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CYCLOPHOSPHAMIDE-GELATIN NANOPARTICLE FORMULATION FOR ENHANCED DRUG DELIVERY-DESIGN AND EVALUATION

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ABSTRACT

Cyclophosphamide (CP), usually known as cytophosphane, is a chemotherapy medication that is also used to suppress the immune system. It has a relatively quick first pass metabolism, a short elimination half-life, and a poor absorption, which results in testosterone decrease only when supplied constantly. Thus, in the current investigation, an attempt was made to include a suitable polymer into nanoparticles carrying the medication. Cyclophosphomide Nanoparticles were produced using Ionic Gelation Method combining acetic acid and gelatin in varied quantities. Various evaluation methods including the entrapment efficiency, SEM analysis, invitro drug release were done to verify the produced nanoparticles were of excellent quality. The generated nanoparticles appear to have a larger potential for drug release than ordinary polymer materials. Due to the manipulation of drug release in in vitro drug release testing, extensive in vivo estimation and drug release study are necessary to build a correct relationship between in vitro and in vivo investigations.

Keywords: Cyclophosphomide, Nano Particles, Gelatin, Drug Release.

INTRODUCTION

Cyclophosphamide (CP), commonly known as cytophosphane, is a chemotherapy drug that is also used to suppress the immune system. Lymphoma, multiple myeloma, leukaemia, ovarian cancer, breast cancer, small cell lung cancer, neuroblastoma, and sarcoma are all treated with it as chemotherapy. It is used as an immunosuppressant in a variety of disorders, including nephrotic syndrome, granulomatosis with polyangiitis, and following organ transplantation. It is given orally or through vein injection. Cyclophosphomide is a nonsteroidal antiandrogenic medication that is commonly used to treat prostate cancer. This medicine has a relatively rapid first pass metabolism, a short elimination half-life, and a low absorption, which results in testosterone reduction only when administered continuously. Cyclophosphomide is hepatotoxic at high doses [2]. Cyclophosphomide is useful in the treatment of prostate cancer only when the appropriate blood concentration is maintained for an extended period of time. Cyclophosphomide liposomes containing egg phosphotidylcholine and cholesterol remained in plasma during 24 hours, whereas the free drug was eliminated from circulation. However, the primary drawback of liposomes was their lack of long-term stability [1]. Nanoparticles have the potential to deliver Cyclophosphomide to the bloodstream for a longer period of time and with greater stability than other particulate dosage forms. Thus, in the current study, an attempt was made to incorporate a suitable polymer into nanoparticles containing Cyclophosphomide using acceptable techniques.

MATERIALS AND METHODS

Preformulation studies

A. Identification of pure drug

Solubility Analysis

Preformulation solubility analysis was done, which included the selection of suitable solvent system to dissolve the drug as well as various excipient used for the formulation of Nanoparticles.

Melting point

Fine powder of Cyclophosphomide was filled in glass capillary tube (previously sealed at one end) and kept in melting point apparatus. The melting point of Cyclophosphomide was found.

B. Compatibility Studies

The spectrums of physical mixes were compared to the original spectra in order to ascertain any molecular interactions between the medication and polymer. FTIR analysis determines the selective absorption of light by certain chemical bonds' vibration modes. The vibration spectrum of an encapsulated medicine is used to determine the type of interaction that occurs between the drug and the polymer.

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Determination of λ max

100 mg of Cyclophosphomide was weighed on an electronic balance and dissolved in 100 ml of 2% tween 80. From this stock solution-I, 10 ml solution was diluted to 100 ml with 2% tween 80 to prepare stock solution-II. Further dilution was made to obtain the concentration of 20µg/ml. The prepared solution was scanned on a UV scanner between 200-400 nm. The maximum obtained in the graph was considered as λ max for the pure drug.

Construction of Standard Curve of Cyclophosphomide

To make a 2 percent tween 80 solution, 5 g of tween 80 was placed in a 250 ml volumetric flask and a little amount of distilled water was added, dissolved effectively, and the volume increased to 250 ml with distilled water. The stock solution was made by dissolving 100mg of Cyclophosphomide in 100ml of Tween 80 solution to get a solution containing 1mg/ml of Cyclophosphomide. 5ml of stock solution was diluted to 50ml with Tween 80 solution to create a standard solution containing 100g/ml. Accurately measured aliquots of standard drug solution (100g/ml) ranging from 1ml to 5ml were transferred to a 10ml volumetric flask and diluted with Tween 80 solution to the specified concentration. As a result, the final concentration fluctuates between 10 and 50 g/ml. At 306 nm, the absorbance of each solution was determined in comparison to a Tween 80 solution used as a blank. The concentration of the medication was plotted against its absorbance.

