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Formulation and Evaluation of Nanospheres of 5-Flourouracil

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ABSTRACT

The aim of the present study is to prepare and evaluate nanospheres containing 5-flourourocil using HPMC, chitosan as the polymer. The 5-flourourocil nanosphers were prepared by Emulsion technique followed by solvent evaporation as an efficient nanodrug preparation technology. Total two batch prepared by the different grades of HPMC. Formulation was optimized and characterization and evaluation test was performed The 5-flourourocil loaded nanosphers were formed by the Emulsion technique followed by solvent evaporation. The physicochemical properties, % drug content, % entrapment efficiency and in-vitro release varied from 245 to 936 nm, 94 to 100%, 92-99% and 9 to 86%, respectively for the formulated sample. Release kinetics showed diffusion-controlled and Non-Fickian release pattern. The zeta potential of three formulations achieved optimum values between -21.1 and 29.6 mV. The FLA5 and FLB5 showed the least particle size, optimum zeta potential range, moderate drug loading efficiency followed by sustained drug release over 12 hours. Hence, this formulation satisfactorily maintain the bioavailability and targeting efficiency towards cancer cell.

Keywords: 5-flourourocil, Nanospheres, Chitosan, Targeted drug delivery system.

1. INTRODUCTION

Nanotechnology is the blend of science, engineering, and technology used in the production of nanoscale material. In pharmaceutical industries, the nanotechnological approach is used for its nano-range, controlled or sustained release drug delivery, improved therapeutic efficacy, improved bioavailability, drug delivery at targeted or affected sites, accurate dose with lesser or no adverse/side effects at other sites of the body, restrain hypersensitivity reaction, improved solubility of lipophilic drugs, improved stability and higher drug permeability [1, 2]. Now day's nanotechnology is the foremost approach used in the pharmaceutical industry since the beginning of the 21st century for its several advantages. The nano approach has been used in the formulation of medications for different routes of administration and treatment of many acute and chronic diseases. Nanotechnology has numerous platforms that include liposomes, polymeric nanoparticles (nanocapsules and nanospheres), nanosponges, nanoparticles, dendrimers, micelles, and nanoconjugates. [3, 4]

Nanospheres are colloidal particles of 10–200 nm that are spherical, polymer matrix-type nanoranged devices that consist of drug molecules present in dispersed phase in the polymer matrix [5]. Nanospheres are amorphous or crystalline in nature, and the drug molecules are dispersed in a solid skeleton formed by a polymer matrix. The Nanospheres are devised in order to tailor (control or sustain) the drug release, reduce the dosing frequency, and deliver the drug at the targeted or affected site. [6]

They may be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of materials is dependent on many factors including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; and (e) drug release profile desired. [7] 5-Fluorouracil (initially 7-12 mg/kg iv for 4 days), a cell-cycle-phase-specific anti neoplastic agent, is indicated in colon, rectal, breast, ovarian, cervical, gastric, oesophageal, bladder, liver, and pancreatic cancer. Fluorouracil exerts its cytotoxic activity by acting as an anti metabolite, competing for the enzyme that is important in the synthesis of thymidine, an essential substrate for DNA synthesis. The hydrophillicity of 5-Fluorouracil allowed it to complex with dendrimers after simply incubating the polymer with the drug. [8, 9] The primary goals of nanoparticles in drug delivery include more specific drug targeting and delivery, reduction in toxicity while maintaining therapeutic effects, greater safety and biocompatibility, and faster development of new safe medicines. [10]

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals: 5-fluorouracil (5-FU, purity 99%) was obtained from Loba Chemie Pvt. Ltd. Chitosan was purchased from Indus valley Bioorganic. HPMC was obtained from SD Fine Chemical Maharashtra, India and other chemicals were of analytical grade obtained from college laboratory.

