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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF 5-FLUROURACIL LOADED HSA NANOPARTICLE FOR CONTROLLED DRUG DELIVERY.

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Abstract

Over the past few decades, there has been considerable interest in developing protein nanoparticles as drug delivery devices. The underlying rationale is their exceptional characteristics, namely biodegradability and nonantigenicity. Herein, simple coacervation method was used to prepare 5fluorouracil-loaded Human serum albumin (HSA) nanoparticles. Drug release was tracked by continuous flow dialysis technique. Morphology of the nanoparticles under scanning electron microscopy was spherical in shape and uniform in size. The mean sizes and entrapment efficiencies of 5-Flurouracil-loaded HSA nanoparticles were in the range of 141.9 nm and 15 - 25 %, respectively. Increasing the amount HSA of added into the formulation led to significant reduction of entrapment efficiency of nanoparticles.

Introduction

The substances with size ranges from 1 to 1000 nm are called nanoparticles. These materials are mainly used in oncology for early detection of malignancy and precise localisation of cancer therapeutics without or with minimal adverse effect to the somatic tissues. These carriers are used to protect drugs, vaccines, nutrients and cosmetics. Nanoparticles exerts its site specific drug delivery by avoiding the reticuloendothelial system, utilising enhanced permeability and retention effect and tumour specific targeting. The formation of nano particles and physicochemical parameters such as pH, monomer concentration, ionic strength as well as surface charge, particle size and molecular weight are important for drug delivery. Further, these nanoparticles have the capability to reverse multi drug resistance, a major problem in chemotherapy.[1] 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.[2].

The limitation in conventional cancer treatment can be alleviated by targeted drug delivery, which is a vehicle that will preferentially carry the drug to the target site in the body and thereby reduce the amount of drug in the rest of the body that can cause undesired side effect. These would increase the range in which a drug is both safe and effective. The distinct capability of nanoparticles to provide access to virtually all cell types may be utilised for the delivery of therapeutic agents to wide array of cellular types and targets[3].

Albumin is the most abundant plasma protein (35–50 g/L human serum) with a molecular weight of 66.5 kDa. Like most of the plasma proteins, albumin is synthesized in the liver where it is produced at a rate of approximately 0.7 mg/h for every gram of liver (i.e. 10–15 g daily); Human serum albumin (HSA) exhibits an average half-life of 19 days. The functions and binding properties of HSA are multifold [4]: a) it acts as the solubilizing agent for long chain fatty acids and is therefore essential for the metabolism of lipids; b) it binds bilirubin, the breakdown product of heme; c) it binds a great number of therapeutic drugs such as

penicillins, sulfonamides,indole compounds, and benzodiazepines to name just a few; d) it binds copper(II) and nickel(II) in a specific and calcium(II) and zinc(II) in a relatively nonspecific manner and acts as the transport vehicle for these metal ions in the blood; e) it is the major protein responsible for the colloid osmotic pressure of the blood; f) when HSA is broken down, the amino acids provide nutrition to peripheral tissue. HSA is used for treating shock, burns, hypoalbuminemia, surgery or trauma, cardiopulmonary bypass, acute respiratory distress and hemodialysis [5.] As an alternative to blood derived albumin, recombinant human serum albumin (Recombumin) has been developed and is a genetically engineered protein expressed in yeast cells that has shown comparable safety, tolerability, pharmacokinetics and pharmacaodynamics to native HSA.

Material and Methods

5-FU was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Human serum albumin is obtained from Central drug house pvt ltd., New Delhi, India. Ethanol, Glutaraldehyde from SD fine chemical ltd, Mumbai, India. All other reagents and Chemicals were of analytical grade.

Preparation of 5-Fluorouracil loaded albumin nanoparticles

5-Fluorouracil loaded albumin nanoparticles were prepared by simple coacervation method. Accurately weighed quantity of 5-Fluorouracil was added to 2% bovine serum albumin solution and incubated for 1 hr[6]. Ethanol was added carefully at a rate of 1 ml/min from an injection under magnetic stirring. The nanoparticles so formed were cross linked by adding 100 µl of 4% glutaraldehyde-ethanol and was stirred continuously at room temperature for 3 hr 80. The nanoparticles suspension was then subjected to freeze drying the dried nanoparticles obtained were then transferred to vials[7].

