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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(2):385-8

Kinetic evaluation of L-Dopa loaded WGA-grafted nanoparticles

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Received 04 October 2019; Accepted 14 January 2020
Available online 2020 with doi: 10.5455/medscience.2019.08.9209

Abstract

Drug release is a critical parameter in the discipline of drug delivery for years. The determination of in vitro release kinetics of active substances from drug systems plays a critical role in predicting and managing of both efficacy and safety. Kinetics is more than a scientific goal; it is a fundamental quality parameter of all type of drugs. Kinetic models are developed to determine drug release from delivery systems which the released drug amount (Q) is plotted versus time; t or as a function of time Q=f (t). Kinetic models facilitate the understanding of release pattern in enabling to design an effective formulation. In this study wheat germ agglutinin (WGA) conjugated, L-Dopa loaded, poly lactic-co-glycolic acid (PLGA) nanoparticles were analyzed for determining the release pattern of L-Dopa. Higuchi, zero order, first order, Hixon Crowell and Korsmeyer-Peppas models were investigated. Hixson Crowell model was found to be the best fitted kinetic model in L-Dopa loaded nanoparticles (r^2 =0.9828).

Keywords: Kinetic evaluation, first order, zero order, hixon-crowell, korsmeyer-pepas, higuchi

Introduction

Over the recent years, drug dissolution of solid dosage forms has become the main issue of profound and cost-effective developments in science. Whenever production of a novel solid pharmaceutical form needs to ensure which active substances release occurs within a proper manner.

Dissolution rate studies are performed in order to ensure the quality of solid dosage forms. The rate of dissolution could be defined as the amount of drug dissolved in dissolution medium per unit time. In controlled release delivery systems, dissolution rate depends on the diffusion of the active substance from the system, by degradation or erosion of the carrier. The amount of active substance released over time is determined by appropriate methods and the suitability of the obtained data to various kinetic models is evaluated. The particle shape and size, the properties of the polymers and excipients used the method of preparation, the properties of the active substance affect the release of drug from matrix and appropriate kinetic models are determined by taking these properties into consideration. Kinetic models were developed to determine drug release from dosage forms which the released drug amount (Q) is plotted versus time, t or as a function of time Q=f (t). Some kinetic explanation of the dissolved drug amount (Q) as a function of time are zero order, first order, Hixson–Crowell, Higuchi, Korsmeyer–Peppas models. Higuchi model describes the drug release from a matrix system. Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles. Korsmeyer and Peppas developed an empiric equation to analyze both Fickian and non-Fickian release of drug from swelling and non-swelling polymeric delivery systems. In this study L-Dopa loaded, WGA grafted PLGA nanoparticles dissolution profiles were analyzed.

Materials and Methods

Materials

HPLC grade dichloromethane and acetonitrile was from Merck (Germany). Distilled, deionized water was used throughout and was obtained from a Millipore water supplier. Resomer® RG 503 H and poly (vinyl alcohol) (PVA) were from Sigma-Aldrich. L-Dopa was a product of Dıvıs' Laboratories and was gifted by ILKO Drug Company (Turkey). Dialysis tubing cellulose membrane was from Sigma-Aldrich with the molecular weight cut-off = 14.000.

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Methods

Preparation of nanoparticles

L-Dopa loaded nanoparticles were prepared using a double emulsion solvent evaporation method using PVA as a stabilizer. Briefly, L-Dopa and PLGA were dissolved in dichloromethane (DCM), and distilled water was added to that solution and by means of using an ultrasonic homogenizer they were mixed to form a primary W/O emulsion. The primary emulsion was further emulsified in PVA solution by homogenization to obtain a double $W1/O/W_2$ emulsion. The resultant double emulsion was then stirred at a certain rate at room temperature (25°C) on a magnetic stirring plate to evaporate the organic solvent. The resulting nanoparticle suspension was kept at 4°C overnight to allow hardening of the PLGA matrix by allowing the DCM to fully partition to the external aqueous phase. Nanoparticles was recovered by ultracentrifugation. The supernatant was removed and nanoparticles sediments was washed twice with Milli-Q water to remove free drug and excess surfactant, and then lyophilized.

