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Research Article

FORMULATION AND *INVITRO* EVALUATION OF AMPRENAVIR NANOPARTICLES PREPARED BY NANOPRECIPITATION METHOD

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ABSTRACT

The development of nanoparticle-based drug formulations has yielded the opportunities to address and treat challenging diseases. Nanoparticles vary in size but are generally ranging from 100 to 500 nm. Through the manipulation of size, surface characteristics and material used, the nanoparticles can be developed into smart systems, encasing therapeutic and imaging agents as well as bearing stealth property. Further, these systems can deliver drug to specific tissues and provide controlled release therapy. This targeted and sustained drug delivery decreases the drug related toxicity and increase patient's compliance with less frequent dosing. Nanotechnology has proven beneficial in the treatment of cancer, AIDS and many other disease, also providing advancement in diagnostic testing. In the presant work antiviral drug amprenivir nanoparticles were prepared and evaluated.

Keywords: Amprenivir, Nanoparticles, Drug delivery.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then to maintain the desired drug concentration. That is, the drug delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to drug delivery namely spatial placement and temporal delivery of a drug [1]. Spatial placement relates to targeting of drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An appropriately designed controlled release drug-delivery system can be a major advance towards solving these two problems [2]. It is for this reason that the science and technology responsible for development of controlled-release pharmaceuticals has been, and continues to be the focus of a great deal of attention in both industrial and academic laboratories [3].

NANOPARTICLES

Nanoparticles are small colloidal particles which are made up of non-biodegradable and biodegradable polymers [4]. Their diameter is from 1-1000 nm. One can distinguish two types of nanoparticles: Nanospheres, which are matrix systems and nanocapsules, which are reservoir systems composed of a polymer membrane surrounding an oily or aqueous core. These systems were developed in early 1970s. This approach was attractive because the methods of preparation of particles were simple and easy to scale-up [5-7]. The particles formed

were stable and easily freeze dried. Due to these reasons, nanoparticles made of biodegradable polymers were developed for drug delivery. Indeed, nanoparticles were able to achieve with success tissue targeting of many drugs (antibiotics, cystostatics, peptides and proteins, nucleic acids, etc.). In addition, nanoparticles were able to protect drugs against chemical and enzymatic degradation and were also able to reduce side effects of some active drugs [8].

Nanoparticles are used for drug targeting both active and passive. The relatively small size of these systems limits their use, as only small quantities of material can be encapsulated. Other types of (non-biodegradable) nanoparticle systems include colloidal sulfur and colloidal gold. Colloidal sulfur is used as a diagnostic agent (labeled with ^{99m}Tc). It is usually protected from aggregation by the addition of gelations as a polymeric stabilizer. Colloidal gold is also used as a diagnostic (^{198}Au) and as a therapeutic agent [9].

Nanoparticles are formulated to target the drug to the specific organ site and to control the rate of release of drug. By encapsulating a drug into nano structures, the existence of the drug in the systemic circulation can be prolonged and thus enhance penetration into target tissue and reduce the toxicity [10].

The main aim of this study is to achieve prolonged release of Amprenavir such that the dosing frequency of the drug can be reduced by which we may reduce the side effects and increase the patient compliance. By formulating Amprenavir as nanoparticles we can directly deliver the drug to the cancer cell and prevent the normal cells from the adverse effects of Amprenavir [11-15].

The objectives of the present study are:

To formulate Amprenavir encapsulated Nanoparticles, To evaluate the influence of surfactant on particle size and PLGA ratio on encapsulation /entrapment efficiency and *in vitro* release of Amprenavir.

Amprenavir is a protease inhibitor used to treat HIV infection, Amprenavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.

