Formulation of Polymeric Multicomponent Systems Containing Cardiovascular APIs

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The main aim of this study was to prepare and characterize polymeric nanoparticles containing two cardiovascular active pharmaceutical ingredients: valsartan (VAL) and amlodipine besylate (AML). Six formulations were evaluated with different ratios of AML:VAL:PLGA (1:16:17, 1:16:34, and 1:16:51) and different stirring speed (1200 and 2400 rpm). Encapsulation efficiency (EE, %) and particle size analyses were performed to characterize and optimize the formulation. All loaded nanoparticles showed a high EE (%), nano-size, negative ξ-potential and a high homogeneity.

Keywords: nanotechnology, cardiovascular drugs, drug delivery systems, medicinal chemistry.

Nowadays, nanotechnology represents the principal domain of interest in biomedicine, and therefore helps research community to improve and develop the technologies for the cardiovascular disease's treatment [1, 2]. The current trend of the development of pharmaceutical industry is represented by super generic drugs. This kind of medication, which mostly represents delivery systems of APIs (Active Pharmaceutical Ingredients) focused on improving the APIs properties (pharmacokinetic properties) previously marketed in other pharmaceutical forms. These new formulations can successfully replace the generic products. In the cardiovascular treatment, super generics can improve solubility in aqueous media, for APIs, like: valsartan, irbesartan, nimodipine, carvediol, amlodipine besylate, etc.; several methods have been developed to dispose this disadvantage, such as the use of surfactants, lipids, permeation promoters, micronization, use of salts, cyclodextrines, nanoparticles and solid dispersions [3, 4]. In contrast to studies that have very good results for a single API and its matrix, this work is geared toward the study of nano-systems that contain two APIs encapsulated simultaneously in a biodegradable polymer. In recent years, nanoparticles (NPs) with polymeric matrix have been used due to the biodegradable property of the polymer, particularly those with a hydrophilic polymer such as PEG or PLGA, recognized as having a high circular distance and thus the ability to travel for a long time to a target organ, as a DNA transporter in gene therapy, and the ability to deliver proteins, peptides or genes [5, 6].

The main objective of this paper is to obtain and characterize the multicomponent systems on nanoscale, because in this way there are prerequisites for improvement the low solubility profile of the therapeutic agents, to reduce the frequency dosing and to increase the bioavailability of APIs with action in cardiovascular pathology. For this purpose, mixed APIs - an angiotensin II receptor antagonist drug (valsartan) and a calcium channel blocker (amlodipine besylate) - and polymeric matrix were chosen suitable: polymer at different concentrations, stabilizer in a constant concentration, at two different stirring speed, in order to obtain l particle size and a good encaspsulation efficiency.

Experimental part

Materials and methods

Poly (D,L-lactide-co-glicolide) (PLGA, 50:50, M_w = 30,000 - 60,000 Da), amlodipine besylate (2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzene sulfonate) with M_w = 567.05 g/mol, valsartan (*N*-(1-Oxopentyl)-*N*-[[22 -(2*H*-tetrazol-5-yl)[1,12 -biphenyl]-4-yl]methyl]-L-valine) with M_w = 435.51 g/mol and Poloxamer 407, known as Pluronic F127 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)) were obtained from Sigma Aldrich (USA). The water used for all experiments was distilled. All other chemicals were of analytical grade obtained from standard sources and used without further purification.

Preparation of NPs

NPs were prepared according to nanoprecipitation method [7, 8]. PLGA at three different concentrations (17, 34, 51 mg) was dissolved in acetone (5 mL), and both drugs were combined in a fixed-dose (AML:VAL – 1:16 mg) in PLGA/acetone solution. Pluronic-F127 (10 mg) was dissolved in distilled water (15 mL). The organic phase was added dropwise into the aqueous phase solution and stirred magnetically at two different stirring speed (1200 and 2400 rpm) at room temperature (25^{0}C) until complete evaporation of the organic solvent (table 1). The final nanosuspension was centrifuged at 10 000 rpm for 30 min at 3^{0}C to separate the drug polymeric aggregates and then it was filtered through 0.22μm Millex®filter membrane.

Evaluation of drug encapsulation efficiency

Encapsulation Efficiency (EE, %) was evaluated using the following equation (1) :

$$EE(\%) = \frac{\text{Initial amount of APIs - Amount of APIs in supernatant}}{\text{Initial amount of APIs}} \times 100 \quad (1)$$

The percentage of encapsulated drugs was determined by using UV-Vis spectrophotometer at 365 nm for AML and 250 nm for VAL (JASCO V-630 Spectrophotometer, Jasco International Co., Ltd., Japan).