Characterization of 5-Fluorouracil loaded albumin nanoparticles

1. Particle size analysis

The particle size was determined by dynamic light scattering, using a Malvern system, with vertically polarised light supplied by an argon-ion laser (Cyonics) operated at 40 mW. Experiments were performed at a temperature of 25.0 ± 0.1º C at a measuring angle of 90º to the incident beam[8].

2. Surface charge analysis

The zeta-potential of the nanoparticles was determined by laser doppler anemometry using a Malvern Zetasizer. Measurements were performed at 25 ± 0.10º C. The nanoparticles was dispersed in 0.1 mM NaCl solution and was taken in clear disposable zeta cell and measured.

3. Surface morphology

Scanning electron microscopy was performed to characterize the surface morphology of the formed nanoparticles and this was done by using a JSM 6100 JEOL Scanning Electron Microscope at 20 kV. Prior to examination, samples were gold-coated to render them electrically conductive and examined under the microscope[9].

4. Determination of process yield.

The process yield of 5-Fluorouracil loaded albumin nanoparticles was determined; as the weight percentage of final product after drying, with respect to the initial amount of drug and polymer used for the preparation[10].

$$\text{Process yield} = \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}$$

5. Determination of percentage drug loading capacity

The percent drug loading was determined by extracting the drug completely from known amount of drug loaded nanoparticles using pH 7.4 phosphate buffercontent was determined spectrophotometrically at a wavelength of 270 nm against blank.

$$\text{Drug loading} = \frac{\text{Actual drug content} \times 100}{\text{Weight of nanoparticles taken}}$$

6. *In vitro* drug release studies

The drug release studies were carried out by dialysis method. 50 mg nanoparticles was placed in a cellulose dialysis bag (cut-off 5 kDa, Himedia, India), and to this a little amount of dissolution media was added, which was then sealed at both ends. The dialysis bag was dipped into the receptor compartment containing the dissolution medium, which was stirred continuously at 100 rpm maintained at 37°C. The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh dissolution medium were measured by UV Spectrophotometer at a wavelength 266 nm against blank.

7. Release kinetics

Data obtained from the (F5) *in vitro* release studies were fitted to various kinetic models such as zero order, first order, Higuchi model and Kosmeyer peppas model. The four models of data treatment are as follows.

Zero-order release equation

$$Q = Q0 – K0t$$

Q is the amount of drug released at time t, K0 is zero-order release rate constant [11]: A plot of fraction of drug release against time will be linear, if the release obeys Zero order release kinetics**.**

First-order release equation

$$In\ Q = In\ Q0 – K1t$$

Q is the amount of drug released at time t, Q0 is the amount of drug remaining in the formulation. Thus, a plot of the logarithm of the fraction of drug remained against time will be linear if the release obeys First order release kinetics.

Higuchi's square root of time equation

It defines a linear dependence of the active fraction released per unit of surface (Q) on the surface root of time.

$$Q = K2\ t1/2$$

K**2** is Higuchi square root of time-release rate constant, A plot of the fraction of drug released against root of time will the linear if the release obeys Higuchi Equation. This equation describes drug release as a diffusion process based on the Fick's Law Square root time dependent.

Korsmeyer-Peppas equation

$$Q/Q0 = K\ t\ n$$

Q/Q0 is fraction of drug release at time, t and K is a constant and n is diffusion exponent indicating the mechanism of drug release. If the value of n is less than 0.45, Fickian mediated release occurs. If n is between 0.45 to 0.89, non-Fickian (i.e. diffusion coupled with polymer relaxation) and erosion (i.e. complete matrix relaxation) mediated release occurs in n= 0.89.

Result and Discussion

Preformulation studies.

Drug-polymer interaction study by FT-IR spectrophotometer

An FT-IR spectroscopy study was carried out separately to check the compatibility between the drug (5-Fluorouracil) and the polymer (human serum albumin) used for the preparation of nanoparticles. The FT-IR was performed for drug, polymer and physical mixture of drug and polymer. The spectra obtained from FT-IR spectroscopy studies at wavelength ranging from 4000 cm-1 to 400 cm-1 are shown in Figures 1 to 3.

