

## Mini-Review

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# Insight into heating method and Mozafari method as green processing techniques for the synthesis of micro- and nano-drug carriers

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**Abstract:** Drug delivery systems, also known as bioactive carriers, are currently an important contribution to the pharmaceutical and biomedical industries. A leading category of these drug carriers is lipid- and phospholipid-based systems including liposomes, nanoliposomes, solid lipid nanoparticles, nanostructured lipid vesicles, archaeosomes, and tocosomes. At present, there are several methods available for the preparation of the lipidic drug carriers at the micro- and nanoscales. There are some misunderstandings and confusion in the literature regarding two of the scalable and environment-friendly (green) techniques developed in our laboratory, namely the heating method and the Mozafari method. These methods are superior to conventional procedures used in the synthesis of drug carriers due to the fact that they do not involve utilization of potentially toxic solvents, detergents, or high-shear homogenizations. This entry is aimed to clarify differences between these methods to the peers and colleagues in academia as well as relevant industries. Some details of the industrially applied patented instrument used in the manufacturing of lipidic carriers are also provided.

**Keywords:** drug delivery, lipidic carriers, manufacturing techniques, encapsulation, Mozafari method

## 1 Introduction

Contemporary pharmaceutical dosage forms benefit from encapsulation techniques particularly with respect to

improving pharmacokinetics and biodistribution of therapeutic and diagnostic agents [1]. In addition, solubility concerns of lipophilic compounds can be addressed by employing lipid-based carrier systems. Furthermore, micro- and nanoencapsulation systems are able to target their load *in vitro* (e.g. targeting contaminating bacteria in food systems [2]) and *in vivo* (e.g. tumour targeting [3–5]). Research and development of polymeric and chitosan-based drug delivery systems is carried out at laboratories worldwide [6–8]. However, the majority of the approved encapsulation systems for human use thus far are based on lipidic drug carrier systems [9,10]. This is due to the versatility and biocompatibility of lipid-based vehicles as well as their ability to encapsulate and/or entrap hydrophilic, hydrophobic, and amphiphilic compounds separately or simultaneously (providing a synergistic effect) [11,12]. Lipidic carriers are not only used in therapeutic and biomedical products but also utilized in the formulation of innovative nutraceutical and cosmeceutical products [13–15].

Physicochemical properties of lipidic carriers are mostly dependent on their composition, size, surface charge (zeta potential – ZP), and the method of preparation [16]. The fluidity/rigidity and the ZP of the lipid bilayers are determined by the choice of the bilayer ingredients. Unsaturated phosphatidylcholine (PC) molecules from natural sources (calf liver, soybean, or egg) result in highly permeable and less stable bilayers, while more rigid and impermeable bilayer structures are obtained when saturated phospholipids (PLs) with long acyl chains (such as DPPC) are employed [13–16]. Lipidic drug carriers possess a great number of beneficial qualities and attributes. Consequently, they can be used for a variety of applications and can serve for the site-specific delivery of medicaments or other macromolecules into human and animal bodies [15,16]. The lipidic carriers can be manufactured in microscale or nanoscale diameters, and as such can offer the advantages of microencapsulation as well as nanoencapsulation technology. These vesicles can be prepared using a wide range of methods and protocols as explained in the following section. In this entry, we try

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to clarify the differences between the methods developed in our laboratory in order to avoid any confusion and future technical misinterpretations.

