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DOI: 10.2478/auoc-2021-0020

Ovidius University Annals of Chemistry Volume 32, Number 2, pp. 132 - 136, 2021

Evaluation of drug release kinetics from polymeric nanoparticles loaded with poorly water-soluble APIs

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Abstract. The aim of this research was to investigate the release behavior of a combination of two poorly water-soluble active pharmaceutical ingredients (APIs) from poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles. Amlodipine besylate - AML, a calcium channel blocker, and valsartan - VAL, an angiotensin II receptor antagonist drug, were used as poorly water-soluble model drugs. PLGA nanoparticles loaded with AML-VAL (1:16 w/w) were obtained by nanoprecipitation using an amphiphilic block copolymer - Pluronic F127 as stabilizer. The drugs release from the PLGA nanoparticles was determined by a dialysis membrane method under sink conditions. Nanoparticles provided a slow release for both APIs and an attenuated burst effect compared to free drug. Five kinetics models such as Zero-order, First-order, Korsmeyer-Peppas, Higuchi and Hixson-Crowell were applied to predict drug release profiles. The Higuchi and Korsmeyer-Peppas models ($R^2 > 0.97$) best described physicochemical release phenomenon for each PLGA formulations.

***Keywords*:** nanoparticles; amlodipine; valsartan; drug release.

1. Introduction

Low water solubility is often the main obstacle to the use of new drugs. This issue leads to a low bioavailability and therefore the drug's active components are not concentrated at the site of action and the treatment fails *in vivo* [1, 2].

Over the past decade, nanotechnology provides solutions to overcome the drawbacks of poorly water-soluble active pharmaceutical ingredients (APIs), including improved solubility, protection of APIs from external medium, controlled drug release, and targeted delivery that can result in an increase of therapeutic efficacy [3-8].

Biocompatible and biodegradable polymeric nanoparticles have been used for drug delivery applications. These are composed of either natural or synthetic materials [9, 10]. One of the most successful polymers in the development of bio-based polymers is the poly-(lactic-co-glycolic acid) (PLGA) due to its numerous advantages, such as biodegradability, biocompatibility, and drug delivery system approval [11-13].

The study of release kinetics from nano-sized systems offers important data for the assessment of safety and therapeutic efficacy. Also, *in vitro* release kinetics can be correlated to the *in vivo* behavior of APIs through predictive mathematical models, resulting in a faster regulatory approval [14-16]. Therefore, the selection of a proper method to assess *in vitro* release kinetics of APIs from nanosystems is critical. Currently, there are no formal regulations or standards for the evaluation of drug release profiles of nanoparticles. However, a review of the literature shows that the drug release profiles can be obtained by various methods, such as flow cytometry, dialysis membrane, and sample and separate methods [17, 18].

In previous studies we evaluated several nanoformulations with different quantities of PLGA and we concluded that in the range of concentrations 5-60 mg PLGA and at a stirring rate of 1200-1500 rpm cardiovascular drugs loaded nanoparticles with good features can be obtained [19, 20]. However, the release behavior has only been studied for the nanoformulations with 5, 7.5 and 10 mg PLGA [20]. The aim of this research was to investigate the release behavior of a combination of two poorly water-soluble APIs from PLGA nanoparticles with higher PLGA concentrations, in the range of 25-50 mg. Amlodipine besylate (AML), a calcium channel blocker, and valsartan (VAL), an angiotensin II receptor antagonist drug, were used as poorly water-soluble model drugs. PLGA nanoparticles loaded with AML-VAL were prepared and characterized in terms of entrapment efficiency (EE), size and polydispersity index (PDI). To evaluate the release behavior of AML-VAL the dialysis membrane approach was applied due to its simplicity and

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avoidance of separating released drug from the nanoparticles. Five kinetics models such as Zero-order, First-order, Korsmeyer-Peppas, Higuchi and Hixson-Crowell were used to predict drug release mechanisms.

