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DRUG RELEASE PREDICTION BY CURVE FITTING FOR SURFACE ENGINEERED NANOSYSTEM DESIGN WITH GREEN BIOACTIVE PAYLOAD

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ABSTRACT

This document contains the formatting information for the papers presented at the International conference on "Engineering Technology International Conference". The conference held at (Bali, Indonesia) during August 10-11, 2015. Scientific interest in cutting edge drug delivery technologies has enhanced considerably to replace expensive drug discovery investigations. Advent of nanotechnology in drug delivery has helped to transform plant bioactives like silymarin or curcumin originally sidelined due to pharmacokinetic limitations into valuable therapeutics. Andrographolide is one such pleotropic plant bioactive having low solubility and short biological half-life. Nanosystem design for the plant bioactive in FDA approved polymer PLGA 50:50 was therefore perceived as probable solution to its pharmacokinetic limitations. However, considerable challenges exist in the pre-formulative stages of nanosystem design. Multiple trial and error runs are required to arrive at a desired release of bioactives from the designed systems leading to time and cost consumptions. Hence an attempt has been made in the present work to predict the drug release from nanosystems at definite time intervals using a simpler mathematical tool by way of curve fitting. The proposed curve fitting, uses a power series to predict the drug release in successive hours, is of the form f(x) = a*x^b+c. The constants a, b and c are predicted by least squares method and the confidence bounds of the prediction is taken to be 99 %. The results obtained from the mathematical curve fitting correlated well with the release data obtained from HPLC experiments. The error percentage of the predicted results from the experimentation is to a tune of 3% in all the data sets.

Keywords: nanosystem, andrographolide, drug release, mathematical tool, curve fitting.

INTRODUCTION

Discovery process has seen a paradigm shift in favour of pharmacokinetic developments of known bioactives rather than the pharmacodymanic one[1]. A renewed thrust is also noticed in favor of plant products, nutraceuticals and active food supplements for quality life requirements[2]. Current technological breakthroughs on the other hand have helped to transform plant bioactives like taxol, silymarin or curcumin into some useful therapeutics[3]. Andrographolide (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)- 5, 8_-dimethyl-2-methylene-1-napthalenyl]ethylidene] dihydro- 4-hydroxy-2(3H)-furanone) is a diterpenoid lactone extracted from the leaves of Andrographis paniculata nees. In spite of its several reported pharmacological activities the pleotropic lactone is sidelined from clinical use due to low solubility (0.5mg/L at 27°C)[4] and short biological half-life of 2 hours[5]. Nanosystem design for the plant bioactive was perceived to improve upon its pharmacokinetic limitations. Colossal technological advancements have taken place in manufacturing of pharmaceutical products. But still considerable hurdles exist in the pre-formulative stages of polymeric nanosystem design[6]. Multiple trial and error runs[7] are required to arrive at a desired release of bioactives from the designed systems. These runs consume high quantities of valuable drugs which further affects the cost of the product. Hence, an attempt has been made in this work to develop a mathematical model by using a curve fitting technique. The proposed curve fitting, uses a power series to predict the drug release in successive hours, is of the form f(x) = a*x^b+c. Power series models are used to describe a variety of data. The rate at which reactants are consumed in a chemical reaction is generally proportional to the concentration of the reactant raised to some power. The constants a, b and c are predicted by least squares method and the confidence bounds of the prediction is taken to be 99 %. The results obtained from the mathematical model were compared with that those of experimental data are in good agreement.

NANOSYSTEM DESIGN AND CHARACTERIZATION

Nanosystem design was approached following by emulsion solvent evaporation technique. Briefly, polymer PLGA and the bioactive Andrographolide were dissolved together in the organic solvent and then emulsified with the aqueous phase containing the surfactant. Reduction in droplet size was done by application of both sonication and high speed homogenization. Solvent removal was achieved by stirring at room temperature and the nanosystems formed subsequently were recovered by ultracentrifugation (30,000 rpm, 25 min at 4°C). Surface engineering of nanosystems was done by pump controlled dropwise addition of the nanosystem dispersion in water to

different chitosan solution in 1% v/v aqueous acetic acid under magnetic stirring at 10°C for one hour. Each batch was prepared in triplicate for future studies.