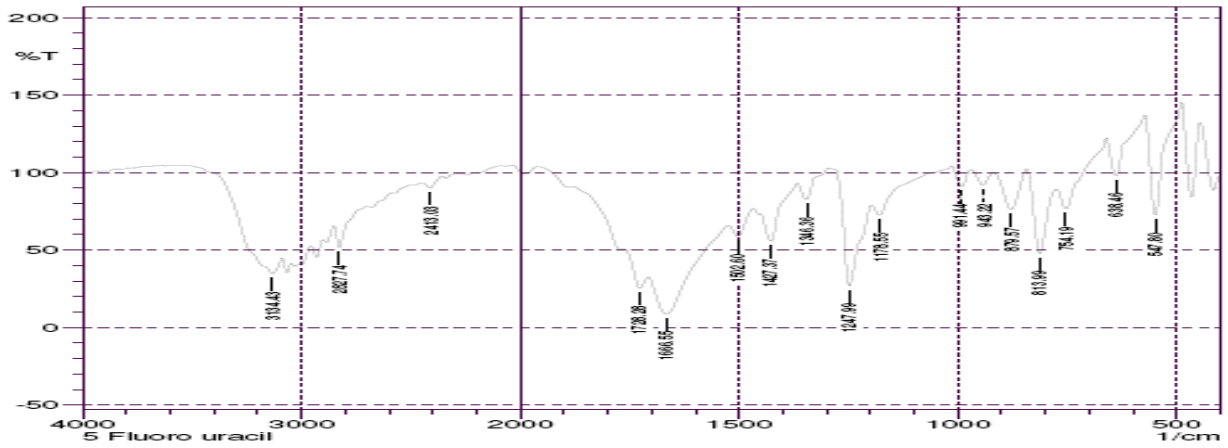


Fig.no:1 FT- IR spectrum of 5-Fluorouracil.

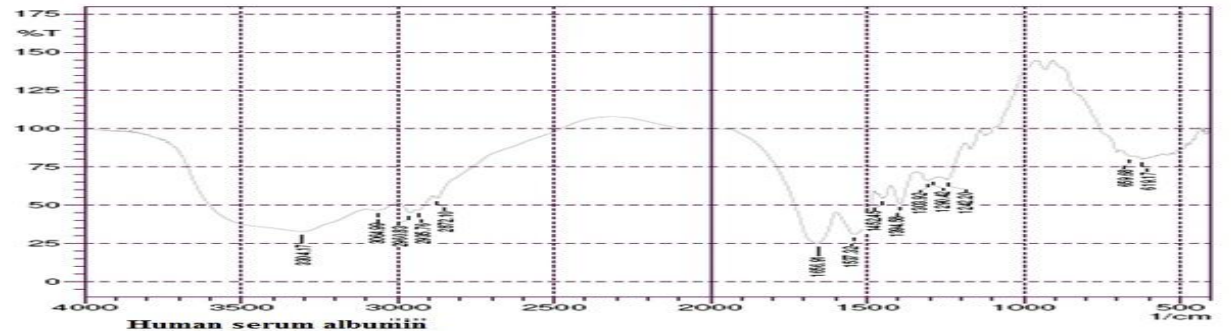


Fig no 2: FT-IR spectrum of Human serum albumin.