2.2 Fourier transform infrared spectral analysis: The FTIR spectrum of 5-flourourocil was observed by preparing potassium bromide pellets. The finely ground 5-flourourocil powder was mixed with powdered potassium bromide and was pressed with a specific hydraulic compression. The prepared KBr pellet was then observed under Fourier transform infrared spectrometer (FTIR) and the spectrum was recorded. [11]

2.3 Determination of absorbance maxima (λ_{max}): 100 mg of 5-flourourocil was accurately weighed by calibrated digital weighing balance and was dissolved in small quantity of methanol. The wavelength at which maximum absorbance was shown by both the dilutions, was recorded as absorbance maximum for 5-flourourocil. [12, 13]

2.4 Formulation Of 5-Flourourocil Nanospheres: Emulsion prepared drug (5-flourourocil) nanospheres followed by solvent evaporation process, and various polymer forms were used.

i) Polymer and Drug Preparedness Solution:

- Weighed the polymer needed and put in a dry beaker.
- Required solvent quantity (methanol) was taken from a measuring cylinder.
- Now, gradually adding methanol to the beaker that contains polymer was applied.
- Then, it was continuously stirred to form a polymer solution with glass pin.
- Attach Linagliptin 300 mg, precisely measured, and blend thoroughly.

ii) Aqueous solution prepared: Weighed the necessary amount of SLS 1 g in 1000mL of water and then retained one side to eliminate air bubbles.

iii) Nanosphere Preparation: 5-flourourocil Nanospheres were prepared using the Emulsion technique followed by solvent evaporation as an efficient nanodrug preparation technology. Polymers dissolved in chloroform then 10 mg of 5-flourourocil drug was fully dispersed in polymer solution and 1% SLS solution applied to this under stirring at 400-500 rpm up to 20 min then beaker put in sonicate to probe for 15min after sonication held for continuous stirring by magnetic stirrer and temperature held at 10 rpm using ice bath. Nanospheres instantly emerged after mixing. [14, 15]

Table 1: Composition of Nanospheres with HPMCK4M

Ingredients	Formulations								
	FLA1	FLA2	FLA3	FLA4	FLA5	FLA6	FLA7	FLA8	FLA9
Drug (gm)	10	10	10	10	10	10	10	10	10
HPMC K4M (mg)	75	150	225	-	-	-	75	150	225
Chitosan (mg)				75	150	225	75	150	225
Ethyl cellulose (mg)	75	150	225	75	150	225			
Chloroform (ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10
SLS 1% (ml)	50	50	50	50	50	50	50	50	50

Table 2: Composition Of Nanospheres with HPMCK100

Ingredients	Formulations								
	FLB1	FLB2	FLB3	FLB4	FLB5	FLB6	FLB7	FLB8	FLB9
Drug (gm)	10	10	10	10	10	10	10	10	10
HPMC K100 (mg)	75	150	225	-	-	-	75	150	225
Chitosan (mg)				75	150	225	75	150	225
Ethyl cellulose (mg)	75	150	225	75	150	225			
Chloroform (ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10
SLS 1% (ml)	50	50	50	50	50	50	50	50	50

2.5 Characterization And Evaluation:

2.5.1 Determination Of Drug Content: 1 mg equivalent of nanospheres dispersion is dissolved in 1 ml of ethanol and the volume is made upto 100 ml to make 10 ug / ml concentration and the absorbance is measured at 345 nm. From the absorbance drug content is measured.

2.5.2 Determination Of Entrapment Efficiency: Entrapment efficiency of 5-flourourocil loaded nanospheres is determined by Centrifugation method. The nanoparticles are separated in a high speed cooling centrifuge at 14,000 rpm for 90 minutes at 4 °C. The sediment and supernatant liquid are separated. The supernatant solution is made up to desired volume with buffer. The amount of drug that is not incorporated in the nanospheres could be obtained by the UV –spectrophotometer. The absorbance of the samples is measured at 345 nm to estimate the percentage entrapment efficiency. The entrapment efficiency is calculated by following formula: [16, 17]

% Entrapment efficiency= Amount taken – Free drug / Amount taken × 100.