Preparation of Cyclophosphomide Nanoparticles using Ionic Gelation Method

We generated gelatin nanoparticles by ionic crosslinking gelatin solution with TPP anions. Gelatin was dissolved in acetic acid solution (6 percent v/v) at concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml. 5ml of 0.25 percent w/v TPP aqueous solution was added dropwise to a 10ml Gelatin solution containing 10mg of Cyclophosphomide dissolved in tween 80 at room temperature. The stirring was maintained for approximately 20 minutes. The suspensions of nanoparticles obtained were centrifuged for 30 minutes at 12000x g using a C24 centrifuge [3,4].

Evaluation of Cyclophosphomide Nanoparticles

A. Determination of Nanoparticles Process Yield

The nanoparticles production yield was calculated by gravimetric method. Fixed volumes of nanoparticles suspensions were centrifuged (16,000×g, 30 min, 15ºC) and sediments were dried.

The percentage process yield (% P.Y.) was calculated as follows:

$$\% \text{ P.Y.} = \frac{\text{Nanoparticles weight}}{\text{Total solids weight}} \times 100$$

B. Particle Size Analysis

SEM microscopy was used to determine the particle size using a JOEL JSM-T330A Scanning Microscope. While the solvent paint was still wet, the pellets were deposited on each stud and allowed to dry. The sample was then viewed and photographed using scanning electron microscopy. Manual diameter measurements of around 20 particles were taken from the resulting images of each batch. Finally, mean average diameters were determined.

C. Determination of % Entrapment Efficiency

The Nanosuspension with known amount of drug (10mg/20ml) incorporated was centrifuged at 5000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100 ml of 2% w/v tween 80 solutions and the absorbance was measured using UV spectrophotometer at 306 nm using 2% w/v tween 80 as blank. The amount of drug unentrapped in the supernatant was calculated. The amount of drug entrapped and percentage entrapment was determined from drug unentrapped. Standard deviation was determined for 3 trials.

D. In vitro Drug Release Studies

The in vitro drug diffusion from the formulation was investigated using a customised equipment and egg membrane – 110 (cut off: 3500 Da). The dissolving media was freshly made tween 80 solution at a concentration of 2% w/v. The egg membrane – 110 was connected to one end of a specially made glass cylinder after being steeped overnight in the dissolving media (open at both ends). This assembly was precisely filled with 5 ml of formulation. The cylinder was mounted on a stand and suspended in 50 ml of dissolution medium maintained at 375oC, just touching the receptor media surface with the membrane. A magnetic stirrer was used to agitate the dissolving media at a low speed. At hourly intervals, aliquots of 5 ml were removed and replaced with an equivalent volume of receptor medium. The aliquots were diluted appropriately with receptor media and examined at 306 nm using a UV-Vis spectrophotometer. For the diffusion research, a dose comparable to ten milligrammes of Cyclophosphomide was administered [5, 6].

RESULTS AND DISCUSSION

Cyclophosphamide nanoparticles were formulated using different drug polymer ratios, the composition of which was shown in table 1. The formulations were evaluated for process yield, surface morphology, particle size, drug entrapment, zeta potential, and *in vitro* drug release.

PREFORMULATION STUDY

Solubility

The solubility of pure drug in 10 mg/10 ml of solvent was carried out and found to be soluble in dichloromethane, acetone, and methanol, soluble in 2%

tween 80 and 2% sodium lauryl sulphate solution and completely insoluble in water.

Identification of pure drug

The purity of Cyclophosphamide was confirmed by comparing its I.R. Spectra with the standard I.R. Spectra of Cyclophosphamide. The Table 2 showed functional groups of Cyclophosphamide and Gelatin at their respective frequencies. Table 5.3 showed the IR Spectrum values of Cyclophosphamide and Gelatin combination.

Determination of λ max for Cyclophosphamide

The absorption spectrum of pure drug was scanned between 200 – 400 nm with 20 µg/ml concentration in 2% tween 80 solutions using UV Spectrophotometer. The maximum peak was obtained at 306 nm that was taken as λ max.