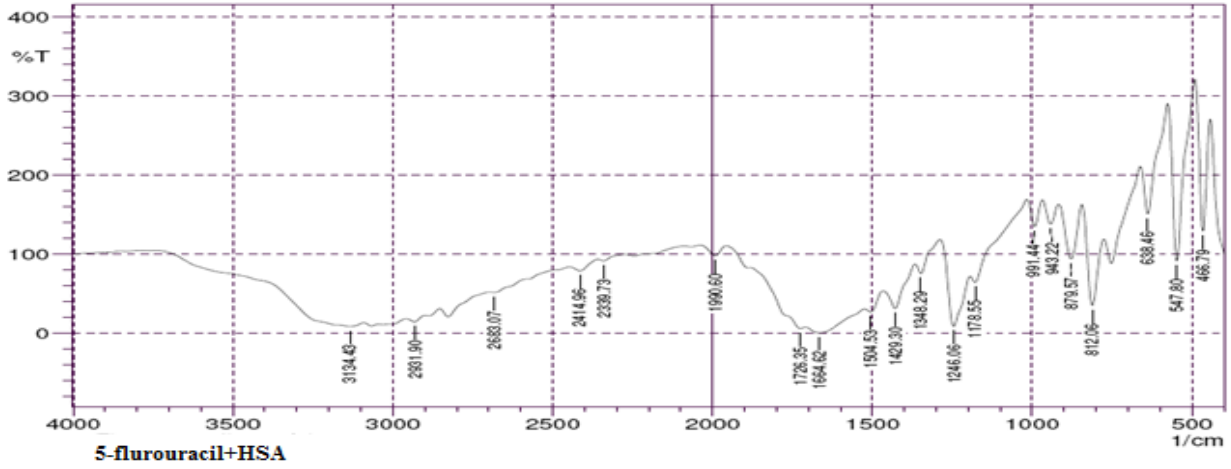


Fig no 3: FT-IR spectrum of physical mixture of 5-Fluorouracil and human serumalbumin.

Preparation of standard graph

Fig no 4: Preparation of standard graph of 5-Fluorouracil in pH 7.4 phosphate buffer Standard graph of drug 5-Fluorouracil was done in pH 7.4 phosphate buffer. Table 5.4 shows the concentrations of 5-Fluorouracil in pH 7.4 phosphate buffer and the respective absorbances.

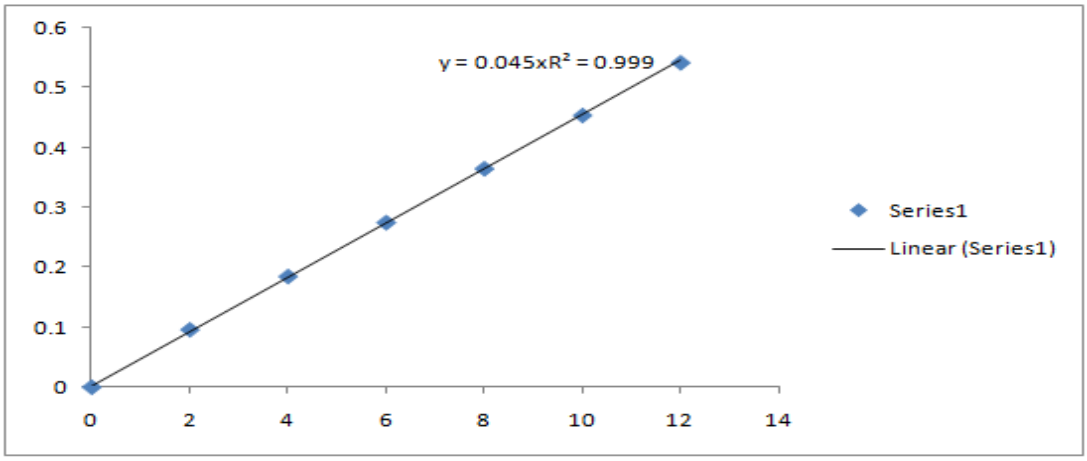


Figure 4 : shows the standard graph of 5-Fluorouracil in pH 7.4 phosphate buffer.

Optimization of process to decrease size:

Langer et al introduced pH, protein concentration, rate of adding Ethanol, temperature, and glutaraldehyde concentration as parameters affecting particles size in desolvation process [Langer,2003].By using the full factorial design method two parameter (protein concentration, a, and rate of adding ethanol, b) 8 tests were done with respect to interaction between parameters and replication. Table 1 shows the composition of tests and their results. As table 1 shows largest particles size is 327.65 nm, when both parameters are at low level (a=0.5 mL/min, b=5 mg/mL) and, in the case of high level of both parameters (a=2 mL/min, b=20 mg/mL), the smallest particles size is 74.1 nm.