2.5.3 Determination of Zeta Potential: Zeta sizer (ZS 90 MALVRN) analyzed the size, size distribution, and zeta potential of the nanospheres. Potential zetapotential samples were held in the zeta sizer analysis chamber for its peak to collect zeta-potential data for analysis. In analyzing these results, monodisperse character is often taken into account instead of polydisperse character. [18]

2.5.4 Particle Size Analysis: The mean diameter of nanospheres in the dispersion was determined by photon correlation spectroscopy (PCS) using a laser light scattering instrument (LS230; COULTER) at a fixed angle of 90° at 25 °C. The particle size analysis data was evaluated using the volume distribution. Before measurement 1 ml of sample is diluted with distilled water. [18]

2.5.5 In Vitro Drug Release: In vitro release of 5-flourourocil from 5-flourourocil loaded nanospheres formulations was determined by dialysis bag method using 0.5 % Sodium lauryl- sulphate solution as dissolution medium. Samples were analyzed for 5-flourourocil drug content spectrophotometrically by measuring the absorbance at 345 nm against a suitable solvent blank. [19]

2.5.6 Kinetic Modeling: In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of nanosphers were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs time), Higuchi's model (cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots. The exact mechanism by which the nanospheres formulations follows is determined by korse-meyer peppas model (log drug release vs log time). [19]

2.5.7 Stability Studies: According to modified ICH guidelines, all formulations of 5-flourourocil -loaded nanospheres were subjected to stability studies at 25±2 °C and 60 ± 5 RH for 1 month also at 4 °C and the entrapment efficiency is estimated for all the formulations at 1 week time intervals for 1 month. [20]

3. RESULTS AND DISCUSSION

3.1 Identification Of The Drug 5-Flourourocil By FTIR Spectra: The FTIR spectra of the given sample showed comparable major absorption bands with that of reference standard of 5-flourourocil. The similarity in the characteristic peaks of obtained drug with that of reference standard confirmed the identity of the drug (Figure 1). The characteristic peaks represented the functional groups present along with the wave numbers associated with the structure. The FTIR spectrum of 5-flourourocil presented the characteristic peaks at 2830.91- 3136.40 cm^{-1} due to –C-H- stretching vibrations. The stretching due to aromatic ring was absorbed by rare pick at wave number at 3069.29 cm^{-1}, –C-O stretching was characterized at 1224.69-1182.09 cm^{-1}, -NH- stretch of amine

group was observed at 1349.35 cm^{-1}, peak at 1661.91 cm^{-1} was due to stretching of –C=O- and spectrum from 752.90 cm-1 indicating C-F stretching respectively.

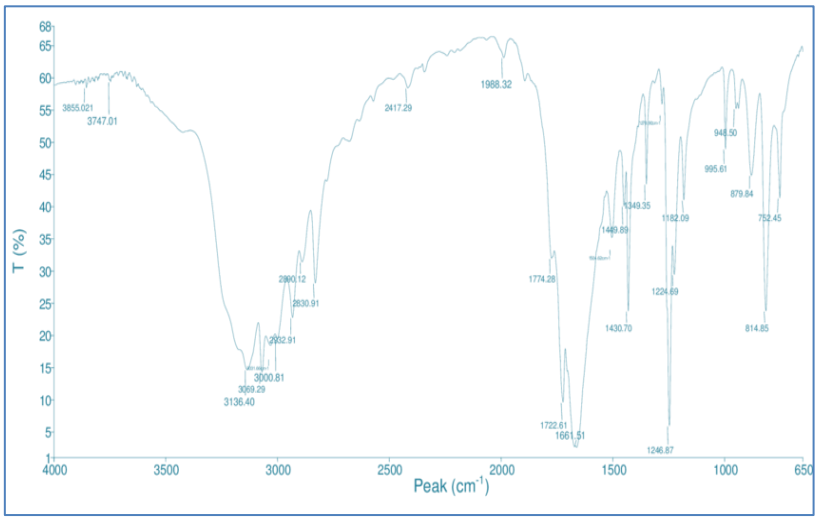

Figure 1. FTIR Spectrum of 5-flourourocil

2.2 Determination Of Absorption Maxima Of 5-Flourourocil: The λmax of 5-flourourocil was found to be 266.4 nm in methanol as solvent. The scanning of the drug was done in the range (200-400 nm) as shown in the Figure 2. The calibration plot of 5-flourourocil was prepared by taking 4, 8, 12, 16, 20 µg/ml. The experiments were performed in triplicate to find the standard deviation and percentage relative standard deviation. Absorbance range was found to be 0.165-0.787. The regression coefficient (R^2 value) was 0.9993 which showed linearity between 4-20 µg/ml concentrations. The Lambert Beer law was obeyed within the linearity range. The standard regression equation was found to be y = 0.0422 x + 0.0042.