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Formulation cod	m_{AML}: m_{VAL} (mg)	m_{PLGA} (mg)	m_{F127} (mg)	$V_{CH3-CO-CH3}$ (ml)	V_{H2O} (ml)	Drop rate (ml/min)	Stirring speed (rpm)	Stirring time (min)
F1	1:16	17	10	5	15	0.5	1200	25
F2	1:16	34	10	5	15	0.5		
F3	1:16	51	10	5	15	0.5		
F4	1:16	17	10	5	15	0.5	2400	
F5	1:16	34	10	5	15	0.5		
F6	1:16	51	10	5	15	0.5		

Table 1
FORMULATION OF POLYMERIC NPs WITH AML-VAL ENCAPSULATED

Measurement of particle size, polydispersity index and æ-potential

Particle size, polydispersity index (PDI) and ξ-potential were determined by Dynamic Light Scattering (DLS - Malvern Zetasizer, Malvern Instruments Ltd, UK) method. Particle size and PDI were measured on samples diluted with distilled water (1:80) at a scattering angle of 90^0, a temperature of 25^0C, solvent refractive index of 1.458 and solvent viscosity of 0.8872 cP. For ξ-potential measurement, all analyses were performed on undiluted samples.

Statistical analysis

Values are represented as mean ± standard deviation (SD) for three replicate samples. Differences were considered significant at $p < 0.05$.

Results and discussions

Formulation and encapsulation efficiency of NPs

The developed formulation is intended to improve the drug solubility of the two selected APIs: amlodipine besylate (solubility 1.91099 g/L (water, 32^0C)) and valsartan (solubility 1.406 mg/L (water, 25^0C), solubility in methanol and ethanol). The fixed-dose combination (AML:VAL = 1:16 (mg)) was chosen based on Hypertension Clinical Guidelines [8-12]. Two parameters were varied: the concentration of PLGA (17, 34 and 51 mg) and the stirring speed (1200 and 2400 rpm). As showed in table 2, a strong relationship could be observed between PLGA concentration and the encapsulation efficiency.

All formulations (F1-F6) showed high EE (%) for both APIs, ranged from 79.35 ± 0.13 % to 81.21 ± 0.13 % for VAL and from 59.90 ± 0.12 % to 67.58 ± 0.11 % for AML. Two parameters were varied, namely: PLGA concentration and stirring speed. Depending of which parameter was modified, the NPs have a different EE (%) and size. Thus, at 1200 rpm and 2400 rpm all formulations revealed a slightly increasing in EE (%) with increasing concentration of polymeric matrix. By increasing the amount of PLGA (17, 34 and 51 mg), the nanoparticle suspension became more and more turbid. Also, as data literature has shown, PLGA can be processed into any size and shape and therefore can encapsulate substances of any size [13]. With the increased amount of PLGA, nanoparticles may encapsulate to a greater extent poorly-water soluble drugs, leading to a higher EE (%) values. These values of EE (%) could be due to the fact that higher amounts of polymer led to more viscous organic solutions. A higher concentration of PLGA leads to larger nanoparticles with a sufficient surface for drugs molecules to be entrapped. Also, an increased viscosity could block the drug's diffusion from the organic phase into the aqueous one, and therefore promote AML-VAL entrapment [14].

Contrariwise, comparing the formulations with same concentration of PLGA, but different stirring speed, it has shown in table 2 better EE (%) for formulations in which the stirring speed was 2400 rpm than those in which preparation was used a stirring speed of 1200 rpm. By increasing the stirring speed, the amount of AML-VAL entrapped slightly increased as well. Higher stirring speed causes smaller droplets and therefore the total surface area of the nanoparticles increase. This fact leads to an extra space for the polymer matrix to encapsulate more AML-VAL molecules; thereby the EE (%) was slightly improved. These results were similar to those reported by Kheradmandnia S et al. [15].

Characterization of PLGA NPs

Mean particle size, PDI and ξ-potential were assessed for all the PLGA formulations with cardiovascular APIs encapsulated. The measured parameters are shown in

Formulation code	Mean particle size (nm)	ζ-potential (mV)	PDI	EE (%)
F1	158.4 ± 1.15	-13.46 ± 0.31	0.091 ± 0.04	79.89 ± 0.13 for VAL 65.35 ± 0.11 for AML
F2	171.1 ± 1.30	-17.07 ± 0.22	0.048 ± 0.03	80.03 ± 0.10for VAL 65.43 ± 0.10 for AML
F3	204.1 ± 1.34	-21.34 ± 0.21	0.102 ± 0.05	80.10 ± 0.14 for VAL 67.58 ± 0.11 for AML
F4	122.1 ± 1.31	-20.24 ± 0.20	0.104 ± 0.08	79.35 ± 0.13 for VAL 58.90 ± 0.12 for AML
F5	130.1 ± 1.30	-20.45 ± 0.24	0.098 ± 0.02	80.02 ± 0.16 for VAL 60.01 ± 0.14 for AML
F6	152.7 ± 1.29	-23.45± 0.33	0.097 ± 0.06	**81.12 ± 0.12 for VAL** 60.83 ± 0.16 for AML

