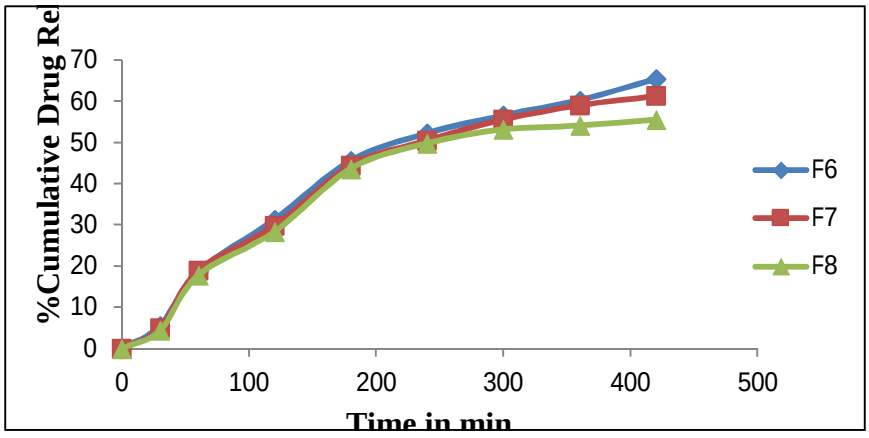


Figure 18: Comparative drug release profile of F6-F8.

The highest drug release i.e. 65.51 % was found for the formulation F6 while the lowest, 55.68 %, for F8. The formulation F6 showed better sustained release (65.51%) at the end of the 7th hour as compared to other batches. Comparative drug release profile of all batches (F6 – F8) is shown in figure 35. The drug release was found to be decreased as the drug: polymer ratio was increased. This is because as a drug: polymer ratio was increased, the amount of polymer available per microsphere to encapsulate the drug becomes more, thus increasing the thickness of the polymer wall. With the smaller drug: polymer ratios the drug release rates were found to be higher due to formation of thinner matrix wall which might lead to a shorter diffusion path.

Conclusion:

The formulation of sustained release microsphere containing Carisoprodol offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing medication for an extended period of time. In the present work sustained release Carisoprodol microspheres prepared by emulsification-solvent evaporation method using an ethyl cellulose polymer. As a final point, from the experiments carried out and the results obtained, it can be concluded that the developed formulations achieved the objective of the investigation.

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