Hydrodynamic particle diameter and polydispersity index (PDI) of surface engineered nanosystems were determined by dynamic light scattering method using Zetasizer (Nano ZS, Malvern Instruments, UK) engaged with 4mw He-Ne laser ray. Surface charges of the designed nanosytems (zeta potentials) were measured following the principle of electrophoretic agility under the effect of an applied electrical field.

Result analysis

Andrographolide nanosystems were designed with US FDA approved polymer PLGA by top down emulsion solvent evaporation approach to provide solution to its pharmacokinetic constraints. Formulation design optimization was done varying the surfactant Pluronic-F-127 concentration. The increase in surfactant concentration favoured the segregation as well as size reduction of emulsion droplets as reported earlier[8] (refer Table-1). Increase of zeta potential was also observed with enhancement of surfactant concentration. Pluronic concentration of 0.3% w/v was selected for further surface engineering as it produced almost the same size and zeta potential than its higher concentration (0.5% w/v). Besides avoiding excess surfactant concentration would also contribute to reduction in toxicity of designed nanosystems.

Table-1. Characterization of Surface Engineered and Non-Engineered nanosystems.

Surface Engineered Nanosystems			
Chitosan Concentration (w/v)	**Particle Size (nm)**	**Zeta potential (mV)**	**Polydispersity Index**
0.1%	200±19.04	+19.18±1.32	0.114±0.01
0.3%	226±17.19	+27.38±1.55	0.121±0.01
0.5%	231±16.34	+27.19±1.83	0.127±0.01

Surface Engineered Nanosystems			
Surfactant Concentration (w/v)	**Particle Size (nm)**	**Zeta potential (mV)**	**Polydispersity Index**
0.5%	200±14.79	−21.7±1.67	0.127±0.01
1.0%	181±12.60	−29.7±1.41	0.115±0.01
1.5%	179±13.91	−30.13±1.53	0.207±0.01

Surface engineered nanosytems with andrographolide payload were designed with the optimized surfactant concentration. Release rate controlling polymer concentration was optimized based on its effect on particle size and zeta potential of surface engineered nanosystems. With the increase in chitosan concentration there was increase in particle size due to covering of the particles by thin chitosan film. The association of cationic chitosan with anionic PLGA surface contributes to the particle size increase. This phenomenon is also reflected in the reversal of zeta potential of the nanosystems[9]. Chitosan concentration of 0.3% w/v appeared as the optimized concentration as it enhanced the stability of the nanosystems to the same extent as its higher concentration. Besides reports also claim that toxicity of chitosan decorated nanosystems is mediated by electrostatic interaction with the negatively charged membrane[10]. So, increasing of chitosan concentration further was not desired because the zeta potential values of 0.3% and 0.5% were almost same[11] indicating the saturation of anionic sites for the attachment of cationic chitosan.

NANOSYSTEM VISUALIZATION IN ATOMIC FORCE MICROSCOPY

Drop of surface engineered nanosytems (100 µL approx) were suspended in water was placed onto fused mica sheets. The surface morphology of nanosystems designed was then visualized in AFM under tapping mode at a scan rate of 1.2 Hz. The resonance frequency varied from 267 to 328 kHz resonance frequency[12] during the capture.

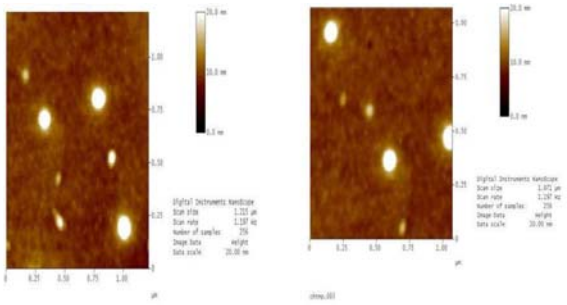

Figure-1. AFM visualization of Surface Engineered nanosystems in different scanning zones.

Surface engineered nanosystems displayed mostly spherical geometry in the scanned area (Figure-1). No coalescence was observed in the zone observation indicating high stability of the nanosystems as reflected from zeta potential values.

BIOACTIVE ENTRAPMENT STUDIES IN HPLC

Bioactive entrapment in nanosystems was evaluated as per our earlier report following reverse phase HPLC method with acetonitrile: 0.1%v/v phosphoric acid in water (40:60v/v) as mobile phase. The flow speed was maintained at 1 ml/min. Andrographolide recorded at a retention time of 4.5 min[12]. Bioactive mass load before and after nanosystem design in the supernatant was detected to calculate entrapment efficiency.