2. Experimental

2.1. Materials

Poly (lactic-co-glycolic acid) (50:50, MW = 30,000 - 60,000 Da), amlodipine besylate, valsartan, and Pluronic F127: poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) were purchased from Sigma-Aldrich (Merck Group, Darmstadt, Germany). The *in vitro* drug release studies were performed in 0.1 M phosphate buffer pH 7.4. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of PLGA nanoparticles loaded with amlodipine-valsartan

PLGA nanoparticles loaded with AML-VAL were prepared by nanoprecipitation method as described elsewhere [19, 20] using PLGA, as biodegradable polymeric and an amphiphilic block copolymer - Pluronic F127 as stabilizer. The composition of prepared PLGA nanoparticles loaded with AML-VAL is presented in Table 1.

Table 1. Formulation of PLGA nanoparticles

Sample Code	$m_{AML:VAL}$ (g/g)	m_{PLGA} (mg)	$m_{Pluronic}$ (mg)	Stirring rate (rpm)
NP1	1:16	20	10	1200
NP2	1:16	35	10	
NP3	1:16	50	10	

2.3. Characterization of PLGA nanoparticles loaded with amlodipine-valsartan

The prepared PLGA NPs were characterized in terms of entrapment efficiency (EE), particle size and polydispersity index (PDI). EE was evaluated using an indirect method as the ratio of the quantity of drugs present in nanoparticles and the initial quantity of drugs using a UV-Vis spectrophotometer (JASCO V-630 Spectrophotometer, Jasco International Co., Ltd., Tokyo, Japan). The quantity of drugs present in nanoparticles was assessed as the difference between the initial quantity of valsartan, respectively amlodipine, used for nanoparticle preparation and the quantity of valsartan (amlodipine) present in the supernatant. Particle size and polydispersity index were evaluated by Dynamic Light Scattering (DLS) using a particle size analyzer (Beckman Coulter N4 PCS Submicron, Coulter Company, France). Measurements were performed on diluted samples (1:20) at a scattering angle of 90° and temperature of 25 °C. For each sample the mean values with standard deviations of 10 determinations were established. The values reported are the mean values with standard deviations for three replicate samples.

2.4. In vitro drug release from PLGA nanoparticles

The *in vitro* drug release studies from the PLGA nanoparticles were carried out using dialysis membrane method under sink conditions. A sample of PLGA nanoparticles loaded with AML-VAL (1 mL) was put in a dialysis cellulose bag with molecular weight cut-off: 14,000 Da (Sigma-Aldrich, Merck Group, Darmstadt, Germany). The ends of the dialysis bag were sealed and then it was immersed into 200 mL 0.1 M phosphate buffer solution of pH 7.4 as release medium. The whole system was kept under magnetic stirring (150 rpm/min) at 37°C. Samples were withdrawn at predetermined intervals and the release medium was refilled with the same volume of fresh medium. The amounts of AML and VAL released were determined by measuring the absorbance at 365 nm (for AML) and 250 nm (for VAL) using an UV-VIS spectrophotometer (JASCO V-630 Spectrophotometer, Jasco International Co., Japan), according to the standard AML and VAL calibration curves. Also, a combination of free AML-VAL was subjected to the same release conditions as control. Release studies were performed in triplicate and average values with standard deviations were reported.

3. Results and discussions

3.1. Characterization of PLGA nanoparticles - EE, size and PDI

The EE, size and PDI of PLGA nanoparticles loaded with AML-VAL were displayed in Figure 1. All formulations presented good EE for both APIs, ranged from 65.35 % ± 0.11 to 67.58 % ± 0.11 for AML, and from 79.89 % ± 0.13 to 80.10 % ± 0.14 for VAL. Also, the formulations NP1, NP2, NP3 had nanometric size bellow 210 nm and a good size-homogeneity with a value of PDI below 0.11. These features are in agreement with results obtained in our previous studies [19, 20]. Also, Verma et al. [21] described loading of losartan, an angiotensin II receptor antagonist drug, in PLGA nanoparticles with a size below 300 nm and 87% entrapment efficiency. Jana et al. encapsulated felodipine, a calcium channel blocker, in PLGA monodispersed nanoparticles with a size of 0.216 to 0.442 and approximately 80% entrapment efficiency [22].