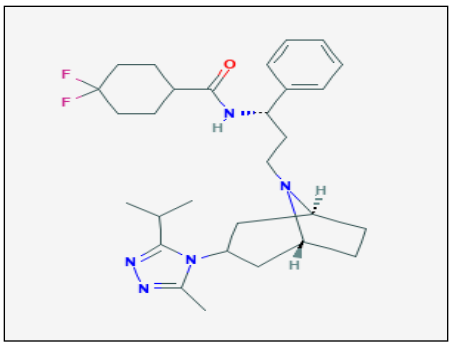
MATERIALS and METHODS

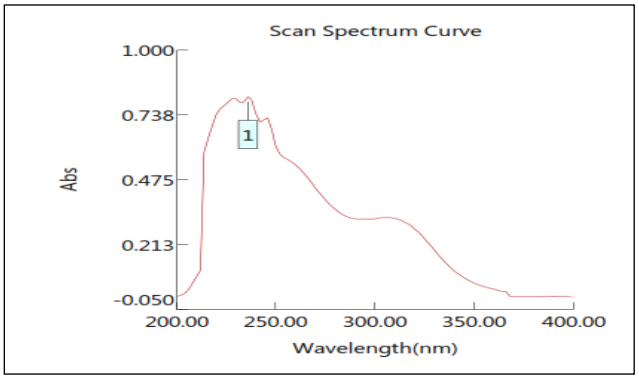
Review of Literature

Nitin Ghuge Attempts were made to develop RPHPLC m ethod for simultaneous estimation of Saquinavir from ta blet. For the RP HPLC method,Younglin (S.K.) Gradi ent system UV detector and C18 (Cosmosil) with 150 mm x 4.6 mm i. d. and 5µm particle size

Jagadeeswaran M A reversed phase high-performance liquid chromatographic method was developed and validated for the quantitative determination of two antiviral drugs viz. lopinavir and ritonavir

Fig 1. Structure of Amprenavir Spectrum Showing of Amprenavir

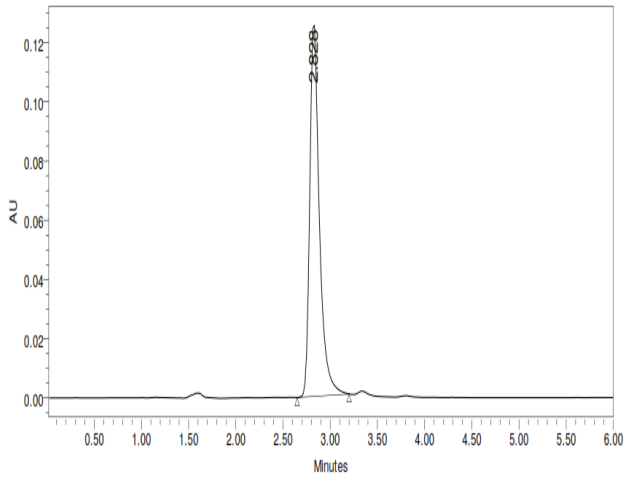




Optimized Chromatographic Conditions For Simultaneous Estimations Of Amprenavir By Rp-HPLC Method

Column : Agilent (5µm, 4.6x150mm)
Column temperature : Ambient
Wavelength : 235 nm
Mobile phase ratio : Methanol: ACN (60:40% v/v)

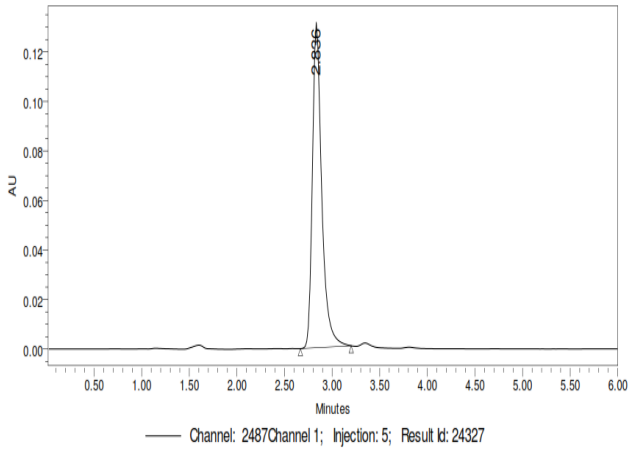
Flow rate : 1.0 ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 10.0 minutes



