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Research Article

FORMULATION AND EVALUATION OF CYTARABINE PLA MICROSPHERES

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ABSTRACT

The present investigation was aimed at developing cytarabine-loaded poly (L-lactide) (PLA) based biodegradable microspheres by a double emulsion solvent evaporation technique which would have sustained release of the drug. The poly (L-lactide) (PLA) microspheres containing Cytarabine as a drug and evaluate the various physicochemical characteristics of the formulations, namely morphology, particle size, FTIR, Cytarabine encapsulation efficiency and in-vitro Cytarabine release profile. Cytarabine-loaded microspheres were prepared by double emulsion solvent evaporation method with different Cytarabine, PLA ratios and at different speeds of homogenization keeping the amount of Cytarabine constant in all the formulations and different amount of salt (NaCl) concentrations Accelerated stability testing was performed with the optimized formulations for a period of two months. The mean particle size and encapsulation efficiency of the microspheres were found to decrease as the speed of homogenization increased and the encapsulation efficiency was increased with increase in salt (NaCl) concentration. The in vitro release study showed a slow and steady release pattern of Cytarabine. Thus a sustained release formulation of Cytarabine loaded PLA microspheres were developed.

Keywords: Poly (L-lactide), Double emulsion, Sodium Chloride, Homogenization.

INTRODUCTION

Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release[1]. Subcutaneous implantable drug pellets using nondegradable polymers have been used for long-term, continuous drug administration. The procedure requires surgical implantation and removal of the drug-containing devices or polymeric matrices[2]. These facts have led to the research and development of novel, controllable, nonirritating, non-carcinogenic, and biocompatible and bioabsorbable drug delivery systems for overcoming the drawbacks of non-degradable implantable pellets for prolonged continuous release [3]. Biodegradable polymeric systems release the drug over a long period of time with simultaneous or subsequent degradation in the tissue of the polymer towards harmless constituents, thus avoiding removal once the therapy is complete. Subcutaneous tissue is essentially a sheet of areolar tissue lying directly underneath the skin. It is rich in fat, but poor in nerve network and hemoperfusion. Therefore, the subcutaneous tissue is an ideal location for implantation and prolonged drug administration because of its easy access, slow drug absorption and low reactivity to the insertion of foreign materials[4].

Cancer chemotherapy is not always effective. Difficulties in drug delivery to the tumor, drug toxicity to normal tissues, and drug stability in the body contribute to this problem. Polymeric materials provide an alternate means for delivering chemotherapeutic agents. When anticancer drugs are encapsulated in polymers, they can be protected from degradation[5]. Cytarabine is used in the treatment of acute myelogenous leukemia and non-Hodgkin lymphoma[6].A number of drug products based upon PLA and PLGA delivery systems have been launched into the global market.

Table 1: Commercial biodegradable drug products

Product	Drug	Company	Delivery technology	Polymeric carrier
Decapeptyl SR	Triptorelin	Ipsen	Microparticles	PLGA
Nutropin Depot	Somatropin	Genetech	Microparticles	PLGA
Risperdal Consta	Risperidone	Janssen	Microparticles	PLGA
Sandostatin LAR	Octreotide	Novaris	Microparticles	PLGA
Trelstar Depot	Triptorelin	Watson Pharma	Microparticles	PLGA
Trelstar LA	Triptorelin	Watson Pharma	Microparticles	PLGA
Vivitrol	Naltrexone	Cephalon	Microparticles	PLGA

MATERIALS AND METHODS

Materials

Cytarabine was obtained from Shang Hai Hengrui International trading co,Ltd. China, poly lactic acid obtained from Evonik roehmgmbh (Germany), Poly vinyl alcohol obtained from S.D. Fine chemicals (Mumbai). All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Preparation of Cytarabine Microspheres

This method for preparation of microsphere was reported to overcome the problem of low encapsulation efficiency of water soluble drug prepared by conventional double emulsion solvent evaporation method. Polymer [Poly (L-lactic acid) (PLA)] is dissolved in organic phase DCM (Dichloro methane). In this organic phase, aqueous drug solution is emulsified using high speed homogenizer (IKA) operating around 10000 rpm for about 5minutes to prepare water /oil (w/o) Primary emulsion. This primary emulsion is added to external aqueous phase containing surfactant (poly vinyl alcohol is used to prepare w/o/w emulsion) at homogenizer speed around 8000 rpm for 3minutes and then stirred at 1000 rpm for 1 hour at 2-8 ^{0}c then next 2 hrs at room temperature to permit evaporation of DCM. The microspheres obtained is collected by centrifugation, filtration and then dried.

