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MDPI

Proceeding Paper

Confinement–Segregation Theory to Explain the Formation Mechanism of Peptide-Containing Particles in Metered Dose Inhalers †

Peili Luo [1,‡], Wenhao Wang [2,‡], Chuanbin Wu [1], Xin Pan [2,*] and Zhengwei Huang [1,*]

[1] College of Pharmacy, Jinan University, Guangzhou 510632, China; stu202235831056lpl@stu2022.jnu.edu.cn (P.L.); chuanbin_wu@126.com (C.W.)
[2] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510275, China; wangwh37@mail2.sysu.edu.cn
* Correspondence: mercurypan@foxmail.com or panxin2@mail.sysu.edu.cn (X.P.); huangzhengw@jnu.edu.cn (Z.H.)
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‡ These authors contributed equally to this work.

Abstract: Understanding how to formulate peptides into metered dose inhalers (MDIs) is a bottleneck issue hampering the clinical translation of relevant products. In our previous studies, a bottom–up method to prepare peptide-containing particles for MDIs was reported. Nevertheless, the formation mechanism of the particles remains unclear. In this work, considering the production workflow, a confinement–segregation theory was put forward as a hypothesis to explain the formation mechanism. Confinement and segregation were two major processes during formation, and their definitions are provided in detail. Based on the theory, some factors influencing particle formation were also discussed, which promoted future formulation design. It is believed that the proposed theory will provide new insights into the study of peptide-containing MDIs and boost their clinical translation.

Keywords: metered dose inhalers; peptide; confinement–segregation theory; formation mechanism

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1. Introduction

Metered dose inhalers (MDIs) are promising delivery systems for peptides due to their rapid adsorption rate, high patient compliance, and low cost [1]. However, the clinical translation of peptide-containing MDIs has not yet been witnessed. This is because formulating peptides with propellant (HFA 134a or HFA 227) [2], a hydrophobic material that is immiscible with most peptides [3], is a complicated task. To facilitate the clinical translation, our group established a bottom–up approach to preparing peptide-containing MDIs [4]. Briefly, peptides and lecithin were dissolved in the aqueous and organic phases, respectively. The aqueous phase was added to the organic phase under the vortex. The system was snap-frozen with liquid nitrogen and then lyophilized. The lyophilizate was washed with an organic solvent to remove excess lecithin and centrifuged to harvest the particles. Ultimately, the particles were suspended in the filled propellant to obtain MDIs. Furthermore, thymopentin was employed as a model peptide for MDI production, and the product possessed high uniformity, lung deposition ratio, and storage stability, which meant it was qualified for general clinical use [5].

2. Hypothesis

The feasibility of the established preparation method has been demonstrated by previous studies. Nevertheless, the formation mechanism of peptide-containing particles is not explicated. Without a clear understanding of this mechanism, we cannot control the potential factors to formulate a wider scope of peptides [6].

Herein, a confinement–segregation theory is put forward to explain the formation mechanism of peptide-containing particles, after referring to the related literature [7–10]. Literally, confinement and segregation are the two critical processes, which are extracted from the above preparation workflow and defined as follows.

I. Confinement process. (1) This involves the construction of a reverse-phase crude emulsion. Peptide aqueous solution and lecithin organic solution act as the inner phase and outer phase, respectively. Lecithin with surface activity constructs an emulsification layer between the two phases [11]. (2) There is an equilibrium of forces to offer a confinement effect. During snap freezing, water in the inner phase converts into ice crystals with expanded volume [12]. Thus, an expanding force vectoring to the outer phase is generated. Correspondingly, lecithin in the emulsification layer imposes a surface tension vectoring to the inner phase. The equilibrium of both counterparts results in a limited size for freezing nucleation. (3) Formation of the transient zone occurs. This is induced mutually by the expanding force and surface tension, where neighboring peptide and lecithin molecules near the emulsification layer collide with each other and yield non-covalent interactions [13]. Subsequently, a transient non-covalent polymer zone is generated.

II. Segregation process. (1) This involves the yielding of an in situ void. During the freeze-drying process, ice crystals in the initial inner phase sublime under extremely low pressure [14], and the volume previously occupied by them turns into a void. In this context, the initial expanding force fades, and the induction force for the transient non-covalent polymer zone becomes insufficient. (2) Next is the segregation of peptides into two microphases. With a substantially reduced induction force, a predominant proportion of non-covalent polymer dissociates, and the free peptides return to the bulk peptide region. At a low temperature, the molecular chains of peptides in this region are folded, and a chain-folded crystal core is formed [15]. Meanwhile, a minor proportion of non-covalent polymers maintain the structure through peptide–lecithin interaction. These species further consolidate into a thin peptide–lecithin 'self-preservation' surface [16]. Noticeably, peptides segregate into two microphases, i.e., a chain-folded crystal core and a 'self-preservation' surface. (3) Next is the removal of excess lecithin. Since merely a minor proportion of non-covalent polymer is preserved, most lecithin molecules are unbonded. Washing the system with organic solvent dissolves the free lecithin [17].

