

Proceedings

# Preliminary Evaluation of Pullulan Nanoparticles Loaded with Valsartan †

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**Abstract:** The objective of this paper is to develop polymeric nanoparticles loaded with a cardiovascular drug, an angiotensin II receptor antagonist drug, valsartan. Polymeric nanoparticles were prepared via nanoprecipitation method using pullulan acetate as biodegradable polymeric matrix and Pluronic F127 as a stabilizer. Pullulan acetate was obtained by chemical modification of pullulan (produced through a fermentation process by *Aureobasidium pullulans* strain) with dimethylformamide, pyridine, and acetic anhydride. Obtained nanoparticles were characterized in terms of entrapment efficiency, size, and polydispersity index using spectrophotometric and dynamic light scattering techniques. The valsartan-loaded nanoparticles showed a good entrapment efficiency of valsartan, nano-metric sizes (lower than 200 nm), and a narrow dispersity (polydispersity index below 0.2). This research revealed that pullulan and pullulan derivatives showed great potential for the production of nanoparticles with application in cardiovascular agents delivery.

**Keywords:** pullulan; nanoparticles; valsartan; cardiovascular disease

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## 1. Introduction

Cardiovascular diseases represent one of the most major cause of mortality worldwide, accounting for an estimated 17.8 million deaths annually globally [1]. A need exists for improving the nowadays technologies in cardiovascular therapy or/and developing new strategies for the delivery of cardiovascular drugs.

Over recent years polymeric nanoparticles have attracted considerable interest in biomedical applications, including cardiovascular therapy, due to their advantages such as: the ability to protect bioactive molecules from premature degradation, the capacity to enhance drugs' bioavailability, absorption and penetration, and to provide controlled release and targeted delivery of therapeutic agents. Both natural and synthetic polymers have been extensively researched as materials for polymeric nanoparticles production [2,3]. The most commonly used polymers for nanoparticles preparation are polysaccharides, as natural polymers, and polymethylmethacrylate, poly(cyanoacrylate) (PCA), polycaprolactone (PCL), poly(lactic acid) (PLA), poly(D,L-glycolic acid)

(PGA), poly(lactide-co-glycolide) PLGA, as synthetic polymers [4,5]. Among polysaccharides pullulan has received a lot of attention from biomedical community.

Pullulan is a microbial exopolysaccharide produced by some strains of the polymorphic fungus *Aureobasidium pullulans*. The structure of pullulan comprises of maltotrose units connected by  $\alpha$  (1 $\rightarrow$ 4) glycosidic bond, whereas consecutive maltotriose units are connected to each other by  $\alpha$  (1 $\rightarrow$ 6) glycosidic linkages. Pullulan has non-mutagenic, non-toxic, non-carcinogenic, non-immunogenic, biocompatible, biodegradable properties and other functional properties like adhesiveness and film formability. Furthermore, pullulan can be easily derivatized in order to widen its applications [6,7]. Mostly hydrophobized pullulan has been used as drug delivery carrier, because pullulan cannot self-associate in aqueous solutions due to its solubility in water. Several pullulan derivatives have been investigated for biomedical applications such as poly(DL-lactide-co-glycolide)-grafted pullulan, cholesterol-bearing pullulan, pullulan acetate, perfluoroalkylated pullulan, etc. Pullulan and its derivatives are used in tissue engineering and drug delivery systems due to their excellent physicochemical properties [8,9].

The aim of this research was to investigate the potential of pullulan for the production of nanoparticles with application in cardiovascular agents delivery. To this end, valsartan was used as model cardiovascular drug. Valsartan is an angiotensin II receptor antagonist drug commonly used for the management of hypertension, heart failure, and type 2 diabetes-associated nephropathy, mainly for patients who are unable to tolerate angiotensin-converting enzyme inhibitors. Several clinical trials revealed that valsartan improves cardiovascular diseases outcome including reducing risk of myocardial infarction, stroke and the progression of heart failure [10,11]. Valsartan has poor solubility in water (1.406 mg/L, 25 °C). It is reported that bioavailability of valsartan is 23–25% after oral dose [12]. A way to improve valsartan poor solubility, reduce frequent dosing and to increase its bioavailability is by preparing nanoparticles. Therefore, valsartan loaded pullulan based nanoparticles were prepared and characterized in terms of entrapment efficiency, size, and polydispersity index using spectrophotometric and dynamic light scattering techniques.