3.2. In vitro release of AML and VAL from PLGA nanoparticles

The *in vitro* release of AML and VAL from PLGA nanoparticles was carried out in 0.1 M phosphate buffer pH 7.4 at 37 ^{o}C, and the experimental data were presented in Figure 2 and Figure 3. The dissolution of free AML and VAL in 0.1 M phosphate buffer pH 7.4 at 37 ^{o}C reached a maximum after 6 h for AML and respectively 7 h for VAL. PLGA nanoparticles provided a slow release for both drugs compared to the dissolution of free drug in the same conditions. The formulation with the smaller amount of PLGA (20 mg) - NP1 had higher CDR, with release reaching a maximum of 72.7% for AML and 85.8% for VAL after 48 hours. As the proportion of polymer increased, the cumulative release of drugs decreased, for example the release from the formulation with medium amount of PLGA (35 mg) - NP2 reached a maximum of 65.3% for AML and 78.1% for VAL in 48 hours, and the release from the formulation with higher amount of PLGA (50 mg) - NP3 reached a maximum of 57.3% for AML and 73.5% for VAL in the same time frame. Also, it can be observed

that the release of drugs from the free AML-VAL exhibited a *burst effect* (67.00 ± 0.28% for AML, and 60.30 ± 0.15% for VAL, respectively, released in the first 30 minutes), while in the case of active substances loaded in polymeric nanoparticles the *burst effect* was reduced (25 % for AML and 40 % for VAL were released in the first 30 minutes from all PLGA-NPs). The burst release of AML-VAL form PLGA nanoparticles could be explained by the diffusion of AML and VAL crystals adhered to the surface of the nanoparticles. The biphasic pattern of cardiovascular drugs release from PLGA NPs was in agreement with the results of other studies [22, 23].