The above theory is schematically illustrated in Figure 1. After undergoing confinement and segregation processes, peptide-containing particles are obtained. A restricted freezing nucleation scope and a solidified particle are successively acquired by these two processes. It is worth mentioning that, with the help of the peptide–lecithin 'self-preservation' surface, particles can be stably suspended in the hydrophobic propellant, as the hydrophobic moieties of lecithin increase the miscibility with propellant [18].

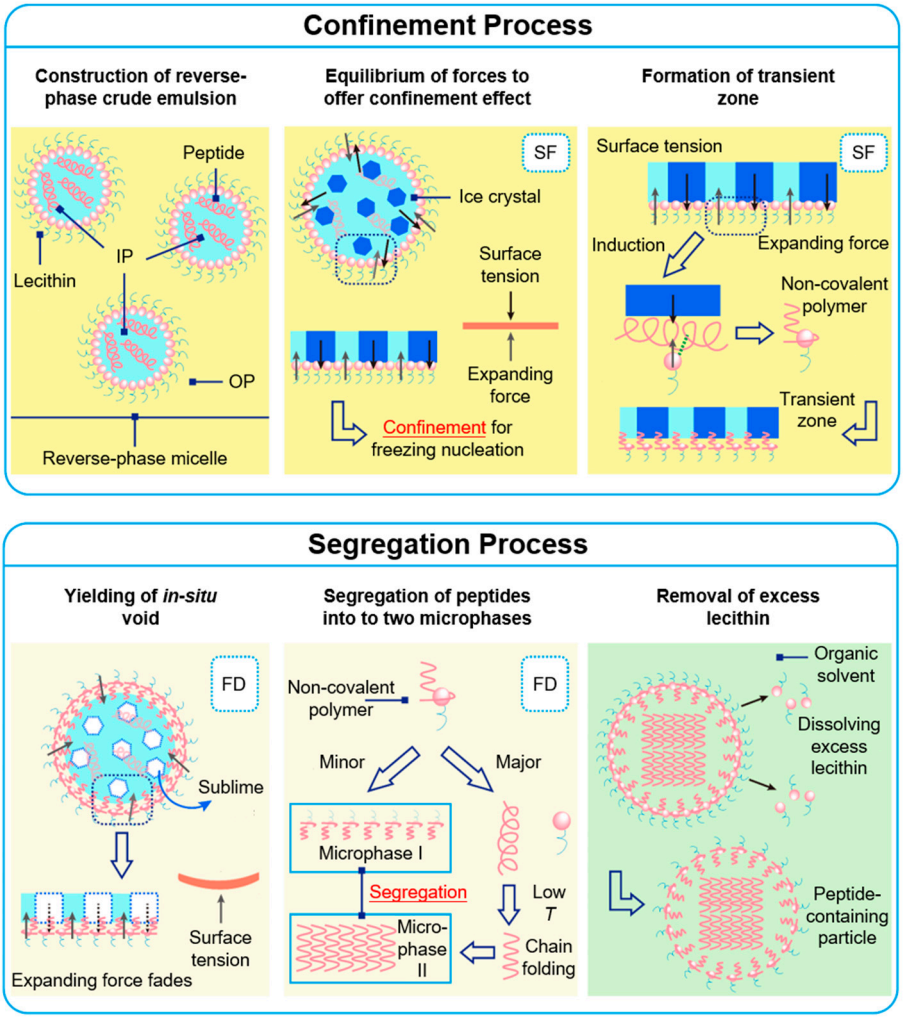

Figure 1. Scheme for confinement–segregation theory. Definition of abbreviations: IP, inner phase; OP, outer phase; SF, snap freezing; FD, freeze drying; and T, temperature.

3. Consequence of Hypothesis

With this proposed theory, we can figure out the factors influencing the formation process of peptide-containing particles and take advantage of them for future formulation design and development [19]. Based on the above rationale, we preliminarily provide one reasonable example here, per one process.

3.1. Snap Freezing Rate in Confinement Process

The equilibrium of expanding force and surface tension determines the size for freezing nucleation that, in turn, dominates the size of the obtained particles, and the expanding force is further determined by the snap freezing rate. As can be expected, a faster rate will result in a faster expansion of ice crystals, and finally a greater expanding force. By adjusting the snap freezing rate, one can probably control the size of the particles.

3.2. Peptide-Lecithin Interaction Force in the Segregation Process

Such a force determines the proportion of non-covalent polymer that is maintained, and then the thickness of the 'self-preservation' surface. Since the 'self-preservation' surface is crucial for stabilizing peptide-containing particles in the propellant, one should match the peptide–lecithin pair with a suitable interaction force.

Hence, under research and development, the snap freezing rate and peptide–lecithin interaction force are worthy of consideration. Formulation screening and optimization should be conducted to find the optimal parameters, according to our theory.

4. Conclusions

After a survey of the relevant documents, a confinement–segregation theory was put forward in this paper to explain the formation mechanism of peptide-containing particles, which were intended for MDI preparation. We will perform experimental validation based on this theory in ongoing studies, using a variety of model peptides. In addition, the significance of tuning the snap freezing rate and peptide–lecithin interaction force will be examined.

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