Table 2: Composition of cytarabine microspheres

Composition		Formulations					
		F1	F2	F3	F4	F5	F6
Drug (cytarabine), mg		50	50	50	50	50	50
Water, ml		2	2	2	2	2	2
Polymer(poly lactic acid), mg		300	400	300	400	800	800
Dichloromethane(DCM), ml		20	20	20	20	20	20
Polyvinyl alcohol(PVA) 0.5%, ml		100	100	100	100	100	100
Sodium chloride (NaCl), %		-	-	2.5	2.5	2.5	5
Homogenization speed (rpm)	Primary(5min)	10000	10000	10000	10000	10000	10000
	Secondary(3min)	8000	8000	8000	8000	8000	8000

Table 3: Compositions of cytarabine microspheres

Composition		Formulations					
		F7	F8	F9	F10	F11	F12
Drug (cytarabine), mg		50	50	50	50	50	50
Water, ml		2	2	2	2	2	2
Polymer(poly lactic acid), mg		800	800	800	800	800	800
Dichloromethane(DCM), ml		20	20	20	20	20	20
Polyvinyl alcohol(PVA) 0.5%, ml		100	100	100	100	100	100
Sodium chloride (NaCl), %		7.5	10	10	10	12.5	15
Homogenization speed (rpm)	Primary(5min)	10000	10000	15000	5000	10000	10000
	Secondary(3min)	8000	8000	8000	8000	8000	8000

Evaluation of Microspheres

Percentage yield

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres multiplied by 100 gives the % yield of microspheres (equation)[7]

% yield = Actual weight of product/ Total weight of excipients and drug × 100.

Drug entrapment efficiency: The amount of drug entrapped was estimated by dissolving the 100mg of microspheres in DCM and water in 3:1 ratio ,under vigorous shaking for 1hr, the resultant solution is centrifuged, both layers were separated, cytarabine was soluble in water but not in DCM. The drug content in aqueous solution was analyzed spectrophotometrically by using UV-Vis spectrophotometer at 272.7nm with further dilutions against appropriate blank. The amount of the drug entrapped in the microspheres was calculated using the formula[8]:

Encapsulation efficiency = Actual weight of drug in sample x 100

Theoretical weight of drug in sample

Scanning electron microscopy

Microspheres were observed and photographed with scanning electron microscopy (SEM) (Using Hitachi-S-3700N). Scanning electron microscopy was carried out to study the morphological characteristics of cytarabine PLA microspheres. The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100A°) before microscopy. Finally the morphology of the microspheres was observed with the scanning electron microscopy[9].

Particle size analysis

Determination of average particle size of cytarabine microspheres was very important character. It was carried out by using malvern instruments, startech labs pvt.ltd.

In-vitro drug release

An in vitro release method using a regenerated cellulose membrane dialysis apparatus (Float-a-Lyzer) was suitable for studying in vitro release of cytarabine-loaded biodegradable microspheres. Microspheres suspension containing known amount of drug was placed in Float-a-Lyzer. The Float-a-Lyzer was placed in beaker containing 50ml of PBS (pH 7.4), maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume and sink conditions. The samples analyzed for drug content by UV-vis spectrophotometer at 272.7nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

***In-vitro* drug release kinetic study**

In order to describe the kinetics of the release process of drug in the different formulations, zero order ($Q_t = Q_0+K_{0t}$), First order ($\ln Q_t = \ln Q_0+K_{1t}$), Higuchi $K_{Ht1/2}$) and Korsemeyer- Peppas ($Q_t/Q_8= K_{tn}$) models were fitted to the dissolution data of all formulations using linear regression analysis. A value of n=0.5indicates case-I (Fickian) diffusion or square root of time kinetics, 0.5<n<1anomalous (non-Fickian) diffusion, n=1 Case-II transport and n>1 Super Case-II transport 12[10].