Table 1: Composition of Cyclophosphomide nanoparticles

S.No	Batch code	Amount of drug (in mg)	Conc. of Gelatin (in mg)	Drug: carrier ratio
1	CGF1	10	10	1:1
2	CGF2	10	20	1:2
3	CGF3	10	30	1:3
4	CGF4	10	40	1:4
5	CGF5	10	50	1:5

Table 2: IR Spectrum Values of Gelatin, Drug and admixture

Vibrations	Wave number(cm^{-1})
Gelatin	
N-H & O-H Stretch	3359.62
C-H Stretch	2879.85
N-H Bending	1656.21
C-H Bending	1419.18
C-N Stretch	1380.03
Cyclophosphamide	
N-H Stretching	3359.27
C-H Stretching	2940.81
C=O Stretching	1715.93
Aliphatic C-H Bending	1469.85
C-N Stretching	1346.27
Aromatic C-H Bending	861.92
Cyclophosphamide and Gelatin Combination	
Aromatic C-H Stretching	3125.28
C-H Stretch	2879.38
C=O Stretch	1718.11
C-O-C Stretch	1148.75

Table 3: Process Yield and % Entrapment efficiency of Cyclophosphamide Nanoparticles

S.No	Batch code	Drug: carrier ratio	Process Yield %	% Entrapment
1	CGF1	1:1	53.25±3.52	64.52±2.11
2	CGF2	1:2	62.58±3.52	67.91±1.46
3	CGF3	1:3	71.84±2.55	69.17±1.46
4	CGF4	1:4	79.81±3.06	76.48±0.98
5	CGF5	1:5	71.09±2.64	72.32±1.13

Table 4: In Vitro Release Profile of Cyclophosphamide Nanoparticles

Time (hrs)	% Cumulative drug released				
	CGF1	CGF2	CGF3	CGF4	CGF5
0	0	0	0	0	0
1	29.07	26.83	25.72	23.70	23.45
2	33.60	30.31	28.53	25.74	28.27
3	39.22	35.90	34.22	30.37	35.42
4	45.25	41.11	38.84	33.33	41.12

6	52.81	46.19	41.72	39.02	44.75
8	58.31	53.67	46.35	44.20	51.36
10	64.88	60.78	53.17	48.87	55.60
12	69.82	67.24	61.97	53.44	58.89

Table 5: Results of Model Fitting of Cyclophosphamide Nanoparticles

Formulation	Zero order	First order	Higuchi	Peppas	'n' values
CGF1	0.8461	0.9980	0.9839	0.9894	0.3801
CGF2	0.8734	0.9947	0.9858	0.9745	0.3921
CGF3	0.8421	0.9706	0.9683	0.9596	0.3561
CGF4	0.8413	0.9992	0.9767	0.9947	0.3552
CGF5	0.8297	0.9779	0.9805	0.9617	0.3955