Particle size and zeta potential analysis

1 µL nanoparticle solution was suspended in 100 mL distilled water, was stirred in the ultrasonic bath for 30 minutes. Particle size, poly dispersite index (PDI) and zeta analysis were tested with zeta-sizer (Malvern, USA).

In vitro dissolution method

Nanoparticles which was suspended in 0.5 mL buffer solution (pH 4.5) was put and sonicated with ultrasonic homogenizer in order to obtain a homogeneous inner phase in a dialysis bag. Both ends were tied. Dialysis bag's cut-off was 14.000 kDa which doesn't prevent free diffusion of L-Dopa due to its low molecular weight (197.1879 g/mol). The dialysis bag was put in 49.5 mL (total 50 mL) buffer solution at pH 4.5 maintained at 37 ± 0.5 °C at fixed time intervals 1 mL aliquots was sampled and replaced with 1 mL fresh pH 4.5 buffer solution.

Analytical method development

Drug concentrations were measured using High Performance Liquid Chromatography (HPLC) (Agilent 1001, USA) which mobile phase was comprised of trifluoroacetic acid (TFA) solution (% 0.05 v/v) pH 3-acetonitrile (95:5). 250 mm × 4.6 mm, 5 µ C18 HPLC column was used for chromatographic separation at 280 nm ± 5 wavelengths. Flow rate was 1 mL/min and running time was 7 minutes with 10 µL injection volume. The method was validated as ICH guideline in terms of linearity, accuracy, precision, detection limit and quantitation limit. The validated method was found to be simple, sensitive, accurate and precise to determine the release of L-Dopa from nanoparticles.

Evaluation of release kinetics

The drug release kinetic is controlled by one or more mechanisms that rely on the combination of the matrix, geometry, preparing method and dissolution medium. This can be described by mathematical models in agreement with the required or needed predictive ability and preciseness of the model. Mathematical models which was used for the parametric declaration of dissolution data in our study was zero order, first order, Hixson–Crowell, Higuchi, Korsmeyer–Peppas models. With the help of the individual unit dissolution data, the best fitted kinetic model preference was built on the comparison of the highest determination coefficient (r^2), lowest residual mean square (RMS) and highest F values of the models. The percentage of drug release vs. time was plotted. t=0 was not included in calculated results [1]. Release constant and RMS, standart deviation (SD), r^2 and F values were calculated with GraphPad® prisim 6 program by using regression analysis for each data.

Results

The basic factors effecting the drug release from the dosage form are the solubility, dosing or content of drug, molecular weight, particle size and shape of polymer. These are factors capable of influencing the release kinetics of a formulation. Particle size, PDI and zeta potential results of the WGA grafted L-Dopa PLGA nanoparticles which was used in the release study is given in Table 1.

Table 1. Particle size, PDI and zeta potential results of the WGA grafted L-Dopa PLGA nanoparticles

	Particle Size	PDI	Zeta Potential
WGA grafted PLGA nanoparticles	329.0 ± 188.3	0.384 ± 0.113	-4.47 ± 0.576

Cellulose can be covalently bonded to active substances due to its structure was contained free coupling ends. Release of L-Dopa without binding to the cellulose membrane was important for accurate monitoring of the release profile. So, 0.5 mL L-Dopa solution was put in dialysis bag, both ends were tied, than in vitro dissolution method was carried out. As shown in Figure 1, L-Dopa was totally released which indicated that L-Dopa didn't bind to the cellulose membrane.