## 2 Manufacturing techniques

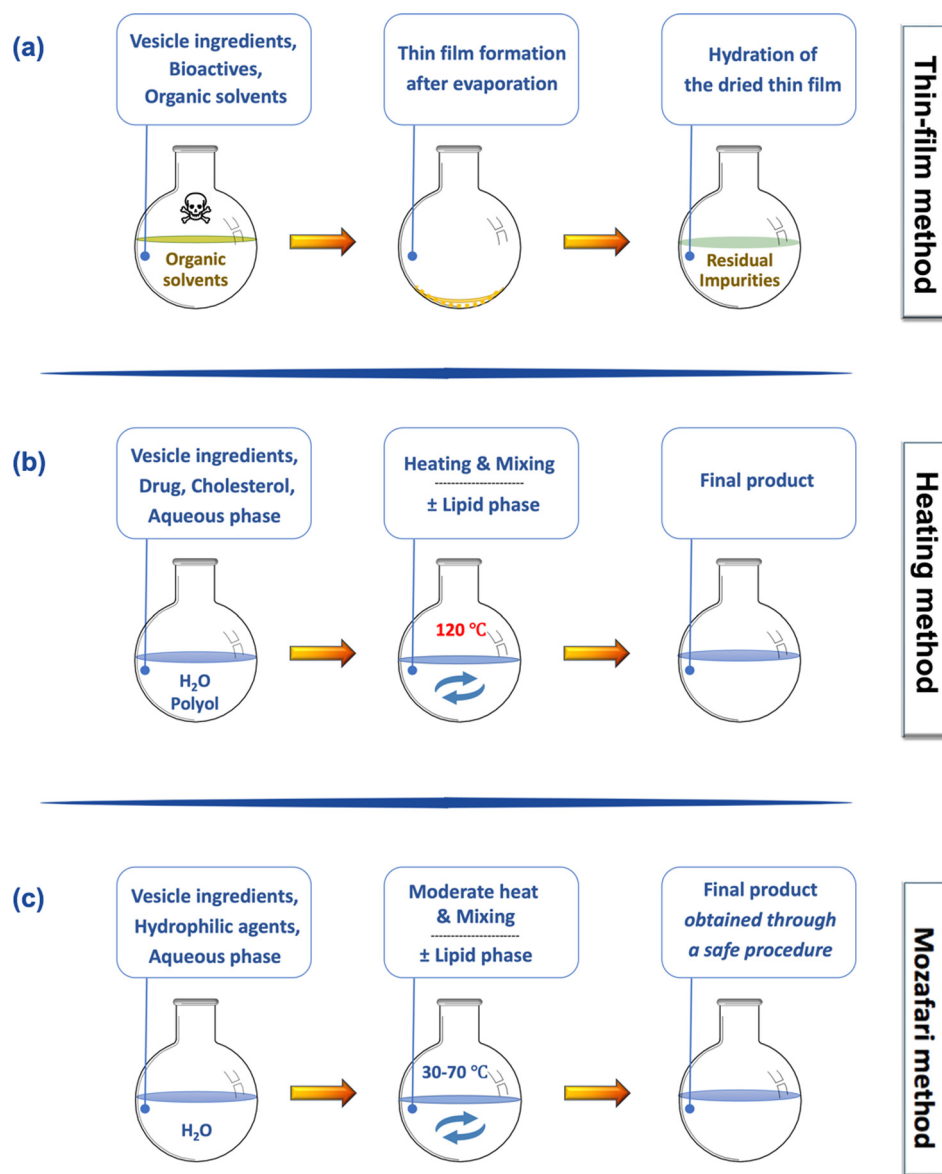
Currently, there are several techniques available for the manufacture of lipidic carriers, including liposomes [17], nanoliposomes [18], solid lipid nanoparticles [19] tocosomes [20], and archaeosomes [21]. A number of these techniques require utilization of potentially toxic solvents, detergents, or harsh treatments such as sonication, microfluidization, or high-pressure homogenization. Issues pertaining to the scale-up of the methodology and cost-effectiveness of the resultant product need also to be considered. Towards this end, safe, robust and scalable methods were developed by our team in order to evade problems associated with the preparation of lipidic carriers. The oldest of these green technologies is the “heating method,” presented to the pharmaceutical community at a conference in Scotland in 2001 [22] and the first article using this method was published in 2002 [23]. As the name implies, this method suffers from the limitation of using high temperatures (i.e. 120°C) in order to solubilize the ingredients of lipidic vesicles (lipids, phospholipids, and particularly cholesterol) in the absence of organic solvents such as chloroform, methanol, or ethanol. In 2007, another method was developed in our lab which did not require organic solvents, detergents, harsh procedures, or high temperatures [2]. Lipid vesicles were manufactured by this mild and robust technique (called “Mozafari method”) at a maximum temperature of 70°C and were used for the encapsulation of sensitive molecules such as anticancer drugs [20], genetic material [24] and omega-fatty acids successfully [25,26]. However, it is noticed that there are some ambiguities in the literature regarding these two methods. For instance, Abbas and colleagues [27] in their article related to the encapsulation of ascorbic acid (vitamin C), referring to Mozafari method, have mentioned that: “This method involves hydration of wall material followed by heating and stirring of material, including active compound, in the presence of glycols.” However, this statement is partially true for the Heating method (not Mozafari method) as explained below. The same error was repeated by other groups including Poudel and co-workers [28]. Therefore, it is necessary to shed light on the practical aspects of these two methods in order to avoid a mistake to be repeated again by scientists in industry and academia.

## 3 Details of heating method and Mozafari method

As explained earlier, these two methods can be used for the manufacture of various drug carriers. Here, for the sake of brevity, details of these techniques are explained by way of example for the preparation of archaeosomes, liposomes, and nanoliposomes. The main ingredients of the lipid vesicles (i.e. lipid/phospholipid molecules) arrange themselves in the form of bilayer structures via van der Waals forces and hydrophobic/hydrophilic interactions when placed in an aqueous medium. In this manner, the hydrophilic head groups of the phospholipid molecules face the water phase while the hydrophobic region of each of the monolayers faces each other in the middle of the bilayer membrane. It should be noted that, contrary to what is stated in some literature, the formation of liposomes and nanoliposomes is not a spontaneous process [29]. Therefore, an adequate quantity of energy must be supplied to the system for the curvature of the bilayer sheets in the form of stable spherical vesicles. Although the vesicular arrangement is at the minimum thermodynamic energy level [30], for vesicle formation to occur, the system has first to be provided with a minimum quantity of energy called “the activation energy.” This required energy input could be either physical, mechanical, thermal, acoustic (e.g. ultrasonication), or a combination thereof [29,30]. The preparation techniques of lipid-based carriers are generally classified as low-energy and high-energy methods. Low-energy consuming procedures include solvent injection, solvent diffusion, and Mozafari method. High-energy consuming techniques include microfluidization, high-pressure homogenization, and sonication [30].