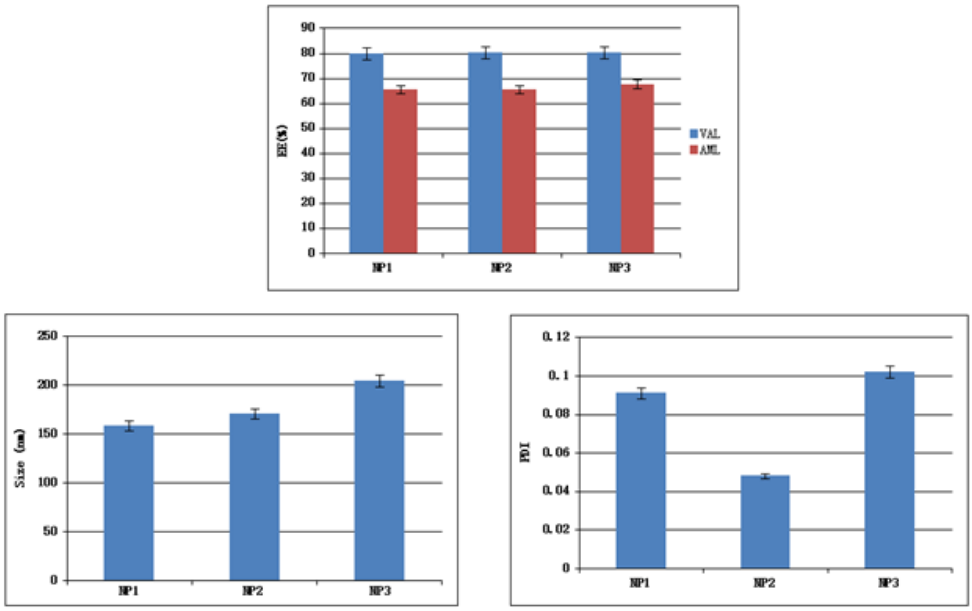

Figure 1. EE, size and PDI of PLGA nanoparticles loaded with AML-VAL

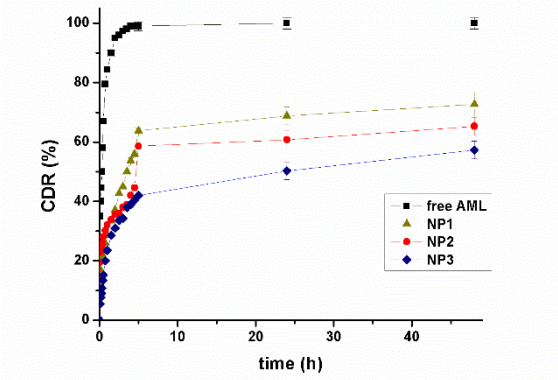

Figure 2. *In vitro* release profile of AML from PLGA nanoparticles

3.3. Analysis of drug release mechanism

In order to understand the release mechanism, experimental data obtained was fitted using Korsmeyer-Peppas, Higuchi, Zero-order, First-order, and Hixson-Crowell models:

$$\frac{M(t)}{M(\infty)} = k_{KP}t^{n} \quad (1)$$

$$\frac{M(t)}{M(\infty)} = k_{H}t^{1/2} \quad (2)$$

$$\frac{M(t)}{M(\infty)} = k_{0}t \quad (3)$$

$$\frac{M(t)}{M(\infty)} = e^{-k_{1}t} \quad (4)$$

$$\frac{M(t)}{M(\infty)} = \left[1-(1-k_{HC}t)^{3}\right] \quad (5)$$

where M(t) represents the amount of AML, respectively VAL released at time t and M(∞) represents the total amount of AML, respectively VAL loaded in the polymeric nanoparticles; k_0, k_1, k_H, k_{KP} and k_{HC} are the constants of the Zero-order, the First-order, the Higuchi, the Korsmeyer-Peppas and the Hixson – Crowell models.

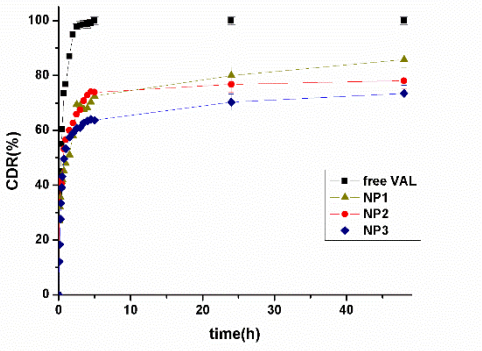

Figure 3. *In vitro* release profile of VAL from PLGA nanoparticles

Table 2. Correlation coefficient for various mathematical models - free versus entrapped AML release from PLGA nanoparticles

Sample	Correlation coefficient (R^2)				
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
Free AML	0.8514	0.9680	-*	-*	0.9345

Sample	Correlation coefficient (R^2)				
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
NP1	0.9348	0.9509	0.9856	0.9836	0.9465
NP2	0.9106	0.9633	0.9759	0.9776	0.9410
NP3	0.8964	0.9282	0.9842	0.9833	0.9200

* Condition for application of Korsmeyer-Peppas and Higuchi model was not met (M (t) / M (∞) < 2/3)

Table 3. Correlation coefficient for various mathematical models - free versus entrapped VAL release from PLGA nanoparticles

Sample	Correlation coefficient (R^2)				
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
Free VAL	0.7207	0.9762	-*	-*	0.9548
NP1	0.8780	0.8654	0.9555	0.9879	0.8320
NP2	0.6534	0.7413	0.9645	0.9886	0.7929
NP3	0.8890	0.8264	0.9677	0.9618	0.7129

* Condition for application of Korsmeyer-Peppas and Higuchi model was not met (M (t) / M (∞) < 2/3)

In Table 2 and Table 3 are listed the correlation coefficients and parameters of mathematical models used for fitting the experimental data of AML and VAL release from loaded polymeric nanoparticles in comparison with drugs solubilization in PBS. The correlation coefficient (R^2) was chosen to compare the models, where a value closer to 1 means a better correlation.

