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Preparation of CaP/pDNA nanoparticles for exogenous gene delivery by co-precipitation method: optimization of formulation variables using Box-Behnken design

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## Title page

# Preparation of CaP/pDNA nanoparticles for exogenous gene delivery by co-precipitation method: optimization of formulation variables using Box-Behnken design

### 1 Abstract:

2 This research focused on optimizing the preparations of pDNA-loaded calcium phosphate  
3 nanoparticles by employing a 3-factor, 3-level Box-Behnken design. Results indicated that a  
4 Ca/P ratio of 189.56, pH of 7.82, and a stirring speed of 528.83 rpm were the optimum  
5 conditions for preparation of the nanoparticles. The size of the optimized CaP/pDNA  
6 nanoparticles was  $61.3 \pm 3.64$  nm, with a polydispersity index of 0.341 and an encapsulation  
7 efficiency of up to 92.11 %. The optimized CaP/pDNA nanoparticles had a high transfection  
8 efficiency and demonstrated good biocompatibility *in vitro*. Therefore, the Box-Behnken  
9 design method was successful in providing desirable CaP nanoparticle pDNA delivery  
10 systems by optimizing the experimental factors.

11

12 **Keywords:** CaP nanoparticles; pDNA; Box–Behnken design; transfection and expression

13

### 14 Introduction

15 Calcium phosphate (CaP) has been widely used as a gene carrier for the delivery of pDNA  
16 into mammalian cells [1, 2] due to its good biocompatibility, biodegradability, easy handling  
17 and preparation [3]. In this work, calcium phosphate/pDNA (CaP/pDNA) nanoparticles for  
18 gene delivery were synthesized through a conventional and economical method - a  
19 co-precipitation reaction of calcium and phosphate ions in the presence of pDNA (Fig. 1).  
20 However, it has been generally agreed that this technique can result in variability in the  
21 pDNA transfection and, consequently, low transfection efficiency. Recent studies have shown

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