Fig: 1 IR Spectrum a. Cyclophosphamide; b. Gelatin; c. Formulation

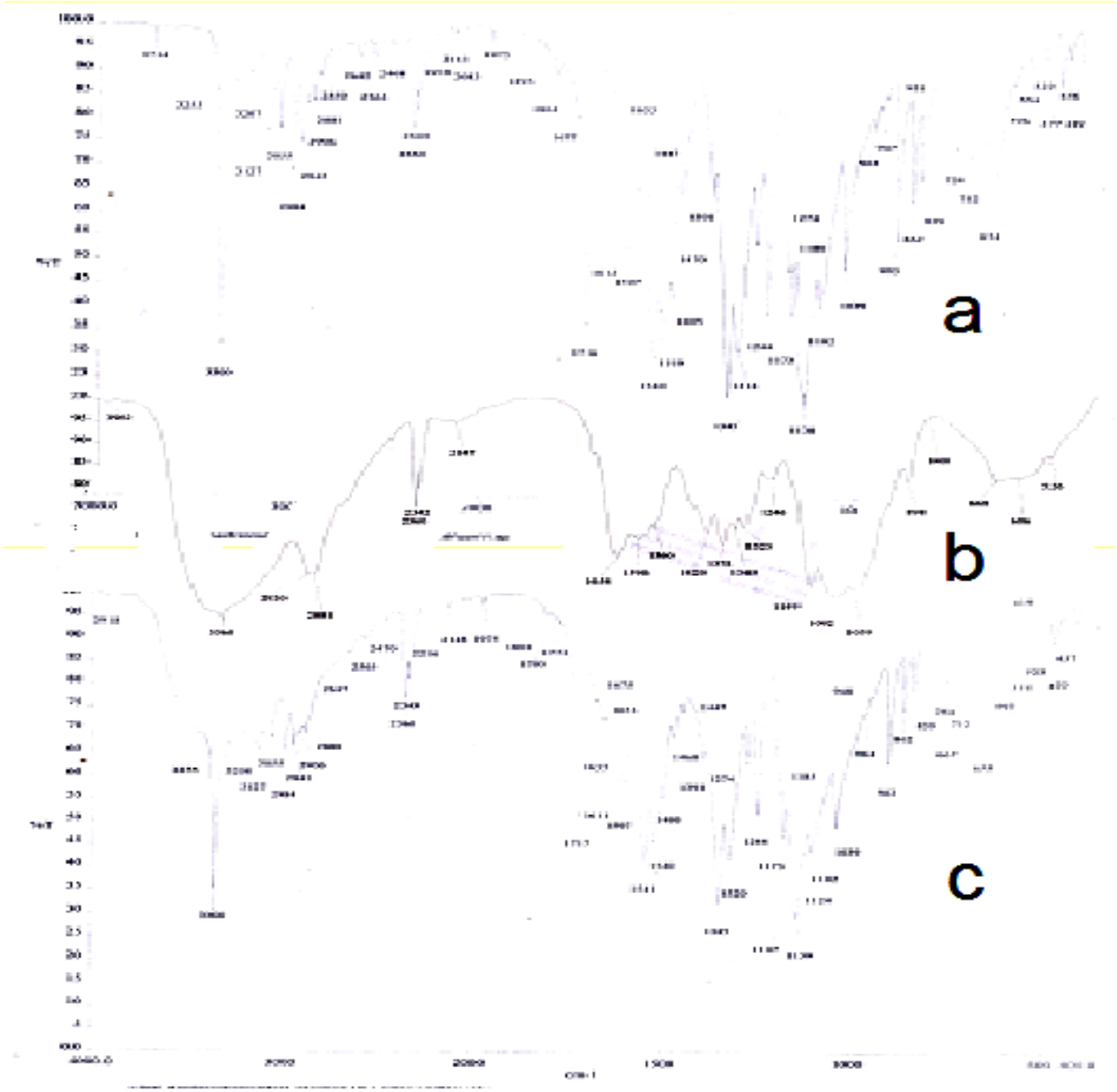


Fig: 2 Standard Curves for Cyclophosphamide by UV Method

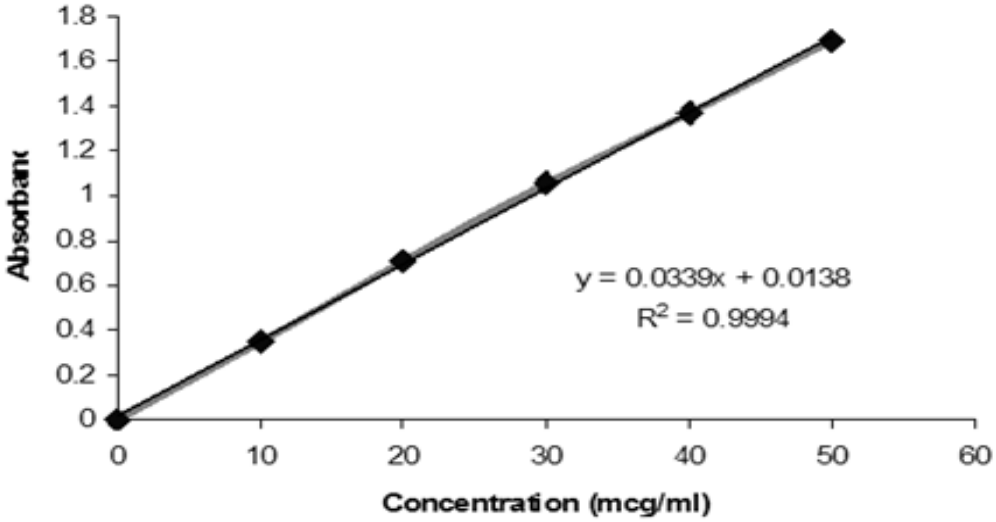


Fig: 3 SEM of formulation CGF4

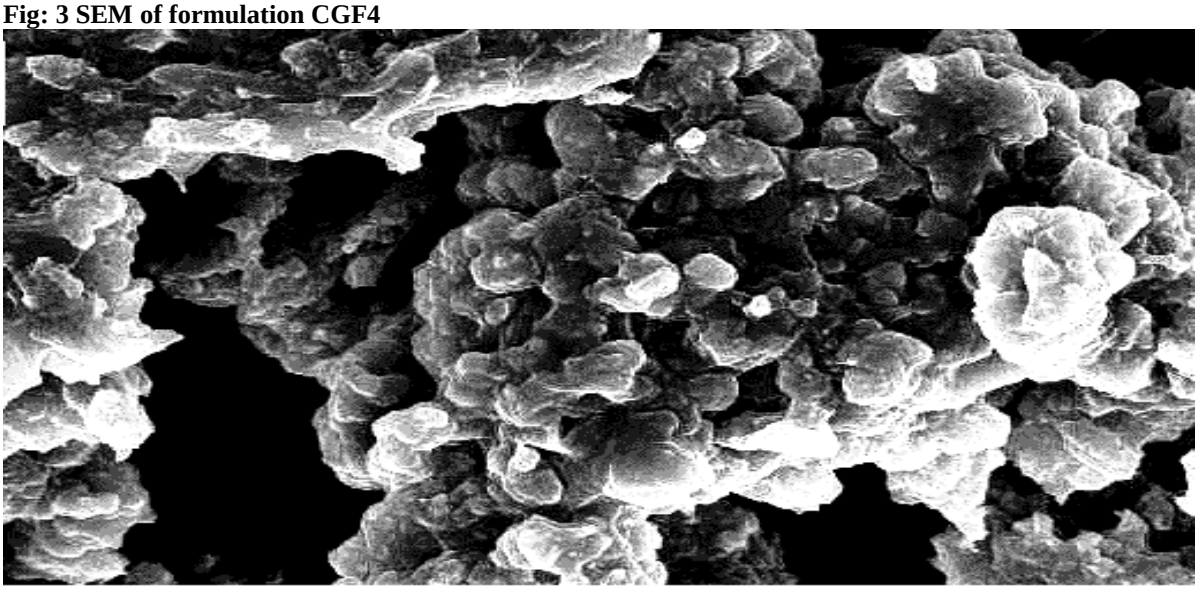
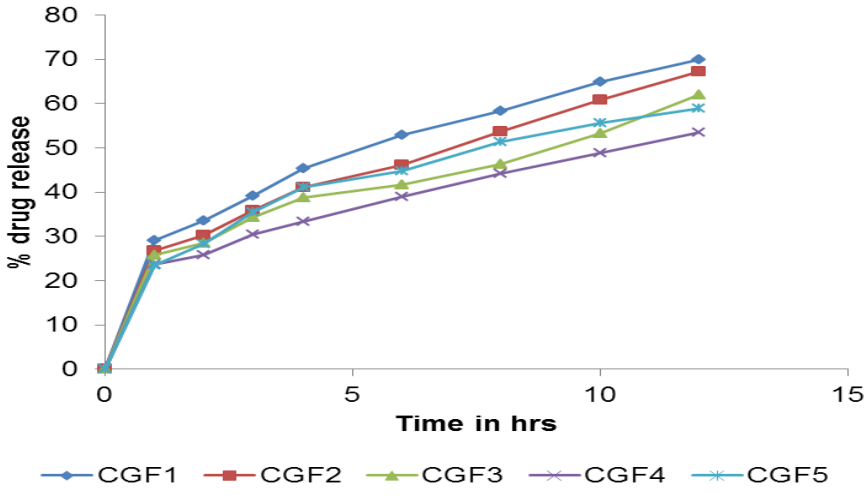


Fig: 4 Comparative In Vitro Release of Cyclophosphamide Nanoparticles



Standard curve of Cyclophosphamide

The absorbance of Cyclophosphamide standard solutions containing 10-50µg /ml of drug in 2% Tween 80 solution was obtained. Figure 5.1 showed the standard calibration curve with regression value of 0.994. The curve was found to be linear in the range of 10-50 mcg/ml at λmax 306 nm.