Table 1. Tests composition and their results

No	Composition of tests	First	Second	Average size
1	1	320	335.3	327.5
2	A	106	141	123.5
3	B	294.4	270	282.2
4	Ab	85.5	62.7	74.1

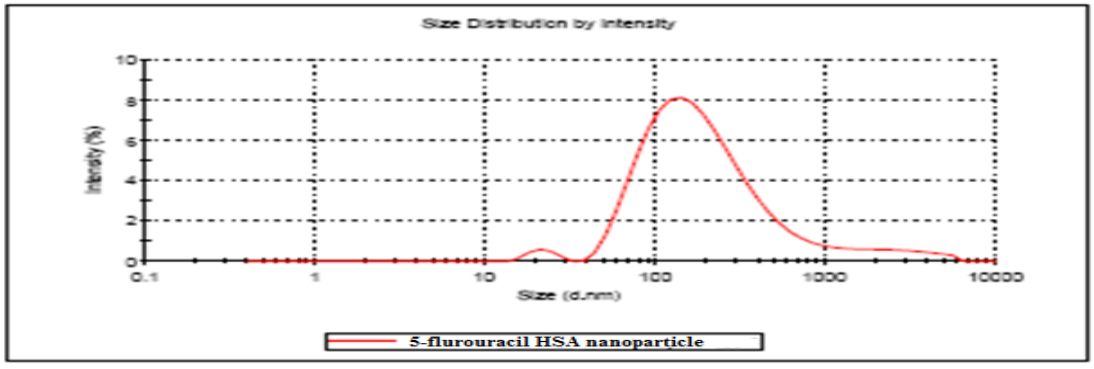
On the basis of the particles formation mechanism, nucleuses form while adding ethanol to protein solution. By adding more ethanol these nucleuses got grow and nanoparticles appeared. Preliminary results indicate protein concentration with 99% assurance is the main parameter and the ethanol adding rate has not much impaction on particle size. After determination of main factor, set of new experiments base of protein concentration was designed. Rate of adding Ethanol was fixed at 1.5mL/min got from conditions determined. In changes of particles size with protein concentration is indicated. Particle size of 5mg/mL protein concentration is 312.9, which is confirmed previous results. In the range of 530mg/mL smaller particles will be gotten in 15mg/mL, From the curve it is estimated that the particle size is approximately 75nm.

Characterization of nanoparticles

5-Fluorouracil loaded albumin nanoparticles were prepared by coacervation method. The nanoparticles were evaluated for particle size, surface charge, and surface morphology.

Particle size analysis and polydispersity index

Particle size analysis of 5-Fluorouracil loaded albumin nanoparticles was done by dynamic light scattering using a Malvern system and the mean particle size of nanoparticles was found to be 141.9 nm. Figure 5 shows the particle size distribution of 5-Fluorouracil loaded albumin nanoparticles. The polydispersity index of prepared nanoparticles was 0.374.



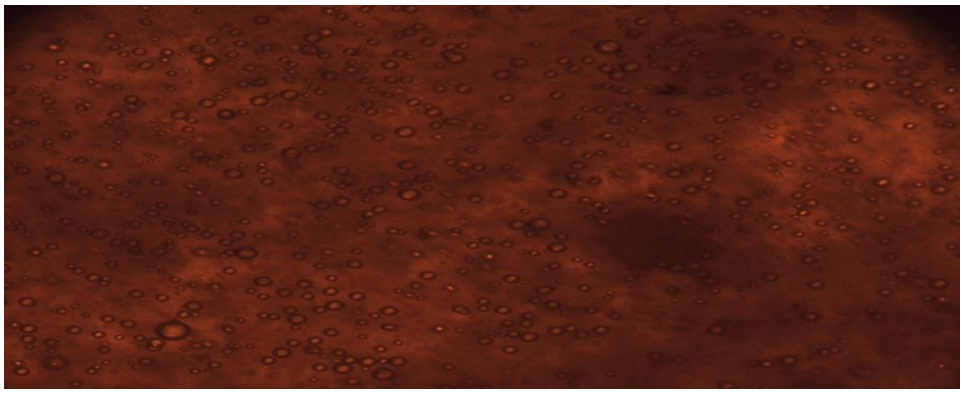


Fig no 5: Particle size of F5 nanoparticles.