Stability studies

To assess the physical and chemical stability of the microspheres, stability studies were conducted for 2 months under various storage conditions mentioned in ICH guidelines. The sample containing optimized formulation were placed in vials and stored at 40±2°c/75±5% RH. After 60 days the formulations were checked for physical appearance and drug content.

RESULTS AND DISCUSSION

Formulation optimization

The Microspheres were prepared by double emulsion technique using homogenizer (IKA). Formulations was optimized for in vitro release profile , particle size and entrapment efficiency. The drug polymer ratio was 1:16 for optimized formulation, PVA concentration was 0.5%, sodium chloride 10%, aqueous phase volume was 2ml and DCM volume was 20ml. The formulation containing cytarabine kept at constant strength was prepared with different excipients DCM, poly l-lactic acid, PVA, NaCl and all other parameters like temperature and rpm were optimized.

Evaluation of Microspheres

Percentage yield and Entrapment efficiency

The percentage yield and encapsulation efficiency were determined for all the formulations from F1to F12 it was in the ranges from,

percentage yield (34.9% - 84.2%) and encapsulation efficiency (6.2% - 91.3%). Among those compositions 6 Formulations are selected as optimized batches for further evaluation based on in vitro dissolution profile and entrapment efficiency.

Table 3: Percentage yield and entrapment efficiency of various formulations

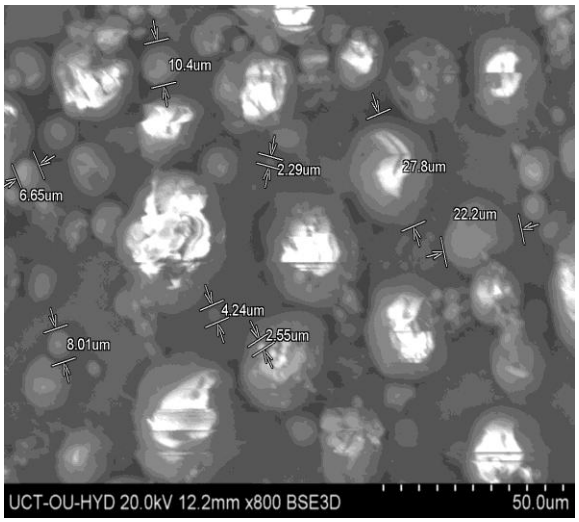
S. No.	Batches	Percentage yield	Entrapment efficiency
1	F1	34.9%	6.2%
2	F2	36.5%	8.3%
3	F3	45.3%	16.7%
4	F4	59.6%	21.3%
5	F5	69.2%	43.6%
6	F6	75.1%	74.7%
7	F7	77.6%	84.2%
8	F8	84.2%	91.3%
9	F9	83.8%	79.4%
10	F10	86.9%	81.1%
11	F11	82.1%	85.6%
12	F12	67.3%	32.5%

Scanning Electron microscopy

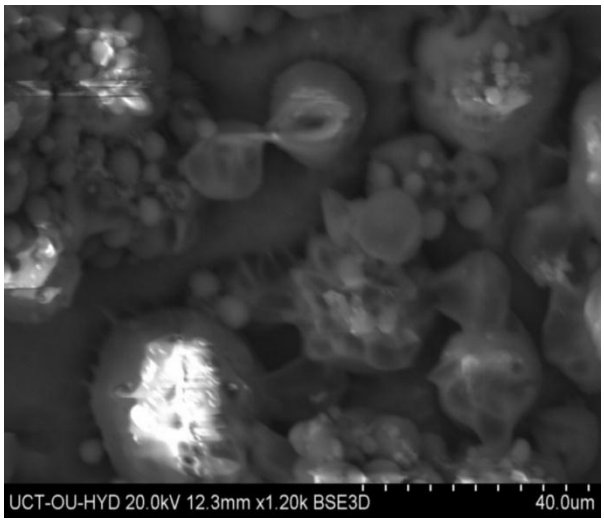
SEM micrographs and typical surface morphology of the microspheres are given in Fig. 1 for F7, F8, F11 formulations. It was observed that microspheres were spherical with smooth surface. Fig. 1(d).