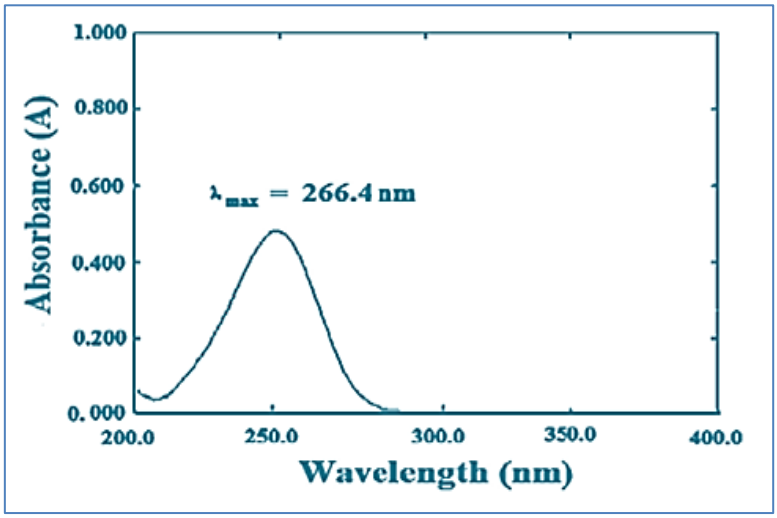

Figure 2. Scan of 5-flourourocil in methanol

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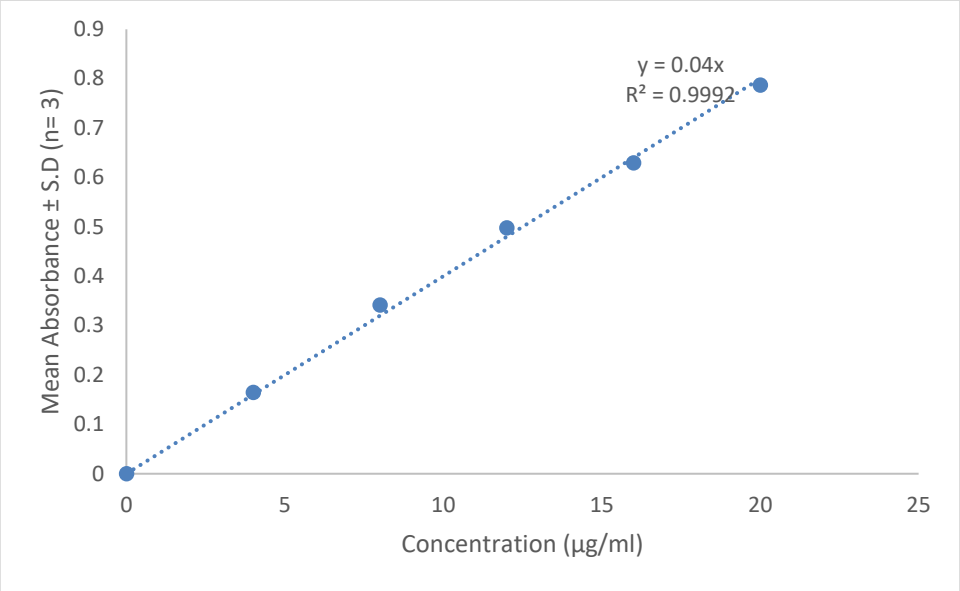

Figure 3. Calibration curve of 5-flourourocil in methanol at 260 nm

2.3 Preparation Of Nanospheres: The emulsion solvent evaporation technique is a method for preparing nanospheres that are particularly adapted for applications requiring materials with high purity and low toxicity, such as for biomedicine or electronics. The composition of the formulations are shown in table 1 and 2. The prepared nanospheres was found to be uniform and homogenous in appearance.

