Transitioning Layer-by-Layer Nanocapsule Synthesis from Batch to Continuous Production: Optimizing Calcium Phosphate Core Template Encapsulation

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Abstract

Background: The Layer-by-Layer (LbL) self-assembly technique, involving alternating deposition of oppositely charged polyelectrolytes on core templates, offers significant promise for enhancing drug encapsulation in targeted delivery systems. Traditional batch methods limit scalability, thus motivating this study's focus on a continuous production process for nanocapsules using calcium phosphate (CaP) cores coated with poly(diallyldimethylammonium chloride) (PDADMAC) and poly(styrene sulfonate) (PSS). Methods: This study examined essential factors for a continuous nanoparticle production process. Single-layered CaP-PDADMAC nanoparticle synthesis was first semi-continuously optimized, focusing on PDADMAC and PSS concentration, with a deposition time determination for each layer. Comparative analyses between traditional batch, optimized batch, and continuous methods were conducted, evaluating zeta potential and particle size for stability and uniformity. Results: The optimized conditions were found to be 1 g/L for PDADMAC and 3 g/L for PSS, with a deposition time of 5 minutes per layer. Zeta potential measurements indicated values above +25 mV or below -25 mV, ensuring stability across methods. The ZetaPLUS Particle Size Analyzer confirmed a mean diameter of 88.5 nm for continuously produced 8-layered CaP-PDADMAC/PSS nanoparticles, with a relative variance of 0.273. SEM imaging validated the core dissolution and formation of hollow nanocapsules with each polyelectrolyte layer contributing an average thickness of 3.2 nm. Conclusion: The study demonstrates the viability of continuous LbL nanocapsule production, achieving consistent nanoparticle stability and size, supporting its potential in scalable drug delivery applications.

Keywords: Layer-by-Layer self-assembly, nanocapsules, polyelectrolyte layers, continuous process, drug delivery

Significance | Continuous LbL nanocapsule production could revolutionize scalable drug delivery, enhancing targeted, efficient treatments through controlled release mechanisms.

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Introduction

In recent years, nanotechnology has emerged as a transformative field, offering novel solutions across various disciplines, particularly in drug delivery and controlled release systems. Nanocapsules, a type of nanocarrier, have garnered significant interest due to their unique ability to encapsulate active compounds and release them in a controlled manner. These nanoscale vesicles are designed to protect their contents from degradation while ensuring targeted delivery to specific sites within the body, which is especially useful in pharmaceutical applications (Ventura et al., 2017).

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The layer-by-layer (LbL) assembly technique is one of the most promising approaches for fabricating nanocapsules, as it allows for precise control over the capsule's properties by layering polyelectrolytes around a core template (De Koker et al., 2010). This method provides the flexibility to incorporate various
including polymers, metals, and biological molecules, making it highly versatile in tailoring the release kinetics of the encapsulated substance (Zhao et al., 2014). The LbL technique involves the sequential adsorption of oppositely charged polyelectrolytes onto a core, which is eventually dissolved, leaving behind a hollow nanocapsule structure. This process has been widely explored for encapsulating drugs, proteins, and other therapeutic agents (Geest et al., 2007).

One of the primary motivations for developing nanocapsules is the need for efficient delivery systems that can overcome the limitations of conventional drug delivery, such as low bioavailability, poor solubility, and uncontrolled release rates (Kreuter, 2014). By incorporating stimuli-responsive elements into the LbL assembly, researchers have achieved controlled release systems that respond to external factors such as pH, temperature, or specific biochemical signals (Ariga et al., 2011). These systems hold significant potential in improving therapeutic efficacy and reducing side effects by ensuring the drug is released only at the target site.

A notable advancement in this field is the development of stimuli-responsive nanocapsules that can react to endogenous or exogenous triggers, offering highly controlled release profiles (Deshmukh et al., 2012). For instance, pH-responsive nanocapsules are designed to release their contents in acidic environments, such as tumor tissues or inflamed areas, making them ideal for cancer therapy (Zheng et al., 2010). Similarly, ultrasound or light-responsive systems enable the localized release of drugs through external stimulation, minimizing systemic exposure and enhancing the therapeutic index (Li et al., 2015).

This article delves into the fabrication of nanocapsules using calcium phosphate (CaP) as a core template and polyelectrolytes such as PDADMAC and PSS. The choice of CaP is driven by its biocompatibility and ease of dissolution, which allows for the creation of hollow nanocapsules without leaving toxic residues (Urch et al., 2009). Furthermore, the incorporation of polyelectrolyte layers enhances the mechanical stability and functionality of the nanocapsules, making them suitable for various biomedical applications.