Particle size of cytarabine microspheres

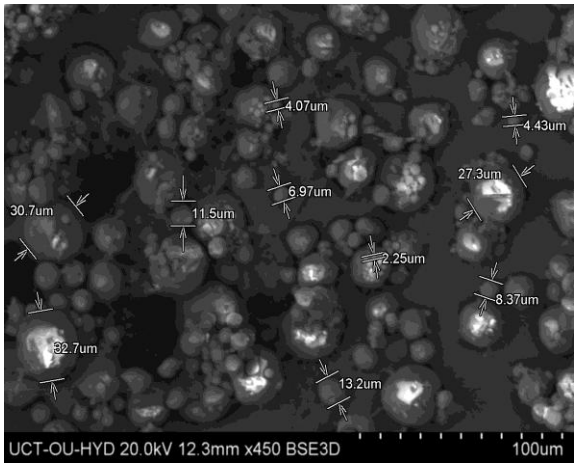
The particle size distribution was analyzed for F7, F8 and F11, formulations of cytarabine by wet method. The particle size was optimum in F8 Formulation, when compared to F7 and F11.



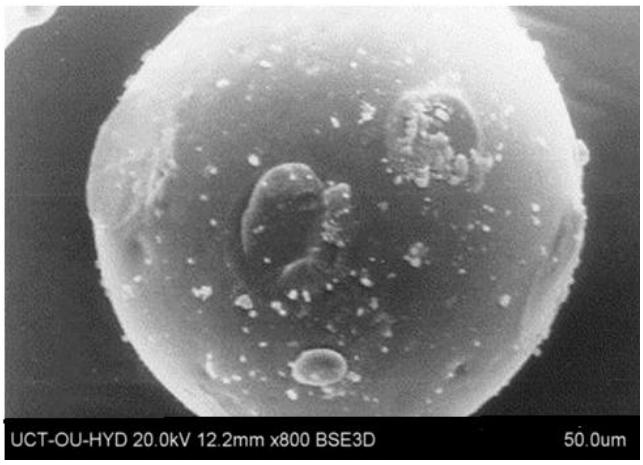
a) SEM photography of microspheres for F7



b) SEM photography of microspheres for F8 formulation



c) SEM photography of microspheres for F11 formulation



d) SEM photography of microspheres for F8 formulation

Fig. 1

Table: 4 particle size of optimized formulations

S. No.	Batches	Particle size (µm)
1.	F7	67.303
2.	F8	45.437
3.	F11	105.786

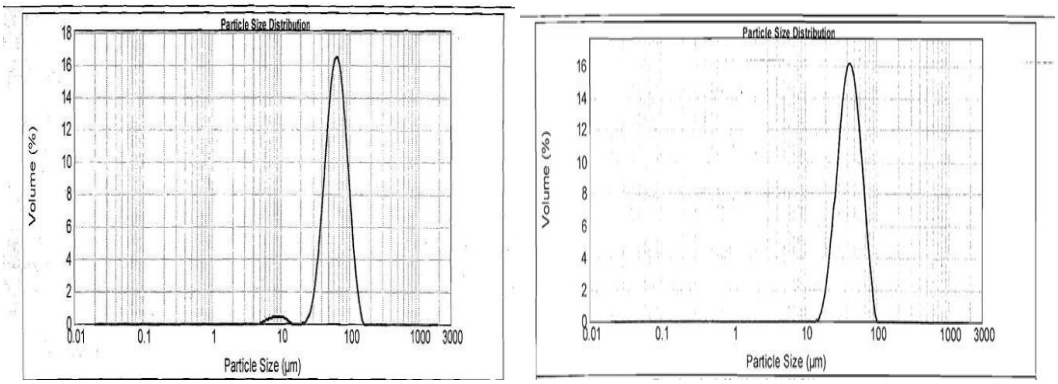
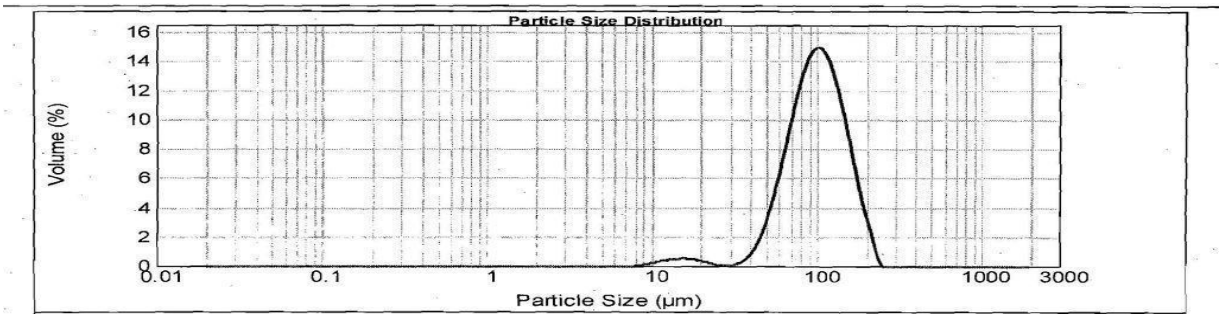