2.4 Characterization And Evaluation

2.4.1 Physicochemical Properties, % drug content and % Entrapment efficiency: The nanospheres was white in color, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 minutes). The drug content of all the formulations varied from 94 to 100 % and are shown in table 3. The results of EE were shown in the table 4. Among the 2 different HPMC used, chitosan showed the highest EE when compared to Ethyl cellulose.

Table 3: % Drug content

Formulation	% Drug content	Formulation	% Drug content
FLA1	94.11	FLB1	94.75
FLA3	95.34	FLB2	97.70
FLA3	95.87	FLB3	97.98
FLA4	96.70	FLB4	98.70
FLA5	99.85	FLB5	100.0
FLA6	99.23	FLB6	99.00
FLA7	98.11	FLB7	97.70
FLA8	99.00	FLB8	98.11
FLA9	99.43	FLB9	99.45

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Table 4: Entrapment Efficiency of All Formulations

Formulation	% Entrapment Efficiency	Formulation	% Entrapment Efficiency
FLA1	90.40	FLB1	92.40
FLA3	95.80	FLB2	95.80
FLA3	94.70	FLB3	92.70
FLA4	97.00	FLB4	96.00
FLA5	98.50	FLB5	99.50
FLA6	99.10	FLB6	45.10
FLA7	89.20	FLB7	95.20
FLA8	92.70	FLB8	97.70
FLA9	94.40	FLB9	96.40

2.4.2 Zeta potential and Particle Size Analysis: Zeta potential is an essential factor to evaluate the stability of nanodispersion. Zeta potential values mainly reflect the electrical repulsion between the particles. The average zeta potential value of 5-flourourocil loaded nanospheres was in the range of −21.1 mV and 29.6 mV respectively. An apparent shift in zeta-potential values to less negative values, which was accompanied by an increase in the particle size. The particle size analysis of the nanospheres formulations was estimated by dynamic light scattering technique. The particle size values are shown in table 5.