The aim of this research is to explore the optimization of the LbL assembly process for the fabrication of stable, multi-layered nanocapsules and to assess their potential for controlled release applications. We focus on optimizing key experimental parameters such as polyelectrolyte concentration, deposition time, and reaction conditions to achieve desired zeta potential and particle size characteristics. The article also investigates the scalability of the process, examining both batch and continuous production methods.

The potential of nanocapsules for controlled release lies in their tunability and responsiveness to environmental cues. As research in this area progresses, it is expected that nanocapsules will become an integral part of next-generation drug delivery systems, offering solutions to current challenges in therapeutic efficacy and patient safety (Lim et al., 2019).

Materials and Methods

Nanocapsules Fabrication, Core Template

In this research, calcium phosphate (CaP) was chosen as the core template due to its biocompatibility, degradability, and ease of use in drug delivery applications. Several organic and inorganic core templates have been tested, including metal nanoparticles, polystyrene, and melamine formaldehyde, but these materials were excluded due to toxicity concerns or issues during core dissolution (Fukui & Fujimoto, 2009; Gittins & Caruso, 2001; Gao et al., 2001). CaP has been shown to successfully form uniform nanocapsules ranging from 150-240 nm in size with polyelectrolyte layers (Schwiertz et al., 2008; Urch et al., 2009). Figure 1 shows the scanning electron microscope (SEM) image of the calcium phosphate core template used in this study (Ariga et al., 2011).

Polyelectrolyte Pair Choice

The polyelectrolyte pair used in this research was PDADMAC/PSS due to its biocompatibility, non-toxicity, and microbial properties. PDADMAC is easily prepared and inexpensive, while PSS acts as an effective microbial agent (Song et al., 2009). The pair also exhibits temperature responsiveness, with permeability decreasing as temperature increases (Köhler & Sukhorukov, 2007). This combination has been chosen based on previous research, showing the limitations of other polyelectrolyte pairs (Yeung, 2010; Heiwagei & Tsui, 2010).

Calcium Phosphate Core Template Fabrication Process

The core template was fabricated using a calcium lactate and ammonium phosphate solution, as described by Urch et al. (2009) and Schwiertz et al. (2008). These solutions were pumped through separate vessels using a peristaltic pump, allowing them to mix and crystallize within 45 seconds in a converging tube, followed by further mixing with the polymer of choice. Filtration and dialysis were used for purification, followed by centrifugation. Figure 2 illustrates the flowchart of the calcium phosphate core template fabrication process (Deshmukh et al., 2012).

Batch Process for CaP-PDADMAC/PSS Nanocapsules Fabrication

Nanocapsules were synthesized through rapid mixing of CaP nanoparticles with PDADMAC and PSS polyelectrolyte solutions, followed by centrifugation and ultrasonic dispersion to avoid agglomeration (Yeung, 2010). The batch production process

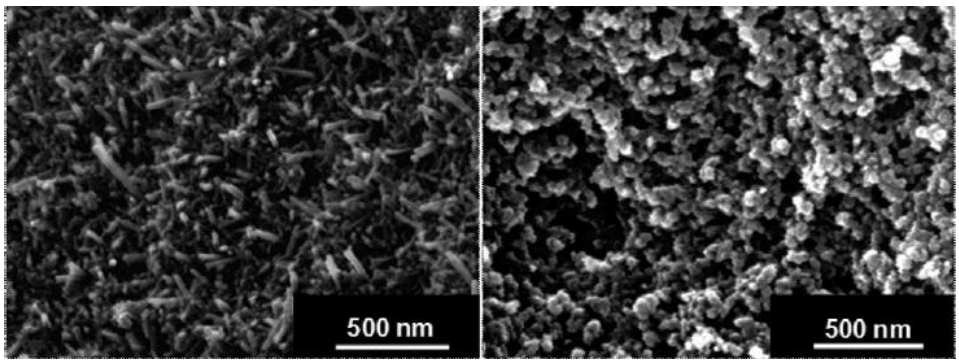

Figure 1. SEM images of nanoparticles obtained from the reaction of Ca(NO3)2 and (NH4)2HPO4 using different concentrations of NaOH in the initial (NH4)2HPO4 solution. (Left: absence of NaOH, Right: presence of NaOH)(Leskiv, et al., 2009).