	Name	RT	Area	Height(µV)	USP Tailing	USP Plate Count
1	Amprenavir	2.828	892717	124236	1.4	3922.9

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5032	1.3
2	***Actual**	**4522**	**1.3**
3	5 % more	3834	1.3

Assay



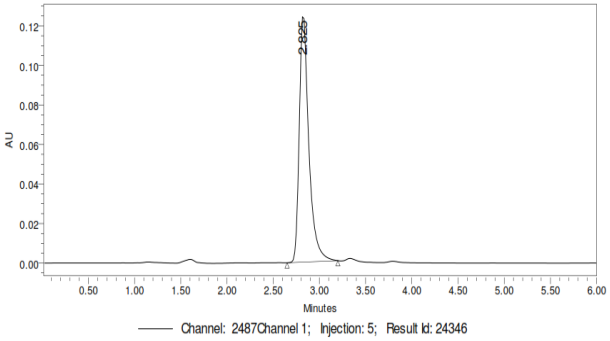
	Name	RT	Area	Height(µv)
1	Amprenavir	2.824	894562	128135
2	Amprenavir	2.827	896754	129139
3	Amprenavir	2.833	893627	132891
4	Amprenavir	2.833	893750	129914
5	Amprenavir	2.836	892682	130515
Mean			894275	
Std.Dev.			1537.7	
%RSD			0.17	

Accuracy

Table 1. Accuracy results for amprenavir

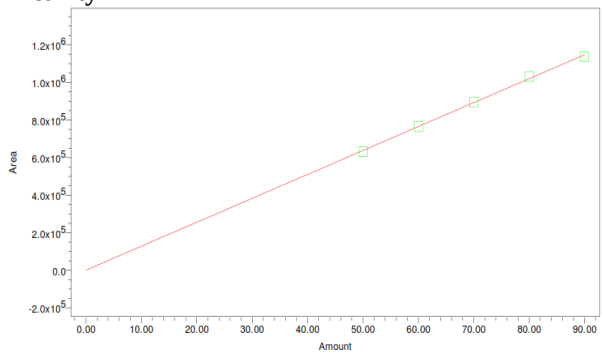
%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1093514.6	5	4.96	99.91%	100.56 %
100%	2246802.7	10	.98	99.18%	
150%	3407885.8	15	15.02	99.60%	

METHOD PRECISSION



	Name	RT	Area	Height(µv)
1	Amprenavir	2.823	895311	125747
2	Amprenavir	2.827	896783	122578
3	Amprenavir	2.828	895237	124365
4	Amprenavir	2.828	894206	124057
5	Amprenavir	2.825	895085	125410
Mean			895324	
Std. Dev.			927.8	
%RSD			0.10	

Linearity



AMPRENAVIR $r^2 = 0.998$

Table 2. Robustness

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4921	1.4
2	**1**	**4600**	**1.4**
3	1.2	4493	1.4

METHODOLOGY

IR spectroscopic study of Amprenavir

Compatibility study (IR spectroscopy) The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Method of Preparation of Amprenavir Loaded Nanoparticles:

Solvent dispersion (Nanoprecipitation):

The nanoparticles are prepared by dissolving the drug in organic phase along with the polymer (PLGA)

and added to the aqueous solution containing TPGS which acts as an emulsifier. The solution of organic phase was added in drop wise into aqueous phase under homogenization at 11,000 rpm. The dispersion was kept under magnetic stirring for 4hrs at room temperature. The solution is kept under reduced pressure for about 2-3min. This process forms nanoparticles loaded with drug.