## 2. Materials and Methods

Pullulan was produced through a fermentation process by *Aureobasidium pullulans* strain. Pullulan acetate was obtained by chemical modification of pullulan with dimethylformamide, pyridine, and acetic anhydride. Valsartan (N-(1-Oxopentyl)-N-[[22-(2H-tetrazol-5-yl)[1,12-biphenyl]-4-yl]methyl]-L-valine) and Pluronic F127 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)) were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetone, analytical grade, was obtained from AdraChim SRL (Bucharest, Romania) and used without further purification. The water used for all experiments was distilled.

### 2.1. Preparation of Pullulan Nanoparticles Loaded with Valsartan

Pullulan nanoparticles were prepared by nanoprecipitation method. Briefly, pullulan acetate was dissolved in acetone (5 mL). Valsartan was accurately weighed and solved in pullulan/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 1000 rpm at room temperature (25 °C) until complete evaporation of the organic solvent. The final nanosuspension was centrifuged at 10,000 rpm for 30 min at 30 °C to separate the free drug from formed nanoparticles. The supernatant was carefully siphoned and the precipitate containing nanoparticles was redispersed in distilled water and filtered through 0.22  $\mu$ m Millex<sup>®</sup> filter membrane. The formulation of prepared pullulan nanoparticles loaded with valsartan are listed in Table 1.

**Table 1.** Formulation of pullulan nanoparticles loaded with valsartan.

Formulation Code	Polymer (mg)	Valsartan (mg)	Pluronic F127 (mg)	Drop Rate (mL/min)	Stirring Speed (rpm)
NP1@VAL	30	5	5	0.5	1000
NP2@VAL	40	5	5	0.5	1000
NP3@VAL	50	5	5	0.5	1000

## 2.2. Characterisation of Pullulan Nanoparticles Loaded with Valsartan

### 2.2.1. Entrapment Efficiency

The entrapment efficiency (EE, %) was evaluated by an indirect method. The amount of valsartan encapsulated in the pullulan nanoparticles was determined as the difference between the initial amount of valsartan used for nanoparticle preparation and the amount of valsartan present in supernatant. The percentage of encapsulated drugs was determined spectrophotometrically using UV-Vis spectrophotometer (JASCO V-630 Spectrophotometer, Jasco International Co., Ltd., Tokyo, Japan) at 250 nm, the maximum wavelength of valsartan.

### 2.2.2. Analysis of Particle Size and Polydispersity Index

Particle size and polydispersity index were determined by dynamic light scattering (DLS) method. Particle size and polydispersity index were measured on samples diluted with distilled water (1:20) at a scattering angle of 90°, a temperature of 25 °C, solvent refractive index of 1.458 and solvent viscosity of 0.8872 cP. For each sample the mean value  $\pm$  standard deviation of ten determinations were established. Values reported are the mean value  $\pm$  standard deviation for three replicate samples.

## 3. Results and Discussion

The objective of this study was to investigate the potential of pullulan for the production of nanoparticles with application in cardiovascular agents delivery. In order to achieve this aim polymeric nanoparticles loaded with valsartan were prepared via nanoprecipitation method using pullulan acetate as biodegradable polymeric matrix and Pluronic F127 as a stabilizer. The pullulan nanoparticles loaded with valsartan were characterized in terms of entrapment efficiency, size, and polydispersity index.