The solubility curve of both amlodipine and valsartan can best be described by an exponential equation, with $R^2 = 0.9680$ for AML and $R^2 = 0.9762$ for VAL. For PLGA-based polymeric nanosystems, it was observed that the release of AML and VAL from all samples is best described by the Higuchi model and the Korsmeyer-Peppas model ($R^2 > 0.95$). The release behavior of nanoparticles with low PLGA concentrations obtained in our previous research [20] was also best described by Higuchi model, and the Korsmeyer-Peppas model was not applied. In the Korsmeyer-Peppas model, k_{KP} is a constant that depends on the characteristics of the system, and the coefficient *n* shows the nature of the release mechanism. When $n \leq 0.5$, the release is dominated by the Fickian diffusion mechanism; if *n* is between 0.5 and 1 then the release follows the mechanism of an abnormal diffusion (non-Fickian diffusion), and if $n > 1$, the release is based on a complex transport mechanism (super-case-II transport). In the Higuchi's model, k_H is a constant proportional to the burst release rate of the release process. The parameters of the Korsmayer-Peppas and Higuchi models for the analysis of AML and VAL release behavior from PLGA polymeric nanoparticles are presented in Table 3 and Table 4. For all the samples the values of the *n* coefficient are below 0.5, indicating a Fickian diffusion.

Comparing with our previous study [20] the samples with high PLGA content (20-50 mg), the coefficient *n* is less than 0.5, indicating Fickian diffusion, while the other samples (5-10 mg PLGA) in this range between 0.5 and 1, indicating a non-Fickian diffusion. Nanoformulations with high PLGA content showed a lower value for k_H than the other polymeric nanoformulations, indicating a less intense burst effect. Also, Jana et al. found the *n* value, the parameter of Korsmeyer-Peppas model, less than 0.5 indicating that the release mechanism of felodipine from the PLGA nanoparticles was diffusion controlled [22].

Table 4. Parameters of Korsmayer-Peppas and Higuchi models for the release behavior analysis of AML from PLGA nanoparticles

Sample	Korsmayer-Peppas		Higuchi
	n	k_{KP}	k_H
NP1	0.1862	27.8001	14.5410
NP2	0.1943	31.6602	17.2252
NP3	0.1957	22.5900	26.1869

Table 5. Parameters of Korsmayer-Peppas and Higuchi models for the release behavior analysis of VAL from PLGA nanoparticles

Sample	Korsmayer-Peppas		Higuchi
	n	k_{KP}	k_H
NP1	0.2311	49.0250	28.8182
NP2	0.2765	55.4978	39.2557
NP3	0.2644	55.8015	39.1066

4. Conclusions

Nanoparticles provided a slow release for both APIs and an attenuated burst effect compared to free drug. Five kinetics models such as Zero-order, First-order, Korsmeyer-Peppas, Higuchi and Hixson-Crowell were applied to predict drug release profiles. The Higuchi and Korsmeyer-Peppas models ($R^2 > 0.95$) best described physicochemical release phenomenon for each PLGA formulations. All the nanoparticles had the values of *n* coefficient below 0.5, indicating a Fickian diffusion.

Acknowledgement: This work was financially supported by ANCSI through PN 1941 04 01.

Conflict of interest

Authors declare no conflict of interest.

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Received: 03.06.2021
Received in revised form: 18.11.2021
Accepted: 19.11.2021