Note: In above all formulations (F1 to F8) 5mg of the drug was added instead of original dose of the API (20mg). The above formulations were prepared and the entrapment efficiency was determined for choosing best formulation.

EVALUATION OF AMPRENAVIR LOADED NANOPARTICLES

1. Particle size
2. Zeta potential
3. Entrapment efficiency
4. In vitro drug release

Particle Size: Particle size was determined by using MALVERN instrument.

Zeta Potential: Zeta potentialwas determined by using MALVERN instrument UK.

Lyophilization: The obtained centrifuged samples were lyophilized and stored at 2-8^{0}C. The samples are lyophilized to attain stability. The obtained lyophilized powder is utilized for determination of entrapment efficiency and in-vitro drug release parameters.

Drug encapsulation

Efficiency: Lyophilized nanoparticles 3mg were dissolved in 1ml of diluents and the drug amount was determined by HPLC analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Amprenavir in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Amprenavir PLGA nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro amprenavir release

10 mg drug equivalent freeze dried Amprenavir loaded nanoparticles were dispersed in 3 ml pH 7.4 phosphate buffer solution which is transferred in dialysis bag and suspended in 100 ml of isotonic pH 7.4 Phosphate buffer solution (PBS). The bag was placed under magnetic stirring in a water bath maintained at 37 ± 0.5° C. At fixed time intervals 5ml of samples were taken out and fresh buffer was replaced. The obtained solution was analyzed by HPLC to determine the drug content.

Mathematical Modeling of The Drug Release

The mechanism of drug release from the formulations during the diffusion in pH 7.4 phosphate buffer was determined using the Zero order, First order, Higuchi equation and Korsmeyer-Peppas plot.

Stability studies

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 250C/60% RH analyzed every month for period of three months.
2. 300C/75% RH analyzed every month for period of three months.
3. 400C/75% RH analyzed every month for period of three months

RESULTS AND DISCUSSIONS

Evaluation Parameters

Optimized formulations

Based on the entrapment efficiency, a set of formulations (F6, F7 and F8) were considered as optimized compositions which can be taken up further studies and evaluated for the diffusion studies.

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 240 hours. Initially the release of drug from all the three batches was found to be about 25-35% in 24 hours. This was due to the release of adsorbed drug from the surface of Nanoparticles. Later on a constant and slow drug release was observed for 240hrs.

The drug diffusion for F6, F7 and F8 formulations was found to be approximately same i.e., 96.4%, 91.5% and 85.4% respectively. Therefore the F8 formulation which had drug polymer ratio of 1:25 was decided to be the optimized formulation.

Diffusion study profile for F6, F7 and F8 formulations

The drug release from the Nanoparticles was found to follow Zero order release based on the "r" value obtained for Zero order (0.952) and first order (0.935) for F8 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.978 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "n" value of 0.774 obtained for Peppa's equation.

IR studies

From the IR spectra it is clearly evident that there were no interactions of the drug. IR spectrum of the pure drug and the Drug polymer mixture (1:1) shows the characteristic peaks at 3386.95 cm^{-1} to 709.35cm^{-1}

This confines the undisturbed structure of the drug (Table 11). This proves the fact that there is no potential incompatibility of the drug with the polymer used in the formulation. Hence the formula for Amprenavir can be reproduced in the industrial scale

with out any apprehension of possible Drug polymer interactions.

STABILITY STUDIES:

There were no significant changes in physical and chemical properties of capsule of formulation F-8 after 2 months. Parameters quantified at various time intervals were shown

Table 3. Materials used

S.No	Materials	Manufacturer / Supplier
	Amprenavir	Scion Pharma, Taiwan
	PLGA	Lactel, Durect corporation Birmingham Division
	TPGS	Eastman company, UK
	Acetone	SRL
	Dialysis membrane	Himedia