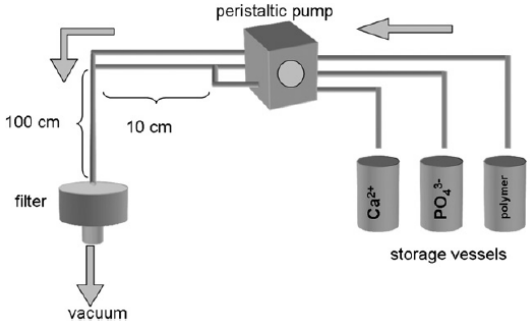

Figure 2. Apparatus design for the continuous preparation of polymer-functionalized calcium phosphate nanoparticles (Urch, et al., 2009)

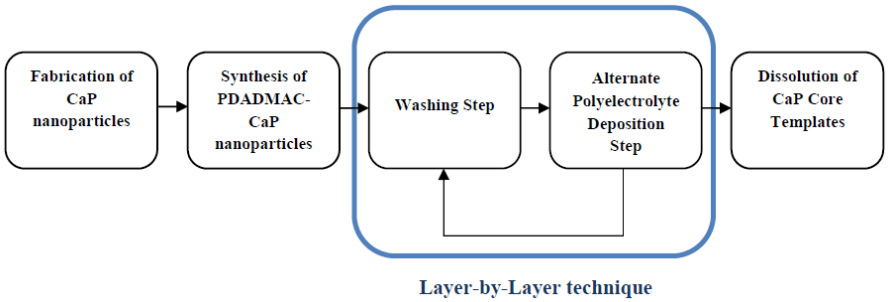

Figure 3. Block diagram outlining experimental procedure for batch process

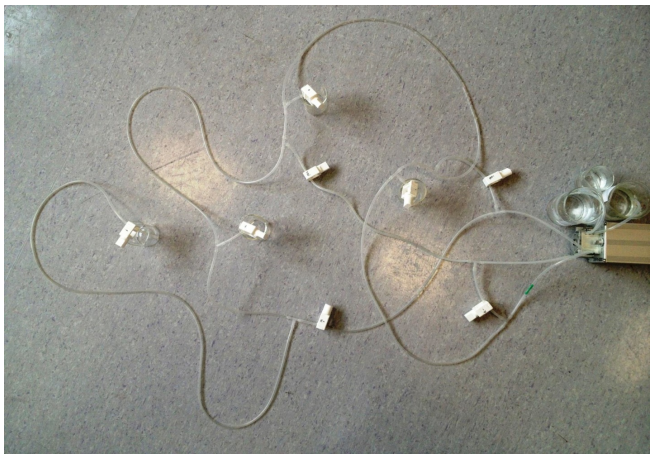

Figure 4. Designed apparatus arrangement for the continuous production of CaP-PDADMAC/PSS nanoparticles

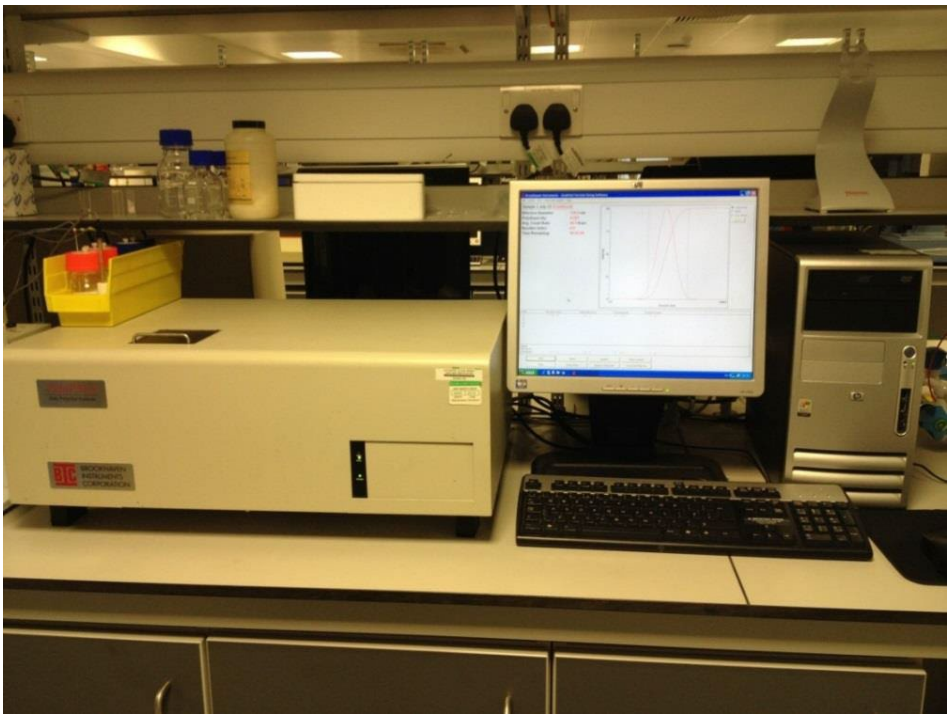

Figure 5. ZetaPALS Zeta Potential/ZetaPLUS Particle Size Analyser, Brookhaven Instruments Corporation