Particle size is a significant characteristic in determining the performance of a nanoparticulate system because it influences circulating half-life, cellular uptake and biodistribution. The homogeneity is also a key factor in assessing the system performance. The polydispersity index is a measure of the heterogeneity of a sample based on size; a polydispersity index value smaller than 0.1 suggest mono-disperse samples, while a value larger than 0.7 are common to a broad size (e.g., polydisperse) distribution of particles. The particle size and polydispersity index of pullulan nanoparticles loaded with valsartan were determined by DLS technique. In Table 2 the values of particle size and polydispersity index of pullulan nanoparticles are presented. As shown in Table 2, all samples were below 200 nm and the particle size slightly increased with the increase of pullulan from NP1@VAL (pullulan 30 mg)  $181.50 \pm 0.61$  nm to NP3@VAL (pullulan 30 mg)  $181.50 \pm 0.61$  nm. A possible explanation of this fact is that as the concentration of pullulan increases, the nanosuspension viscosity increases leading to a decrease of efficiency of stirring, thus the diffusion of the organic solvent (acetone) into the aqueous phase is hindered, producing larger droplets, which in turn provide larger particles [13–15]. The polydispersity index values were below 0.2 with little variability between different samples meaning a good homogeneity of the systems.

The entrapment efficiency of pullulan nanoparticles loaded with valsartan are presented in Table 3. All formulations showed high entrapment efficiency ranging from  $(75.13 \pm 0.13)$  % to  $(90.24 \pm 0.14)$  %. The entrapment efficiency is influenced by the characteristics of the polymer, drug, surfactant etc. A high entrapment efficiency value is due to the high affinity of therapeutic agent and

polymer to the same solvent while a low entrapment efficiency value results from high affinity of therapeutic agent and polymer to the different solvents [16]. In the present study it can be observed an increase in entrapment efficiency with the increase of pullulan acetate concentration in the formulations. The increased entrapment efficiency may be due to the higher proportion of polymer present in the formulation.

For the stability of loaded nanoparticles assessment, the samples were kept in amber-coloured glass vials and stored at 4 °C for 3 months (Table 3). To assess the stability of samples, entrapment efficiency parameter was evaluated after different storage periods of time (0, 1, 2, 3 months). The pullulan nanoparticles loaded with valsartan were stable for at least three months, with approximately the same amount of drug entrapped after 3 months storage. No aggregation of pullulan nanoparticles loaded with valsartan was observed during storage, probably because the brownian motion and the diffusion rate for nano-sized formulations are higher than the gravitational-induced sedimentation rate.

**Table 2.** Particle size and polydispersity index of pullulan nanoparticles loaded with valsartan.

Formulation Code	Size (nm)	Polydispersity Index
NP1@VAL	170.23 ±0.52	0.184
NP2@VAL	173.66 ±1.41	0.185
NP3@VAL	181.50 ±0.61	0.187

**Table 3.** Entrapment efficiency of pullulan nanoparticles loaded with valsartan.

Formulation Code	EE, %	EE, % (1 months)	EE, % (2 months)	EE, % (3 months)
NP1@VAL	75.13 ± 0.13 %	74.53 ± 0.12 %	74.13 ± 0.34 %	74.03 ± 0.53 %
NP2@VAL	80.25 ± 0.53 %	80.05 ± 1.50 %	79.85 ± 0.72 %	79.25 ± 1.21 %
NP3@VAL	90.24 ± 0.14 %.	89.84 ± 0.24 %.	88.24 ± 1.02 %.	88.04 ± 0.14 %.

#### 4. Conclusions

Polymeric nanoparticles were prepared via nanoprecipitation method using pullulan acetate as biodegradable polymeric matrix, valsartan as model drug and Pluronic F127 as a stabilizer. Nanoparticles were characterized in terms of entrapment efficiency, size, and polydispersity index using spectrophotometric and dynamic light scattering techniques. The valsartan-loaded nanoparticles showed a good entrapment efficiency of valsartan, nano-metric sizes and a good homogeneity of system. This research revealed that pullulan and pullulan derivatives showed great potential for the production of nanoparticles with application in cardiovascular agents delivery.

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