Fig. 2: a) Particle size distribution of Cytarabine microspheres for F7formulation.

b) Particle size distribution of Cytarabine microspheres for F8 formulation.



c) Particle size distribution of Cytarabine microspheres for F11 formulation

Table 5: In vitro drug release profile of cytarabine microspheres

Time	Cumulative % drug released					
(Days)	F6	F7	F8	F9	F10	F11
0	0	0	0	0	0	0
1	36.1	34.6	31.1	0	0	28.2
3	38.2	36.8	35.4	32.4	34.2	36.8
7	46.8	43.2	42.2	37.2	36.4	45.3
14	62.7	65.6	65.7	41.8	45.7	69.1
21	75.2	78.1	76.5	68.2	65.3	79.4
28	79.6	83.8	88	78.9	76.6	87.1
30	81.3	91.9	93.3	83.7	85.2	91.6

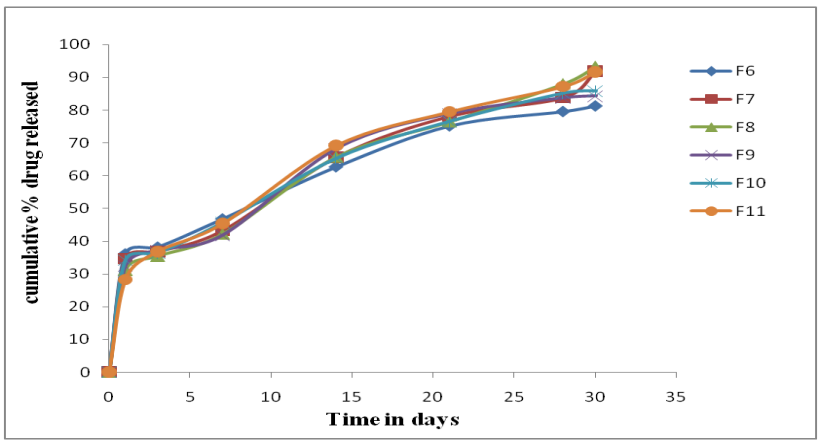


Fig. 3: Comparative release profiles of cytarabine microspheres

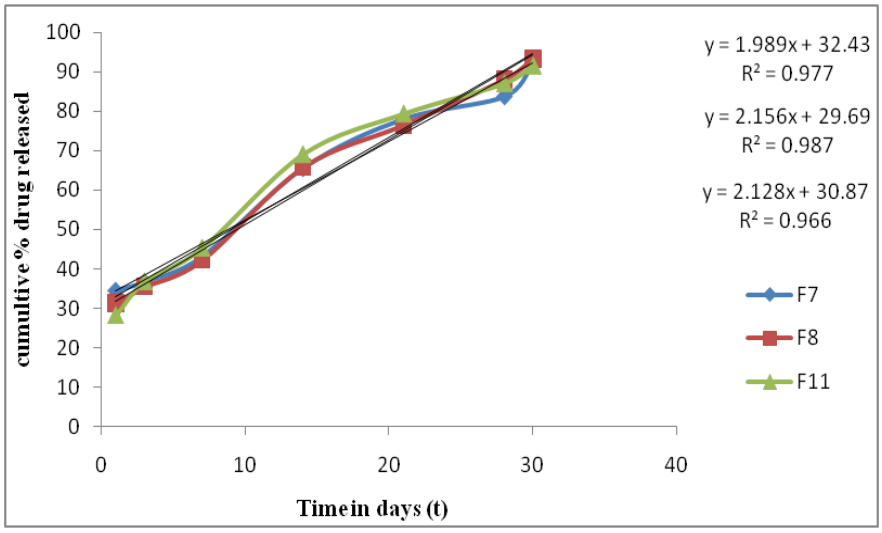


Fig. 4: Comparison Zero order release studies for optimized formulations F7, F8 and F11.

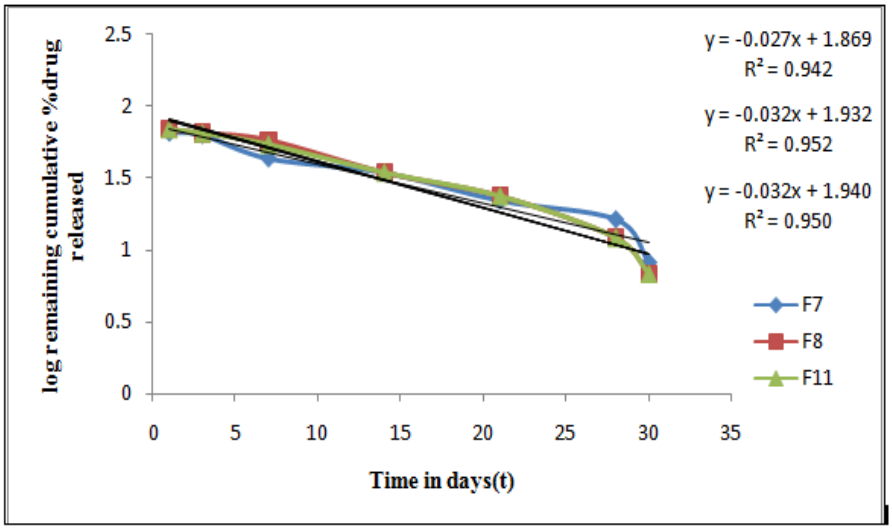


Fig. 5: Comparison of First order release studies for optimized formulations F7, F8 and F11.

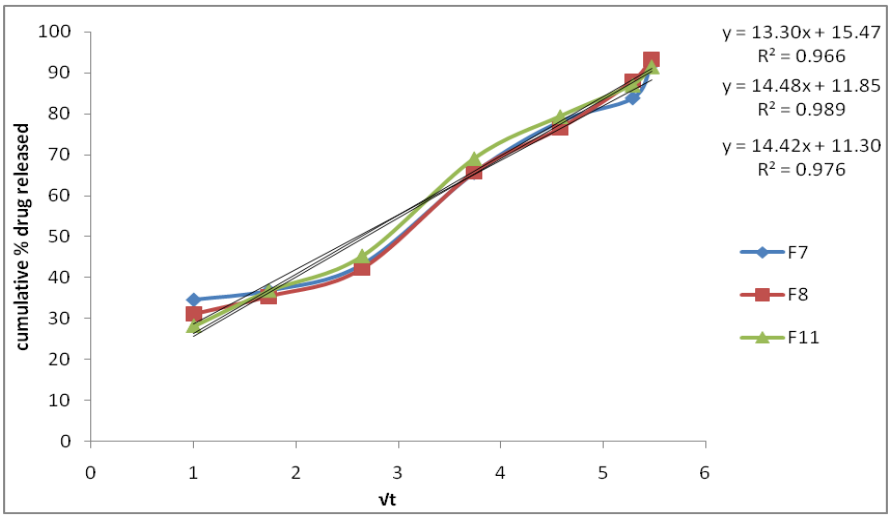


Fig. 6: Comparison of Higuchi model release studies for optimized formulations F7, F8 and F11