***In vitro* drug release studies**

The drug release from the nanoparticles was studied by dialysis method. The *in vitro* release profile of 5-Fluorouracil from albumin nanoparticles are shown in Table 2. The cumulative percentage release of 5-Fluorouracil from albumin nanoparticles varied from 75.12 to 97.26 % depending on the drug concentration in each formulation for 24 h.

Determination of process yield and percentage drug loading capacity

The process yield and percentage drug loading of different batches are shown in Table 3. The process yield ranged between 96.8 to 98.19% w/w depending on the drug polymer ratio. The drug loading capacity ranged between 4.22 to 19.8% w/w.

Release kinetics.

Data obtained from *in vitro* release studies (F5) were fitted to various kinetic models such as zero order, first order, Higuchi model and Korsmeyer-Peppas model and the results are shown in Table 4.

Table no:2 *In vitro* release profile of 5-Fluorouracil loaded albumin nanoparticles of F1-F5.

	Batch code				
Time (h)	**F1**	**F2**	**F3**	**F4**	**F5**
0.5	5.15	10.14	28.01	26.50	41.00
1	12.12	19.54	37.52	44.25	52.90
2	23.21	26.50	49.90	52.31	58.57
3	34.81	32.05	55.31	61.85	67.27
4	43.47	39.62	60.21	70.23	74.06
6	57.56	45.48	69.18	72.39	77.75
8	59.31	47.98	72.57	75.46	82.62
10	61.81	57.24	76.96	79.28	85.63
12	64.21	63.98	78.54	83.15	87.44
24	75.12	81.60	89.45	94.61	97.24

Table 3: Data of process yield and percentage drug loading capacity of 5-Fluorouracil loaded albumin nanoparticles for F1-F5.

Batch code	Process Yield (%)	Drug loading (%)
F1	98.19	4.22
F2	97.72	9.22
F3	97.39	12.74
F4	97.08	14.42
F5	96.8	19.8

Table 4: Release kinetics of 5-Fluorouracil loaded albumin nanoparticles (F5).

Time (h)	Square root of time	Log time	CDR	%CDR	Log of %CDR	Log Cu% of drug remaining
0.5	0.7071	-0.3010	4.0588	41.00	1.6128	1.7709
1	1	0	5.2367	52.90	1.7235	1.6730
2	1.4142	0.3010	5.7984	58.57	1.7677	1.6173
3	1.7321	0.4771	6.6594	67.27	1.8278	1.5149
4	2	0.6021	7.3323	74.06	1. 8696	1.4140
6	2.4495	0.7782	7.6977	77.75	1.8907	1.3473
8	2.8284	0.9031	8.1791	82.62	1.9171	1.2400
10	3.1623	1	8.4776	85.63	1.9326	1.1575
12	3.4641	1.0792	8.6561	87.44	1.9417	1.0990
24	4.8990	1.3802	9.6286	97.26	1.9879	0.4378

Conclusion

In the present study, an attempt was made to develop nanoparticulate delivery system for water soluble drug 5-Fluorouracil. FT-IR studies were carried out to find the possible interaction between the selected drug and polymer. This study revealed that there was no interaction between the selected drug and polymer.

Albumin nanoparticles of drug 5-Fluorouracil was prepared by coacervation method. This method was able to produce desired size and shaped uniform nanoparticles. All the formulations showed good process yield and drug loading capacity. Among the different batches, formulation F5 was selected as the ideal formulation, after considering its drug loading capacity and *in vitro* drug release. Particle size analysis showed that the formed particles were in nano size and possess a negative surface charge. All the formulations were able to sustain the drug release for a period of 24 h. Release kinetics showed that the 5-Fluorouracil release from the nanoparticles was first order diffusion controlled. The n value of Korsmeyer-Peppas equation indicated the release mechanism was Fickian.

Based on the observations, it can be concluded that the formulated nanoparticulate delivery system of water soluble drug 5-Fluorouracil using widely accepted and physiologically safe polymer was capable of exhibiting sustained release properties for a period of 24 h. This may reduce concentration of drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability, and increase the effectiveness of the drug.

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