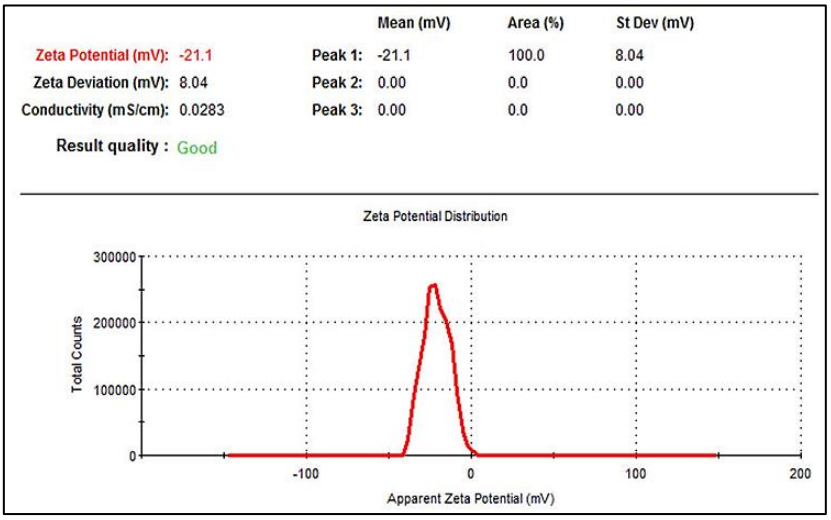

Figure 4: Zeta potential of formulation FLA5

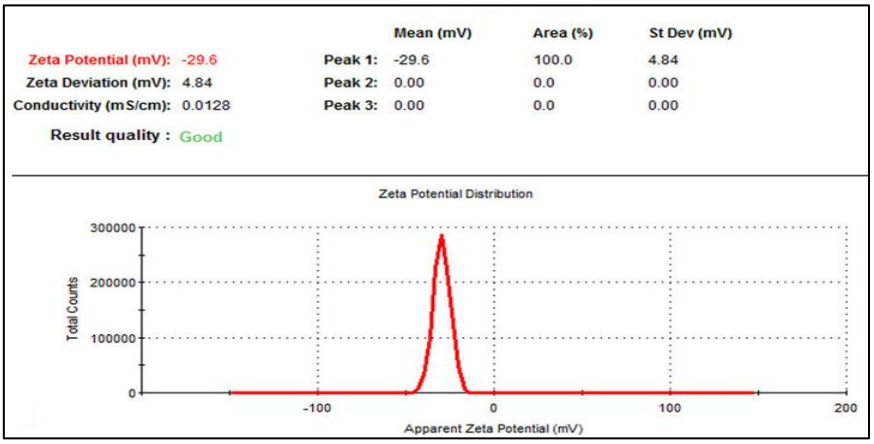

Figure 5: Zeta potential of formulation FLB5

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Table 5: Comparison of Particle Size

Formulation	Particle Size	Formulation	Particle Size
FLA1	895 nm	FLB1	899 nm
FLA3	503 nm	FLB2	509 nm
FLA3	936 nm	FLB3	564 nm
FLA4	276 m	FLB4	521 nm
FLA5	247 nm	FLB5	746 nm
FLA6	536 nm	FLB6	653 nm
FLA7	245 Nm	FLB7	543 nm
FLA8	807 nm	FLB8	805 nm
FLA9	654 nm	FLB9	487 nm

2.4.3 In Vitro Release Studies: Nanospheres containing two different HPMC concentration displayed a similar biphasic drug release pattern with a burst release within 30 minutes followed sustained release afterwards. The reason for burst release is possibly due to the drug associated with the surface of nanoparticles.

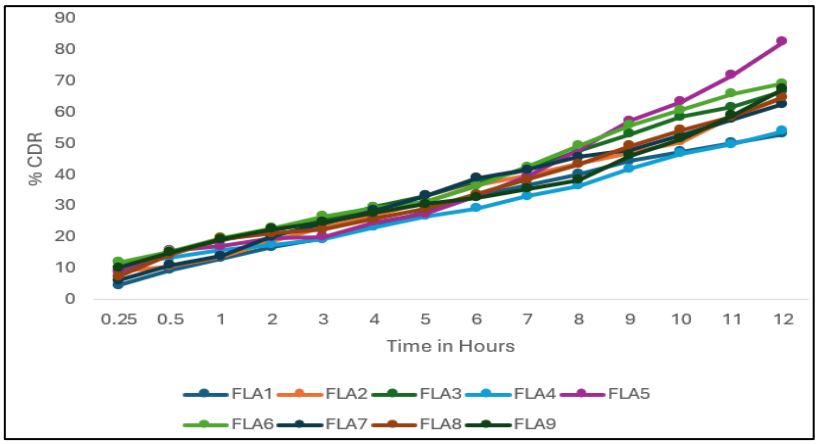

Figure 6: Release Profile Of nanospheres HPMC K4M

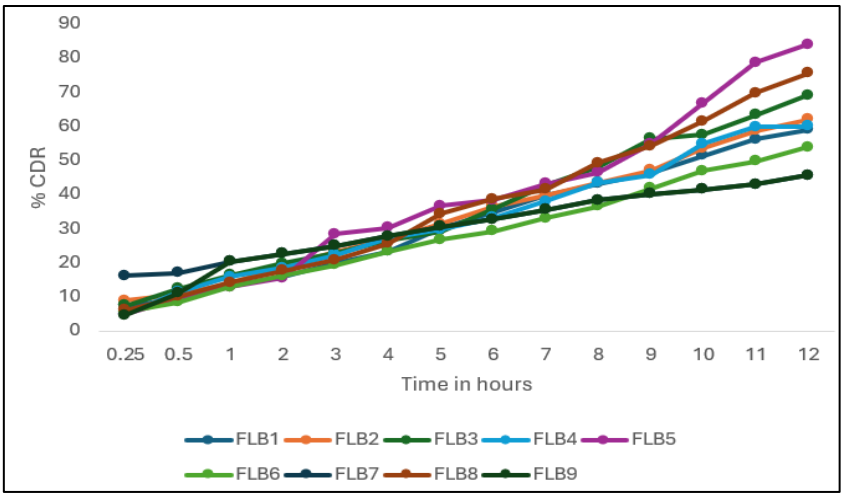

Figure 7: Release Profile Of nanospheres HPMC K100

2.4.4 Release Kinetics: The kinetics and mechanism of drug release were studied by release kinetics, the n, k and r^2 values are indicated in the table 6. All the formulations showed first-order release which had higher linearity than the zero-order or Higuchi model. The exact mechanism of the release kinetics was determined by Korsemeyer-Peppas model. Results indicated that all the nanospheres formulations followed non-fickian model of release kinetics.