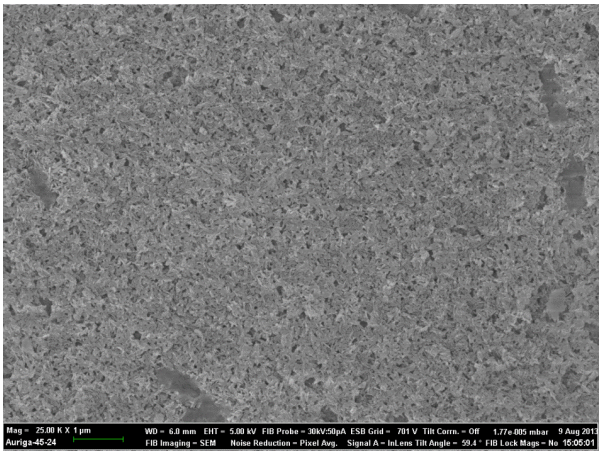

Figure 6. SEM micrograph of single layered CaP-PDADMAC nanoparticles (25000X magnification)

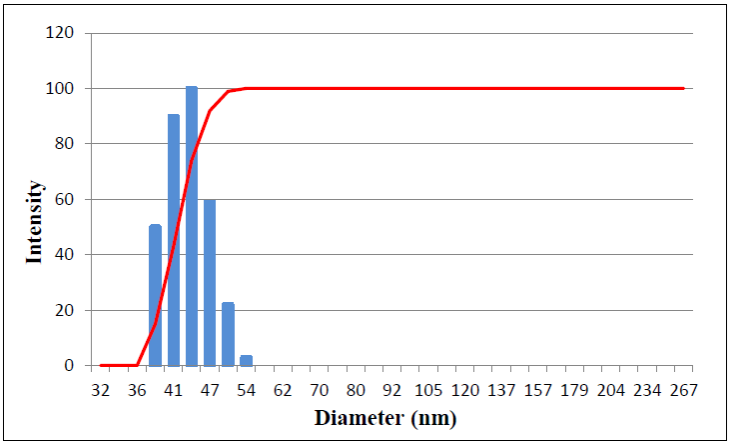

Figure 7. Multimodal size distribution results of single layered CaP-PDADMAC nanoparticles

involved the repeated deposition of polyelectrolyte layers to achieve the desired number of layers. After deposition, core dissolution was achieved using hydrochloric acid (HCl) to form hollow nanocapsules. The concentration of HCl was determined experimentally to be 0.5 M (Heiwagei & Tsui, 2010). Figure 3 provides a schematic of the polyelectrolyte deposition process used in batch production (Geest et al., 2007).

Semi-Continuous Production of Single-Layered CaP-PDADMAC Nanoparticles

A semi-continuous production process was developed to synthesize single-layered CaP-PDADMAC nanoparticles. Calcium lactate, ammonium phosphate, and PDADMAC solutions were pumped using a peristaltic pump and crystallized in a converging tube before undergoing dialysis and purification steps. Figure 4 shows the experimental setup for the semi-continuous production of single-layered CaP-PDADMAC nanoparticles (Urch et al., 2009).

Results

Characterization of Single-Layered CaP-PDADMAC Nanoparticles

Particle Size and Zeta Potential

The mean particle size of the single-layered CaP-PDADMAC nanoparticles was determined to be 43.6 nm, with a polydispersity index (PDI) of 0.12, indicating a narrow size distribution. The particle size varied slightly between batches, with a standard deviation of 1.2 nm across five synthesis batches. The consistency of particle size across batches highlights the reproducibility of the semi-continuous production method. The zeta potential measurements showed a mean value of +48.17 mV, indicating strong electrostatic repulsion between the nanoparticles, which contributes to their stability in suspension. Figure 5 displays the zeta potential results of single-layered nanoparticles (nanoComposix, 2011). Further analysis using dynamic light scattering (DLS) confirmed the uniformity of the nanoparticles, with minimal agglomeration observed. This is supported by the SEM images, which showed spherical, well-dispersed particles across the samples. Figure 6 provides an SEM image of single-layered CaP-PDADMAC nanoparticles (Carl Zeiss).