Table 4. Equipments used

S. No.	Instrument	Manufacturer / Supplier
	Homogenizer	Kinematica AG(Poly tron PT2100)
	Rotary evaporator	Super fit
	Analytical balance	ShincoDeshi .,Ltd, Japan
	pH meter	Polmon, LP-139S
	Microscope	Olympus, CH20
	HPLC	Waters
	Sonicator	Enertech electronic Pvt. Ltd
	Lyophilizer	Lyophilisation systems India PVT LTD
	Particle size analyzer	MALVERN
	Zeta potential analyzer	MALVERN
	Magnetic stirrer	Rimek

Table 5. Composition of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
PLGA (50:50)(mg)	13	13	13	25	50	75	100	125
TPGS(%g/ml)	0.015	0.03	0.06	0.03	0.03	0.03	0.03	0.03
Amprenavir (mg)	5	5	5	5	5	5	5	5
Acetone (ml)	3	3	3	3	3	3	3	3
Water (ml)	10	10	10	10	10	10	10	10

Table 6. Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size, Zeta Potential and Drug Loading

z	Particle size (nm)	Zeta potential (mV)	Drug Loaded (mg)	Entrapment Efficiency (%)
F1	250.8	-0.272	0.2	4
F2	152.5	-5.16	0.23	4.6
F3	539.9	-1.92	0.21	4.2
F4	---	---	1.05	21
F5	106.8	-24.1	1.9	38
F6	132.3	-24.7	3.25	65
F7	155.5	-25.6	4.1	82
F8	122.4	-27.2	4.9	98

Table 7. Formulations used for in vitro diffusion study

Ingredients (mg)	F6	F7	F8
PLGA (50:50)	75	100	125
TPGS%(g/ml)	0.03	0.03	0.03
Amprenavir (mg)	5	5	5

Acetone (ml)	3	3	3
Water (ml)	10	10	10

***In- vitro* drug release of Amprenavir loaded Nanoparticles:**

Table 8. Diffusion study profiles for F6, F7,F8

Time (Hr)	Cumulative % drug release		
	F6	F7	F8
0	0	0	0
24	34.2	31.6	26.4
48	41.2	38.8	32.1
72	48.6	44.5	39.4
96	56.4	50.3	46.3
120	62.9	56.4	49.7
144	69.3	64.1	55.3
168	75.6	69.7	64.4
192	81.3	75.4	69.3
216	88.5	82.6	74.6
240	96.4	91.5	85.4

Table 9. DATA FOR IR SPECTRA OF AMPRENAVIR

FUNCTIONAL GROUPS	FREQUENCY (cm^{-1})
(--O—H) Stretching in alcohols	3386.95
(--C—H) Stretching in alkanes	2928.19&2860.73
(>C=O) stretching	1709.49
(–NH) Stretching(2^0 Amine)	1504.64
(–C–H) bending	1453.26
(–C–O) stretching 3^0 Alcohols	1168.16
(–C–O) stretching 2^0 Alcohols	1103.28
(–Phenyl) stretching	709.35

Table 10. DATA FOR IR SPECTRA OF MIXTURE OF AMPRENAVIR AND PLGA:

FUNCTIONAL GROUPS	FREQUENCY (cm^{-1})
(--O—H) Stretching in alcohols	3388.21
(--C—H) Stretching in alkanes	2979.22 & 2934.29
(>C=O) stretching	1710.28
(–NH) Stretching (2^0 Amine)	1503.07
(–C–H) bending	1448.8
(–O–) stretching	1257.98

(–C–O) stretching 3^0 Alcohols	1169.61
(–C–O) stretching 2^0 Alcohols	1098.59
(–Phenyl) stretching	709.32

Table 11. Results of stability studies of optimized formulation F8:

Formulation code	Parameters	Initial	1st Month	2nd Month	Limits as per specifications
F8	25˚C/60%RH % Release	96.20	96.27	96.78	Not less than 85%
F8	30˚C/75%RH %Release	97.12	96.79	96.80	Not less than 85%
F8	40˚C/75%RH %Release	97.25	97.48	96.83	Not less than 85%
F8	25˚C/60%RH Assay value	97.65	98.19	98.31	Not less than 90% Not more than 110%
F8	30˚C/75%RH Assay value	98.16	98.21	98.32	Not less than 90% Not more than 110%
F8	40˚C/75%RH Assay value	98.20	98.16	98.22	Not less than 90% Not more than 110%

Fig 2. Diffusion study profile Cumulative % release Vs Time (hrs)

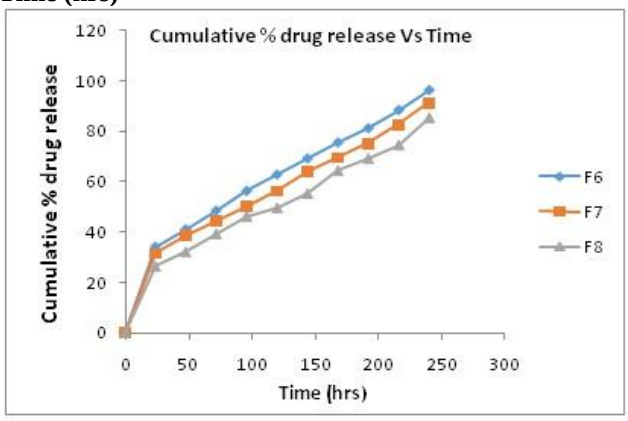
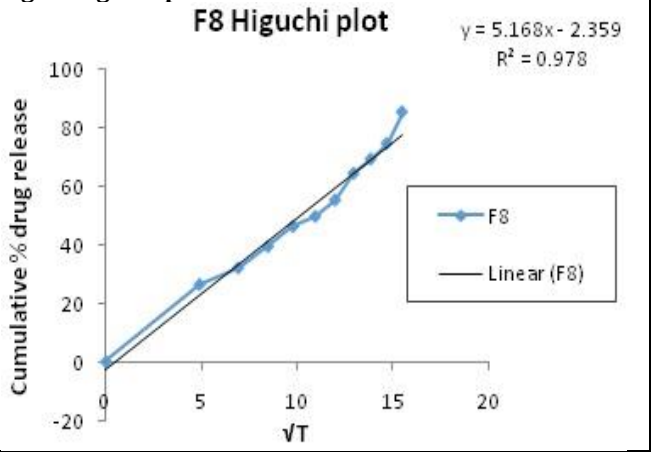


Fig 3. Higuchi plot For F8 Formation



DISCUSSION

Assay

The amount of Amprenavir present in the taken dosage form was found to be 99.54 % respectively.

Accuracy

The percentage mean recovery of Amprenavir is 100.56% respectively.

System Suitability

The % RSD for the retention times and peak area of Amprenavir were found to be less than 2%.

Linearity and Range

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Amprenavir is 0.998.

Precision

Test results for Amprenavir are showing that the %RSD of Assay results are within limits.

Robustness

The system suitability parameters were within limit at all variable conditions.

Ruggedness

The %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

The validated method is found to be Specific, Linear, Precise, Accurate, Robust and Rugged for the estimation of Amprenavir in tablet dosage form.

Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity for routine analysis as well as for stability analysis.

The present research proposed a novel formulation by applying Vitamin E TPGS as an emulsifier to fabricate Nanoparticles by solvent dispersion/nanopresipitation for controlled release of antineoplastic drug Amprenavir. Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimised. Our results demonstrated

that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric nanoparticles, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution and in vitro release kinetics of the nanoparticles. In this research, a drug encapsulation efficiency as high as 98% has been achieved. The particle size and size distribution strongly depends on the amount of TPGS added in the fabrication. Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as these plots showed the highest linearity (r^2=0.978) but a close relationship was also noted with Zero order kinetics (r^2 =0.952)

ACKNOWLEDGEMENT
Nil

CONFLICT OF INTEREST
No interest

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