Result analysis

Bioactive load in nanosystems is a vital parameter which regulates the performance of these newer systems *in-vivo*. Andrographolide mass load in surface engineered nanosystems varying in chitosan concentration as analysed by RP-HPLC (Figure-2). Loading percentage

was 85.37%±4.9%, 84.86±3.94 and 81.19%±4.12% respectively with change in coating polymer concentration 0.1%, 0.3% and 0.5%. However in case of non-engineered nanosystems drug load decreased with increase in surfactant concentration 85.97 ±4.94 (0.5%), 84.86±3.94 (1.0%) and 80.13±5.04 (1.5%). This phenomenon is reported by other researchers where drug load decreases due to decrease in particle diameter as a result of higher surfactant concentration[13].

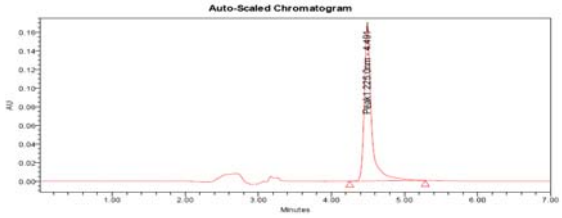

Figure-2. HPLC Chromatogram of andrographolide.

ANALYSIS OF SURFACE ENGINEERED POLYMER COATING

Quantitative analysis of rate controlling polymer coating in nanosystems was based electrostatic association of polymer chitosan with colorimetric dye Alizarin Red[11]. A standard graph was plotted from chitosan alizarin reaction at 571 nm and was used to determine polymer mass content. The nanosystem associated polymer concentration was determined by deduction of the free polymer left over in the supernatant after ultracentrifugation from the initial concentration of the polymer in the stock during nanosytem design. All experiments were done in triplicate.

Results analysis

Standard curve developed with concentration (x) versus absorbance (y), y =0.0037x + 0.1649, R^2 = 0.9863, at acidic pH environment (pH 5) was used for further analysis of the polymer mass content. Percentage of chitosan association calculated on a weight basis for surface engineered nanosytems designed with varying chitosan concentration (0.1%, 0.3% & 0.5%) were respectively 71.86 ± 4.13, 75.94 ± 3.22 and 77.67± 4.72. Results are expressed as mean ± standard deviation obtained from triplicate studies.

TRACKING DRUG RELEASE *IN-VITRO* IN HPLC

For drug release studies, nanosystems equivalent to 2 mg of drug payload, was solubilized in phosphate buffer (100 mM, pH 7.4, 1ml) and transferred into dialysis bags with molecular weight cut-off. The bags were placed in glass vials containing phosphate buffer (10ml, 37°C) in a shaker unit[9]. Drug release at definite time intervals was analysed by HPLC. The release medium was replaced by fresh buffer to maintain sink conditions. Release studies were conducted for both surface engineered and non-engineered nanosytems.

Result analysis

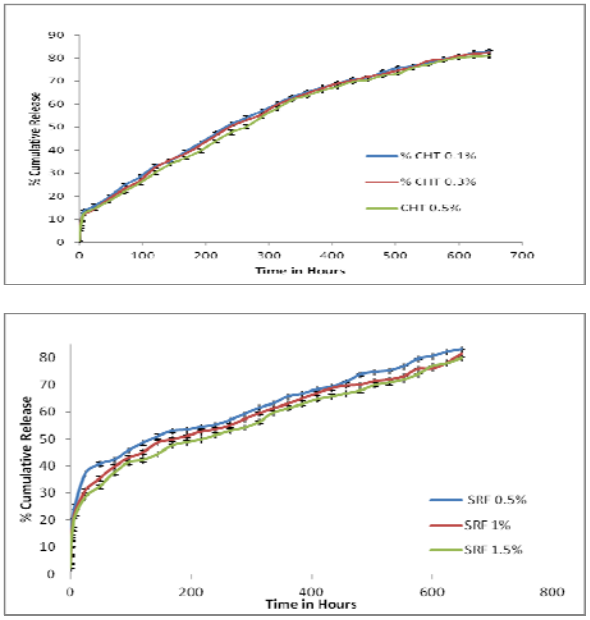

Figure-3. Drug release profile of (A) Surface Engineered.