Table 6.: Kinetics Release of Formulation FLA1-FLA9

F. Code	Zero order	First order	Higuchi's plot	Kormeyer's and Peppas plot	
	Regression coefficient (R^2)	Regression coefficient (R^2)	Regression coefficient (R^2)	Regression coefficient (R^2)	Exponential value (n)
FLA1	0.811	0.988	0.923	0.908	0.063
FLA3	0.859	.973	0.910	0.811	0.063
FLA3	0.900	0.992	0.940	0.811	0.072
FLA4	0.900	0.985	0.975	0.842	0.089
FLA5	0.951	0.997	0.989	0.779	0.091
FLA6	0.934	0.981	0.993	0.806	0.098
FLA7	0.655	0.925	0.827	0.982	0.047
FLA8	0.819	0.987	0.925	0.912	0.061
FLA9	0.895	0.992	0.958	0.837	0.072

Table 7: Kinetics Release of FLB1-FLB9

F. Code	Zero order	First order	Higuchi's plot	Kormeyer's and Peppas plot	
	Regression coefficient (R^2)	Regression coefficient (R^2)	Regression coefficient (R^2)	Regression coefficient (R^2)	Exponential value (n)
FLB1	0.900	0.985	0.975	0.842	0.089
FLB2	0.951	0.997	0.989	0.779	0.091
FLB3	0.934	0.981	0.993	0.806	0.098
FLB4	0.655	0.925	0.827	0.982	0.047
FLB5	0.819	0.987	0.925	0.912	0.061
FLB6	0.895	0.992	0.958	0.837	0.072
FLB7	0.947	0.994	0.988	0.785	0.084
FLB8	0.955	0.990	0.976	0.759	0.084
FLB9	0.985	0.987	0.961	0.676	0.109

2.4.5 Stability Studies: Nanospheres formulations were examined for stability studies. One set were stored at refrigeration temperature (4 ± 2°C) and the other set were stored at 25 ± 2 °C and 60 % ± 5 %RH at the stability chamber for 1 month and the entrapment efficiency were determined at 1 week intervals. From the results, lowered entrapment efficiency were observed on storage, this may be due to drug expulsion during lipid modification. Hence it was concluded that at 4°C and 25 °C the formulations showed no significant change in entrapment efficiency.

Table 8: Stability Study (4 ± 2°C)

FORMULATION	0	1	2	3	4	5
FLA5	98.50	98.32	98.04	97.64	97.23	96.08
FLB5	99.50	99.23	98.89	98.56	98.12	97.85

Table 9: Stability Study (25 ± 2 °C and 60 % ± 5 %RH)

FORMULATION	0	1	2	3	4	5
FLA5	98.50	98.01	97.75	97.45	97.23	97.03
FLB5	99.50	98.10	98.69	98.30	98.87	97.60

3. CONCLUSION

The success of the studies on the release of drugs in vitro recommends the product for further in vivo studies which may improve patient compliance. From the tests, formulation FLA5 and FLB5 containing 5-Flourourocil nanospheres using polymers combination evolved as the optimized formulation and releases over 86.6 and 84.3 percent drug in 12 hrs. IR spectroscopic experiments have shown that the optimized formulation does not interfere with drug-excipients. The optimized formulation FLA5 can be regarded as a nanospheres nano-sphere drug delivery system delivering almost zero-order drug release over a 12-hourspan.