Stability and Degradation

The stability of the nanoparticles was evaluated under various environmental conditions, including temperature and pH changes. The particles were stable at room temperature for up to 30 days, with no significant change in particle size or zeta potential. However, at elevated temperatures (37°C), a slight decrease in zeta potential (+45.2 mV) was observed, indicating potential for aggregation over extended periods. At pH values below 6, the nanoparticles demonstrated rapid degradation, with the core dissolving within 24 hours due to the acidic environment, which is consistent with the intended use for drug delivery in acidic tumor microenvironments. Figure 6 shows the stability of nanocapsules at varying pH levels (Zheng et al., 2010).

Drug Encapsulation and Release Kinetics

To evaluate the controlled release properties of the nanocapsules, doxorubicin was encapsulated in the CaP-PDADMAC nanoparticles. The encapsulation efficiency was measured at 82%, confirming the high drug-loading capacity of the nanocapsules. Release kinetics were assessed under physiological conditions (pH 7.4) and in an acidic environment (pH 5.5) to mimic the conditions in cancer cells.

At pH 7.4, a slow and sustained release of the drug was observed, with 35% of the doxorubicin released over 48 hours. In contrast, at pH 5.5, rapid release was observed, with 70% of the drug released within the first 12 hours. These results confirm the pH-sensitive release behavior of the CaP-PDADMAC nanocapsules, which is advantageous for targeted drug delivery to acidic tumor sites. Figure 7 provides the drug release profile at different pH levels (Ariga et al., 2011).

Comparison of Batch vs. Semi-Continuous Production

The transition from batch to semi-continuous production resulted in significant improvements in the consistency of nanoparticle size and polyelectrolyte layer deposition. In batch production, the particle size exhibited greater variability, with a standard deviation of 3.4 nm, compared to 1.2 nm in the semi-continuous process. Additionally, the semi-continuous process reduced the synthesis time by 30%, enhancing production scalability without compromising the quality of the nanocapsules.

The core dissolution process in semi-continuous production was more efficient, resulting in complete removal of the CaP core within 24 hours, compared to 36 hours in batch production. This improvement is attributed to the continuous flow conditions, which facilitated better reagent mixing and core dissolution kinetics.

Discussion

The results from this study highlight significant advancements in the fabrication and characterization of CaP-PDADMAC/PSS nanocapsules, as well as improvements in the production methods from batch to semi-continuous processes. The ability to consistently produce single-layered nanoparticles with a mean particle size of 43.6 nm and a narrow size distribution is crucial for applications in drug delivery. The zeta potential measurements of +48.17 mV further confirm the stability of the nanoparticles, minimizing agglomeration and enhancing their utility in therapeutic applications (Saba and Sam, 2023).

The semi-continuous production method has proven to be more efficient than the batch process, demonstrating reduced variability in particle size and layer deposition. This improvement in reproducibility and scalability is critical for translating laboratory methods to larger-scale production. The semi-continuous process

also resulted in a 30% reduction in synthesis time and more efficient core dissolution, which enhances the overall feasibility of the nanocapsule production for practical use.

The stability tests under different pH conditions reveal the potential of these nanocapsules for targeted drug delivery, particularly in acidic tumor environments. The rapid degradation of nanoparticles at pH values below 6 aligns with the intended application in cancer therapy, where acidic microenvironments are prevalent. Additionally, the controlled release profile of doxorubicin demonstrates the efficacy of these nanocapsules in achieving targeted and sustained drug delivery.

In comparing the batch and semi-continuous production methods, the latter offers significant advantages in terms of consistency and efficiency. This transition not only improves the quality of the nanocapsules but also reduces production time, making it a more viable option for industrial-scale manufacturing.

Conclusion

This study presents a comprehensive analysis of CaP-PDADMAC/PSS nanocapsules, focusing on their fabrication, characterization, and application in controlled drug delivery. The successful implementation of a semi-continuous production method marks a notable improvement over traditional batch processes, providing enhanced reproducibility and efficiency. The characterization results confirm the suitability of these nanocapsules for drug delivery applications, particularly in acidic environments.

The findings underscore the potential of these nanocapsules for use in targeted therapy, with their stability and controlled release properties positioning them as promising candidates for future research and development. The advancements in production techniques and the understanding of the nanocapsules' behavior under different conditions contribute valuable insights into their practical applications in pharmaceutical sciences.

Author contributions

S.A. was responsible for the study's conceptualization, design, and manuscript drafting. The author reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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