The hydrophobic drug Cyclophosphamide was successfully entrapped by dissolving it in 2% tween 80 prior to inclusion into the gelatin solution. The formulation conditions were chosen based on the findings of early research that examined the influence of the solvent volume and concentration of the gelatin solution on the nanoparticles' physicochemical properties. The % yields of nanoparticles were shown in Table 3. CGF4 batch, which comprises drug and polymer in a 1:4 ratio, demonstrated the highest percentage production of nanoparticles. The percentage particle yield rose as the polymer concentration increased.

Entrapment efficiency of Cyclophosphamide nanoparticles

The entrapment effectiveness of Cyclophosphamide nanoparticle formulations CGF1, CGF2, CGF3, CGF4, and CGF5 containing Drug:Polymer in various ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 was measured and tabulated. The entrapment efficiency increased steadily when the polymer content in the formulation was increased. CGF4 had the greatest entrapment rate of 79.81 percent and was determined to be the best formulation.

Particle size and surface morphology

Scanning electron photomicrographs of formulation CGF4 was shown in Fig 3. Particles of all formulations were in nanoparticles having smooth surface. The particle size was in the range of 400 nm.

***In vitro* drug release profile of gelatin nanoparticle**

The release data for formulations CGF1, CGF2, CGF3, CGF4, and CGF5 were tallied, resulting in graphs showing cumulative percent drug released as a function of time for all five formulations.

After 12 hours, the cumulative percentages of CGF1, CGF2, and CGF3 released were greater than the cumulative percentages of CGF4 and CGF5. After 12 hours, the cumulative percentage release of CGF1, CGF2, CGF3, CGF4, and CGF5 was 69.82 percent, 67.24 percent, 61.97 percent, 53.44 percent, and 58.89 percent, respectively. Cyclophosphamide in vitro release was seen to exhibit a fast first burst followed by a very sluggish drug release. A rapid first release shows that some medicine was localised on the nanoparticles' surface. CGF4 had a prolonged release profile when compared to other formulations, and it was deemed the best formulation.

To characterise the release kinetics of each of the five formulations, the corresponding dissolution data were fitted using several kinetic dissolution models, including zero order, first order, and Higuchi. The calculated regression coefficients are tabulated for each formulation. For the model and drug equations, these values were compared to one another. As evidenced by greater R2 values, all formulations release the medication according to the first order and Higuchi models. Because it was proved to be a Higuchi model, the release mechanism was regulated via swelling and diffusion.

CONCLUSION

A successful attempt was made to make and analyze Gelatin-based nanoparticles integrated with Cyclophosphomide. Numerous ratios have been found to be near optimum for Nanoparticles. The produced nanoparticles appear to have a greater potential for drug release than standard polymer materials. Due to the modulation of drug release in in vitro drug release tests, comprehensive in vivo estimate and drug release research are required to establish a correct connection between in vitro and in vivo studies.

REFERENCES

1. Krishna RSM, Shivakumar HG, Gowda DV, and Banerjee S. Nanoparticles: a novel colloidal drug delivery system. *Indian J Pharm Edu Res* 40(1), 2006, 15-21.
2. Monach PA, Arnold LM, Merkel PA, *et al*. "Incidence and prevention of bladder toxicity from cyclophosphamide in the treatment of rheumatic diseases: a data-driven review". *Arthritis and Rheumatism*. 62(1), 2010, 9–21.
3. Shabouri EI MI, *et al*. Positively charged nanoaprticles for the oral bioavailability of cyclosporine-A. *Int J Pharm* 2003, 249, 101-08.
4. Pandey R, Ahmad Z, Sharma S, Khullar GK, *et al*. Nanoencapsulation of azole antifungals: Potential applications to improve oral drug delivery. *Int J Pharm* 301, 2005, 268-76.
5. Joseph Nisha M, Palani S, Sharma PK, Gupta MK, *et al*. Development and evaluation of nanoparticles of mitomycin-c. *J Pharma Res* 5, 2006, 53-6.
6. Sivakumar T, Prabal Kumar M, Sundar Rajan T, Mahmoud A, Rajappan M, *et al*. Design and evaluation of diclofenac sodium megaloporous matrix system aimed for colonic drug delivery. *Iran J Pharm Sci*, 3, 2007, 1-12.