Table 2
CHARACTERISTICS OF PLGA NPs WITH A MIXTURE OF CARDIOVASCULAR APIs ENCAPSULATED

table 2. By increasing of stirring speed, the particle size of NPs was decreased from F1 (PLGA: 17 mg, 1200 rpm): 158.4 ± 1.15 nm to F4 (PLGA: 17 mg, 2400 rpm): 122.1 ± 1.3 nm, from F2 (PLGA: 34 mg, 1200 rpm): 171.1 ± 1.30 nm to F5 (PLGA:34 mg, 2400 rpm): 130.1 ± 1.30, from F3 (PLGA: 51 rpm, 1200 rpm): 204.1 ± 1.34 nm to F6 (PLGA: 51 rpm, 2400 rpm): 152.7 ± 1.29 nm. Increasing the stirring speed resulted in the formation of smaller particles. Moreover, as the content of PLGA was increased, the particle size of NPs was also increased; at 1200 rpm from F1 (PLGA 17 mg) 158.4 ± 1.15 nm to F3 (PLGA 51 mg) 204.1 ± 1.34 nm, and at 2400 rpm from F4 (PLGA: 17 mg, 2400 rpm): 122.1 ± 1.3 nm to F6 (PLGA: 51 rpm, 2400 rpm): 152.7 ± 1.29 nm. The particle size increased with the increasing of PLGA concentration was also reported in literature [16]. As the concentration of PLGA increases, the viscosity of the nanosuspension increases proportionally; thereby these phenomena lead to an increase in the size of the nanoparticles [16]. Particle size represents a critical factor for drug delivery systems as nanoparticles; it influences the circulating half-life, biodistribution, cellular uptake and the drug's release kinetics (the smaller the particle size, the faster the release rate) [17].

The effect of PLGA concentration on particle size can be explained by the viscosity of the organic phase, as well as the presence of number of polymer chains per unit volume of organic solvent [18]. Increasing PLGA concentration leads to an increased of the organic solution viscosity and therefore, the diffusion of the organic solvent into the aqueous phase is slowed down, forming larger droplets, which in turn provide larger nanoparticles [19]. Furthermore, Song X. et al. demonstrated that higher PLGA concentration favors some polymer-polymer interactions, thus more polymer chains remain associated during the solvent's diffusion into the aqueous medium [17].

Polydispersity indices were low and showed little variability between different samples, ranging from 0.048 ± 0.03 to 0.104 ± 0.08. The values of PDI had no significant differences no matter what concentration of PLGA or stirring speed were used in the preparation. All six samples showed a PDI less than 0.15, which means a significantly higher homogeneity of the systems. For all six formulations, the electric charge was negative, which could be due to the terminal carboxylic groups of PLGA present on the surface of the nanoparticles [20]. The zeta potential ranged between -13.46 ± 0.31 mV and -23.45± 0.33 mV, and according to literature [20], all samples indicated a moderate stability. With the increasing of the amount of PLGA, the NPs had became more and more turbid and the æ-potential becoming more and more negative. Dinh, Tran et al. demonstrated that PLGA NPs showed a significant reduction in æ-potential related to the poloxamer coating on NPs surface of Pluronic F127 which reduced the electrophoretic mobility [20].

Conclusions

In this study, we have demonstrated that the AML-VAL-PLGA NPs represent promising dual-drug delivery systems for encapsulating poorly water-soluble drugs as combined therapy in cardiovascular disease. All six formulations had significant encapsulation efficiency (EE, %): 79.89 ± 0.13 - 81.12 ± 0.12% for VAL and 58.90 ± 0.12 - 67.58 ± 0.11 for AML% for AML. According to this characteristic and depending of the two variable parameters (PLGA concentration and stirring speed), the best formulation was F6 (AML:VAL:PLGA = 1:16:51 w/w, 2400 rpm). AML-VAL NPs with mean particle size about 122.1 ± 1.31 to 204.1 ± 1.34 nm were successfully prepared by nanoprecipitation method. All nanoformulations showed a negative æ-potential ranging between -13.46 ± 0.31 mV and -23.45± 0.33 mV, indicating a moderate stability, and a PDI values smaller than 0.15, which means a significantly higher homogeneity of the systems. These data reveal that amlodipine besylate and valsartan encapsulated in PLGA NPs could be used as nano-therapeutic delivery systems in combined therapy for cardiovascular disease's treatment. However, further *in vitro* studies are needed to demonstrate the pharmacokinetic and pharmacodynamic effect.

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