4. REFERENCES

1. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez Torres MDP, Acosta Torres LS. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnology. 2018;16(1):71. doi: 10.1186/s12951-018-0392-8, PMID 30231877.
2. Gagliardi A, Giuliano E, Venkateswararao E, Fresta M, Bulotta S, Awasthi V. Biodegradable polymeric nanoparticles for drug delivery to solid tumors. Front Pharmacol. 2021;12(1):601626. doi: 10.3389/fphar.2021.601626, PMID 33613290.
3. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. Exp Mol Pathol. 2009;86(3):215-23. doi: 10.1016/j.yexmp.2008.12.004.
4. Mohanraj VJ, Chen Y. Nanoparticles-a review. Trop J Pharm Res. 2006;5(1):561-73. doi: 10.4314/tjpr.v5i1.14634.
5. Nair SK. An overview on nanosphere drug delivery. Eur J Pharm Res. 2018;1:192-8.
6. Lu XY, Wu DC, Li ZJ, Chen GQ. Polymeric nanoparticles. Prog Mol Biol Transl Sci. 2011;104:299-323
7. Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. Pharm Res. 2006;23(7):284-8.
8. Aprahamian M, Michel C, Humbert W, Devissaguet J P and Damge C; Transmucosal passage of poly alkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. Biol Cell 1987; 61: pp 69-76.
9. Banker G S; Pharmaceutical applications of controlled release – an overview of the past, present and future. In Medical Applications of Controlled Release; Langer, R. S. Wise, D. L. Eds; CRC Press: Boca Raton, Florida, 1984; 11, Chapter 1.
10. Wim H De Jong, Paul JA Borm. Drug delivery and nanoparticles: Applications and hazards. Int J Nanomedicine. 2008;3(2):133-49.
11. Ganta S, Paxton J W, Baguley B B and Garg S; Formulation and pharmacokinetic evaluation of an asulacrine nanocrystalline suspension for intravenous delivery, International Journal of Pharmaceutics 367 (2009) pp 179-186.
12. Vijaykumar N., Venkateswarlu V., Raviraj P.,Development of oral tablet dosage form incorporating drug nanoparticles Research Journal of Pharmaceutical, Biological and Chemical Sciences Vol-1(4) 2010, 953-963.
13. Gupta D.K., Razdan B.K., Bajpai M., Formulation and Evaluation of Nanoparticles Containing Artemisinin HClInternational Journal of Research and Development in Pharmacy and Life SciencesVol-3(2), 2014, 925-934.
14. S.Dalvi, R.Dave. Using ultrasound and stabilisers in antisolvent precipitation technique to monitor particle size of a poorly water-soluble drug, Int. J.Pharm. Sci and science. 48 (2009), paragraph 7581–7593
15. Ying Xu, and Xiaoyi Liu, and Ruyue Lian, and Siji Zheng. Enhanced dissolution and oral bioavailability of aripiprazole nanosuspensions based on acid – base neutralization Int. prepared by nanoprecipitation / homogenisation. P. 287 – 295 of Pharm 438 (2012).
16. Amir Maghsoudi, Seyed Abbas Shojaosadati, Ebrahim Vasheghani Farahani. 5-Fluorouracil-loaded BSA nanoparticles: formulation optimization and in vitro release study. AAPS PharmSciTech 2008;9:1092-6.
17. Amrit Rai, Josephine Jenifer, Ravi T Prakash. Nanoparticles in therapeutic applications and role of albumin and casein nanoparticles in cancer therapy. Asian Biomed 2017;11:3-20.
18. Min-Soo Kim, Shun-Ji Jin, Kim Jeong-Soo, Hee Jun Park, Song Ha-Seung. Preparation, characterisation, and in vivo examination of nanospheres of amorphous atorvastatin calcium using supercritical antisolvent (SAS) methods. Eur. Eur. J Pharm and 69 (2008) 454–465.
19. Marazban Sarkari, Judith Gray, Steve Swinnea and Xiaoxia Chen. Better removal of the drug through evaporative precipitation to aqueous solution. European pharmaceutical journal 243 (2002) 17–31.
20. Suman K, V S R Chandrasekhar P, Balaji S. Drug Nanocrystals: A Novel Method for Formulation of Poorly Soluble Drugs. Int J Res1 PharmTech 3(2009), 682-694

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Conflict of Interest Statement: All authors have nothing else to disclose.