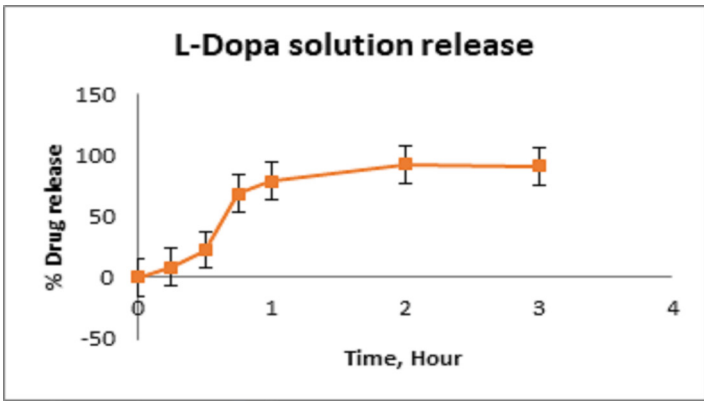

Figure 1. Free L-Dopa release from dialysis membrane

In pharmaceutical drug application, zero order release profile design is a need for prolonged release dosage forms. It points to the case of constant drug release from a drug delivery system independent of the concentration. Simply, zero order release could be presented as; $Q = Q_0 + k_0 t$ (Equation 1).

Where Q is the amount of drug released or dissolved, Q_0 is the primary amount of drug in dissolution medium, and k_0 is the zero-order release constant [2-4]. The fitting of the dissolution data to the zero-order kinetic model is seen in Figure 2 & Table 2.

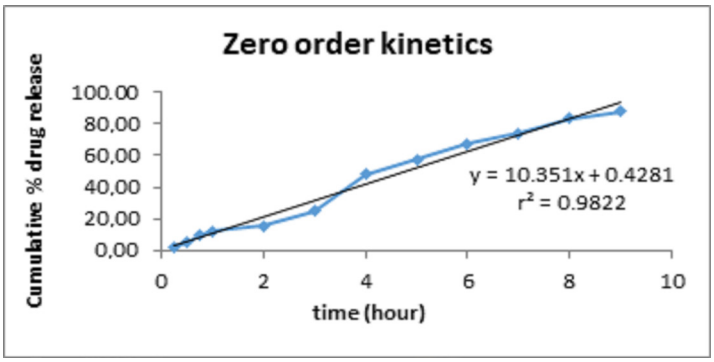

Figure 2. Zero order release profile of L-Dopa from nanoparticles

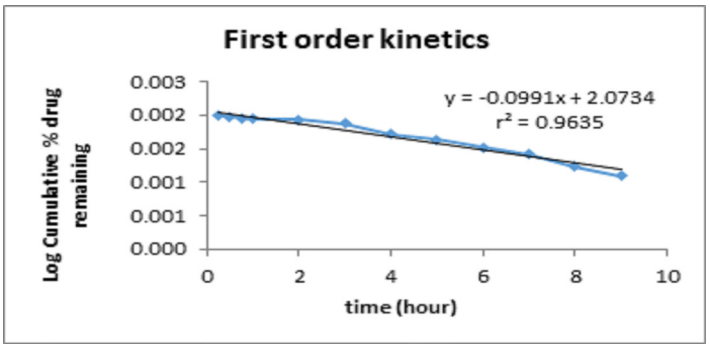

Figure 3. First order release profile of L-Dopa from nanoparticles

The first order equation defines the release from system where release rate is concentration reliant, expressed by the equation:

$dC / dt = - k_t$ (Equation 2)

Where k is first order rate constant represent in units of time. This equation can be demonstrated as:

$Log\ C_t = Log\ C_0 – k\ t / 2.303$ (Equation 3).

Where, C_0 is the initial concentration of active substance and C_t is the concentration of drug in solution at time t. The equation intimates a first order hinge on the concentration gradient (C_o - C_t) among with the stable liquid layer by the side of the solid surface and the bulk liquid. The fitting of the dissolution results to the first order kinetic model is seen in Figure 3 & Table 2.