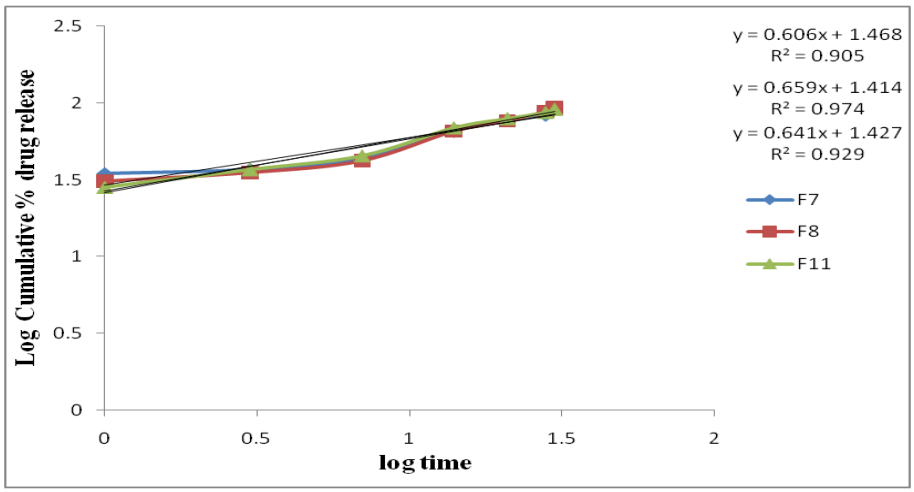


Fig. 7: Comparison of korsemeyer - peppa's model release studies for optimized formulations F7, F8 and F11.

Table 6: Curve fitting data of release rate profile of Formulations F7, F8 and F11

Formulation code	Zero-order (R^2)	First-order (R^2)	Higuchi (R^2)	Korsemeyer – Pappas (n)
F7	0.977	0.942	0.966	0.606
F8	0.987	0.952	0.989	0.659
F11	0.966	0.95	0.976	0.641

The release kinetics of F7, F8, F11 formulations was studied. All formulations follow Zero order release kinetics and follow Non-Fickian diffusion when it applied to the Korsemeyer-Peppa's Model for mechanism of drug release.

Table 7: Short term stability data for cytarabine microspheres at 40±2⁰c/75%RH

Test	Effect of stability at 40±2⁰c/75%RH				
	0 days	15 days	30 days	40 days	60 days
Description	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white
Assay of F7 Formulation	84.2±0.65	84.3±0.47	83.9±0.72	83.94±1.0	83.71±0.65
Assay of F8 Formulation	91.3±0.96	89.9±0.92	90.9±0.62	90.6±0.58	90.8±0.53
Assay of F11 Formulation	85.6±1.3	84.5±0.65	84.8±0.69	84.8±0.86	84.4±0.74

In-vitro cumulative % drug release profile

The *in vitro* dissolution profile of prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 30 days in 7.4 pH phosphate buffer.

The cumulative percent release of F6, F7, F8, F9, F10 and F11 formulations at various time intervals was calculated and tabulated in Table No: 5 For F8 formulation 93.3% drug release was achieved on 30th day. Drug release profile increases with increase in drug to polymer ratio.

In-vitro release kinetics

The release kinetics of F7, F8, F11 formulations was studied.

Stability studies

Accelerated stability studies of cytarabine microspheres (F8) at temperature 40⁰C/75%RH as per ICH guidelines were studied for 60 days. The physical appearance of the formulation was a White to off-white and it was observed that there was no color change indicating physical stability. The drug content was analyzed and data is presented in table No7. From the data, it is observed that there was negligible change in the drug content indicating chemical stability.

CONCLUSION

From the executed experimental results, it could be concluded that the poly lactic acid, and sodium chloride were suitable for preparation of Cytarabine microspheres. Though the preliminary data based on *in-vitro* dissolution profile, release kinetics and stability studies proved that the suitability of such formulations .F8 formulation showed best particle size, better drug entrapment efficiency, and better sustained release profile for 30 days.

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