Nanosystems (B) Non-Engineered Nanosystems

The release studies were of surface engineered nanosystems were conducted varying the concentration of rate-controlling polymer chitosan on the naked nanosystem surface. Surface engineering of polymeric nanosystems with chitosan is reported to improve drug payloads, induce bioadhesive property, and prolongs drug release compared to non-engineered nanosytems[14]. The drug release of engineered nanosystems was biphasic in nature and exhibited a sustained release up to 648 hours. Initial drug release showed an accelerated rate which lasted for nearly 8 hours followed by constant sustained release phase. About 80% of the plant bioactive payload was accountable from the engineered nanosystems (Figure-3). On the other hand in case of the naked PLGA nanosytems drug release showed a different release pattern. Initially up to 24 hours the drug payload release was significantly high ranging for 28 to 35% compared to approximately 15% for the engineered nanosystems. This burst release is expected for polymeric nanosystems due to surface adsorbed drug moieties. Surface decoration with rate controlling polymer is an excellent strategy to minimize this effect.

DRUG RELEASE PREDICTION BY CURVE FITTING

Curve fitting technique involves in finding coefficients (parameters) for one or more models that fit experimental data[15]. The experimental data obtained is divided into two arrays: a deterministic array and a random array.

data = deterministic array + random array

The deterministic array is given by a parametric model and the random array is often described as error associated with the data.

data = model + error

The model is a function of the independent (predictor) variable and one or more coefficients. The error represents random variations in the data that follow a specific probability distribution (usually Gaussian). The variations can come from many different sources, but are always present at some level when you are dealing with measured data. Systematic variations can also exist, but they can lead to a fitted model that does not represent the data well. In the present investigation, a number of trails have been done to fit the experimental drug release by choosing exponential series, Gaussian, interpolant, rational, Fourier, smoothing spline, sum of sine functions Weibull and power law. Out of all the chosen functions power law best fits the data and with 99 % confidence and an R-Square error of 0.9935.

Results analysis

General model of the power law adopted using curve fitting technique is of the form

f(x) = a*x^b+c

The coefficients are predicted with 95% confidence bounds as a = 10.01, b = 0.3137 and c = 3.313 for the set of data CHT 0.3% (indicated as CHT 2 % in Mathematical Model). The predicted results with the experimental data and with 99 % confidence bounds is plotted in Figure-4.

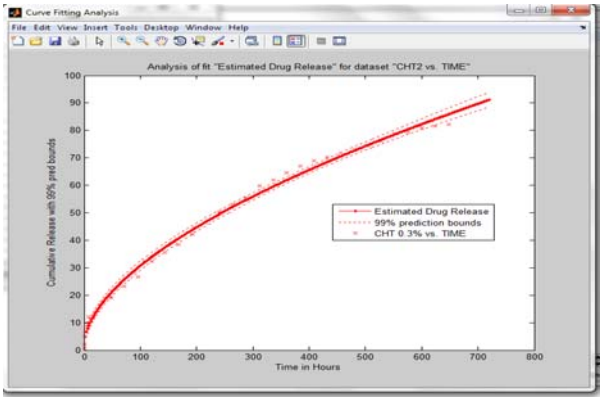

Figure-4. Predicted and experimental drug release profile of Surface Engineered nanosystems.

A similar equation is of the power law is used for prediction of the next set of experimental data and the coefficients are predicted with 95% confidence bounds as follows a = 8.912 , b = 0.3294 and c = 2.204 for the set of data SRF 1.0 % and a = 6.485, b = 0.3733 and c = 3.696 for the set of data SRF 1.5 %. Similarly, the same power equation is used for prediction of the drug release in cumulative hours for with coefficients as a = 2.21, b = 0.5612 and c = 2.486 and a =2.015 b = 0.5757 and c = 2.2 and a = 1.639, b = 0.6058 and c = 2.499 for the sets of data where CHT 0.1, 0.3 and 0.5 % respectively.

CONCLUSIONS

Surface engineered nanosystems for pleotropic bioactive andrographolide was successfully designed in PLGA. The drug release pattern is predicted by curve fitting method using a power series function. The predicted curve fitting method enables to predict the cumulative response in terms of hours. This method will be useful during the preformulation stages to predict the drug release at specific time intervals and thereby have a definite impact on the cost effectiveness in the manufacturing of such newer nano-scale systems. The present study has a promising potential to make advanced therapeutics affordable throughout the society. It also paves the way to meet the challenges that are being encountered during formulation development, drug release and drug loading by developing a suitable mathematical model which the authors are working on presently.

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