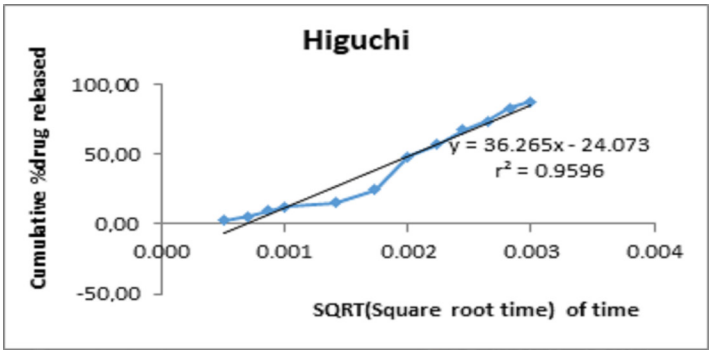

Figure 4. Higuchi release profile of L-Dopa from nanoparticles

Higuchi formulated numerous hypothetical models to study the release of water soluble and low soluble drugs integrated in semi-solid and/or solid matrixes. Release of the active substance from homogeneous and heterogeneous matrices produced which biodegradable polymers is compatible with the kinetics proposed by Higuchi. According to this model, the amount of released active substance immediately is compatible to the square root of time as shown in Equation 4 where, k_H is the Higuchi dissolution constant [2-4]. The fitting of the dissolution data to the Higuchi kinetic model is seen in Figure 4 & Table 2.

$f_t = Q = k_h\ t^{1/2}$ (Equation 4)

Nanoparticles that having uniformed size particles, Hixson and Crowell derived the equation which shows rate of drug release built on cube root of particles' weight and the radius of particle is not accepted to be constant.

This is stated by the equation,

$M_0^{1/3} – M_t^{1/3} = \kappa\ t$ (Equation 5).

Where, M_0 is the initial amount of drug in the dosage form, M_t is remaining amount of drug in the dosage form at time 't' and κ is proportionality constant [2-4]. The adjustment of the dissolution data to the Hixson Crowell kinetic model is seen in Figure 5 & Table 2.

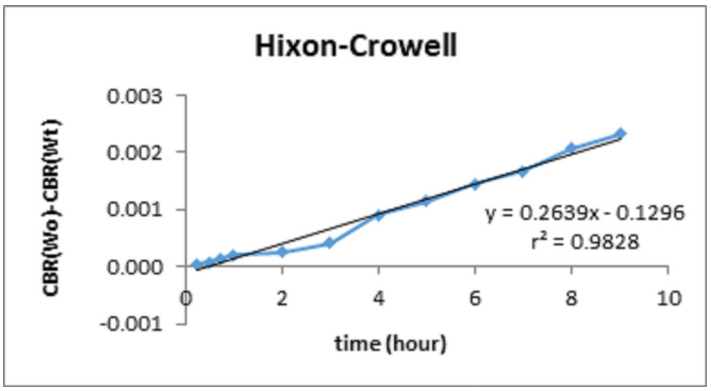

Figure 5. Hixon Crowell release profile of L-Dopa from nanoparticles

Korsmeyer gathered a simple correlation which defined drug dissolution from polymeric systems and Korsmeyer and Peppas developed an empiric equation to examine both Fickian and non-Fickian release of active ingredient from swelling as well as no swelling polymeric systems. To determine the mechanism of drug release, first 60% drug release results was suited in Korsmeyer – Peppas model.

$M_t / M_\infty = K_p\ t^n$ (Equation 6).

Where Mt/Mα is the proportion of drug released at time t, k is the rate constant (having units of tn) integrating morphological and three-dimensional specifics of the delivery system. n is the release proponent indicative of the transport mechanism of active ingredient through the polymer. The n value is used to describe different release mechanisms [2,4]. The adapted of the dissolution

data to the Korsmeyer-Peppas kinetic model is seen in Figure 6 & Table 2.

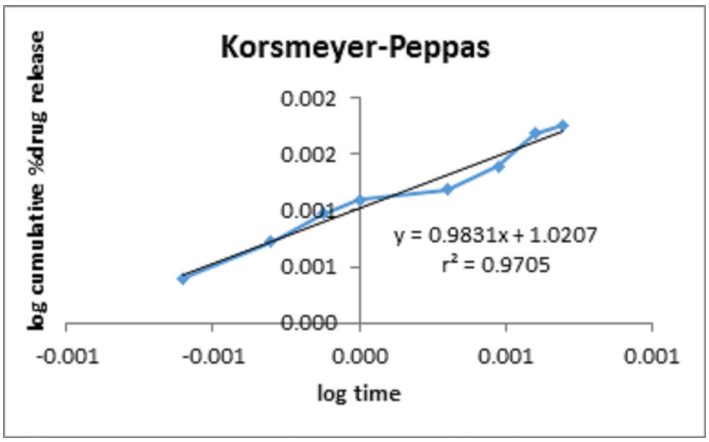

Figure 6. Korsmeyer- Pepas release profile of L-Dopa from nanoparticles

Release parameters of WGA grafted L-Dopa nanoparticles were listed in Table 2.

Table 2. Release parameters of WGA grafted L-Dopa nanoparticles

Release kinetics		PDI
Zero order	k_r^0 (µg/h)	10.35
	*SD	0.2386
	**RMS	613.7
	***F	1882
First order	k_r^1 (h^{-1})	-0.09910
	*SD	0.003305
	**RMS	0.1178
	***F	899.0
Higuchi	k ($h^{-1/2}$)	36.27
	*SD	1.276
	**RMS	1397
	***F	807.7
Hixon Crowell	k_h	0.2639
	*SD	0.005984
	**RMS	0.3861
	***F	1944
Korsmeyer-Pepas	n	1.021
	kp	0.9831
	*SD	0.03653
	**RMS	0.1321
	***F	724.1

*SD: standard deviation, **RMS: residual mean square, ***F: F value for regression analysis

Discussion

As shown in Figures 1-5 and Table 2 plots were drawn, in accordance with several kinetic models, were giving linear relationship. In zero order plot (Figure 2 & Table 2) the r^2 value obtained is 0.9822 and first order (Figure 3& Table 2) gave 0.9635 describing the drug release rate relationship with concentration of drug. In Higuchi plot (Figure 4 & Table 2), the r^2 value was calculated as 0.9596, where Korsmeyer-Peppas model gave a r^2 result of 0.9705 (Figures 5,6 & Table 2). The best linearity was found in Hixson Crowell equation plot (Figure 5) (r^2 = 0.9828) [5]. Although r^2 is a goodness-of-fit measure for linear regression models, it sometimes is not enough to evaluate the difference if the r^2 values give close results. In our study, zero order and Hixson Crowell kinetics r^2 values were also found so close to each other. As a result, it was thought it would not be possible to make an accurate assessment only based on this parameter. Therefore, it was decided to include F and RMS parameters in the kinetic evaluation of the investigated models. RMS; is the standard deviation of the residuals (prediction errors). Residuals are a measure of how far from the regression line data points are. Meanwhile F value; is a value you get when you run an ANOVA test or a regression analysis to find out if the means between two populations are significantly different. Low RMS and high F values express the best fitted model. Hixson Crowell equation showed the lower RMS and higher F value than zero order equation which supports the drug release mechanism of PLGA nanoparticles depend on degradation of polymer and diffusion of drug from nanoparticles.

Conclusion

Drug release kinetics of WGA grafted L-Dopa nanoparticles correspond best to fit Hixson Crowell kinetic and drug release mechanism of PLGA nanoparticles depend on degradation of polymer and diffusion of drug from nanoparticles.

Acknowledgment
This poster presentation is a part of our project which is supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey; Project No:116S639)

Conflict of interest
We declare that we have no conflict of interest.

Financial Disclosure
This poster presentation is a part of our project which is supported by TUBİTAK (The Scientific and Technological Research Council of Turkey; Project No:116S639)

Ethical approval
Ethics committee approval was obtained

References

1. Anderson N, Bauer M, Boussac N, et al. An evaluation of fit factors and dissolution efficiency for the comparison of in vitro dissolution profiles. J Pharm Biomed Anal. 1998;17:811-22.

2. Yuksel N, Kanık AE, Baykara T. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and-independent methods. Int J Pharm. 2000;209:57-67.

3. Sathe MP, Tsong Y, Shah VP. In vitro dissolution profile comparison: stastics and snalysis, model dependent approach. Pharm Res. 1996;13:803-1799.

4. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;2001:123-33

5. Park TG. Degradation of poly (lactic-co-glycolic acid) microspheres: effect of copolymer composition. Biomaterials. 1995;16:1123-30.