In both the heating method and Mozafari method, energy input is in the form of mechanical energy (mixing) and thermal energy, as depicted in Figure 1. The following criteria need also to be taken into consideration in the preparation of archaeosomes, liposomes, nanoliposomes and other drug carriers:

- i) Physicochemical characteristics of the drug or other bioactive compounds to be encapsulated.
- ii) Acceptable range of drug encapsulation efficiency.
- iii) The route of drug administration.
- iv) The stability of the formulation (certain methods may be less harmful to the encapsulated material).
- v) Physicochemical properties of the medium or solvents in which the vesicles and other excipients of the formulation are dispersed.
- vi) Desired shelf-life, size, polydispersity index, zeta potential, and release profile of the carriers.



**Figure 1:** Comparison between three different methods employed for the manufacture of lipidic drug delivery systems. (a) Thin-film hydration method, which requires utilization of potentially toxic solvents such as chloroform and methanol. (b) Heating method, which does not require employment of toxic solvents, but in some cases necessitates the use of high temperatures (e.g. to dissolve cholesterol). (c) Mozafari method, which is a single-pot technique and does not use toxic solvents, detergents, extreme pH values, elevated temperatures, or harsh treatments such as sonication or homogenization.

- vii) Potential toxicity and influential concentration of the encapsulated bioactive ingredients in the formulation.
- viii) Number of steps and vessels involved in the manufacturing process.
- ix) Scalability of the methodology, in order to ensure both consistent quality (with respect to batch-to-batch variations) and efficient manufacture yield [29–31].

Ideal preparation method should cover all of the above-mentioned criteria. Among these, particle size is a critical

parameter governing the bioavailability and targetability of carrier systems for drug delivery. Different preparation techniques can be employed to control the size of vesicles based on the intended application. Moreover, the encapsulation and loading of drugs with different solubilities can be tuned by selecting the appropriate preparation method [13–15,30,31]. A comparison of the steps involved in each of the heating method and the Mozafari method is provided in Table 1.

As mentioned in Table 1, Mozafari method does not involve the initial step of the heating method (i.e.

**Table 1:** Comparison between Mozafari method and heating method [2,22,23,29]

	Mozafari method	Heating method
Process Steps	<ol style="list-style-type: none"><li>1. Adding carrier ingredients and the active agents to a preheated (40–70°C) mixture of the bioactive agent and a polyol</li><li>2. Stirring the mixture at 40–70°C at 1,000 rpm under an inert atmosphere (such as nitrogen) until all materials are dissolved/dispersed</li><li>3. Keeping the product at temperatures above the phase transition temperature of the phospholipids (Tc) under an inert atmosphere for 1 h to allow the vesicles to anneal and stabilize</li></ol>	<ol style="list-style-type: none"><li>1. Hydrating the ingredients in an aqueous medium for 1–2 h under nitrogen at RT</li><li>2. Mixing the dispersion with the material to be encapsulated after adding glycerol at the temperature of 120°C. In the absence of cholesterol, a lower temperature could be used</li><li>3. Mixing the sample (800–1,000 rpm) at a temperature above the Tc* of the lipids until all the lipids and other excipients are dissolved/dispersed</li><li>4. Leaving the product above Tc* under nitrogen for 1 h to allow the sample to anneal and stabilize</li></ol>
Advantages	<ul style="list-style-type: none"><li>– No degradation of the lipid ingredients and encapsulated bioactives occur</li><li>– Green, robust, and versatile technique</li><li>– Single-pot method</li><li>– Feasibility of a reproducible and scale-up manufacture with ease</li></ul>	<ul style="list-style-type: none"><li>– No toxic solvents or detergents required</li><li>– No need for sterilization.</li><li>– Possibility of scale-up</li></ul>
Disadvantages	<ul style="list-style-type: none"><li>– N/A</li></ul>	<ul style="list-style-type: none"><li>– Requirement for high temperatures in some instances</li><li>– Multistep technique</li></ul>

\*Tc: Phase transition temperature.

hydration of the ingredients and excipients of the formulation for 1–2 h). In addition, the required temperature of the Mozafari method does not exceed 70°C, and as such, there is no risk of damage to the structure and function of the encapsulated material [29,31,32]. This method should be preferably performed in a specially designed and patented reaction vessel particularly for the manufacture of encapsulation systems on the industrial scales (Figure 2) [33,34]. Specifications and attributes of the Mozafari method vessel are given in the next section.

## 4 Specifications of the Mozafari vessel

In order to facilitate fast and reproducible manufacture of drug delivery carriers using green technology on the industrial scale, a simple but efficient apparatus was designed and patented [32–34]. The rationale behind this invention was to present the machine for the large-scale preparation of micro- and nanoencapsulated products without the need to use toxic solvents/detergents or harsh procedures including homogenization or microfluidization. The invention also relates to a new method for the preparation of

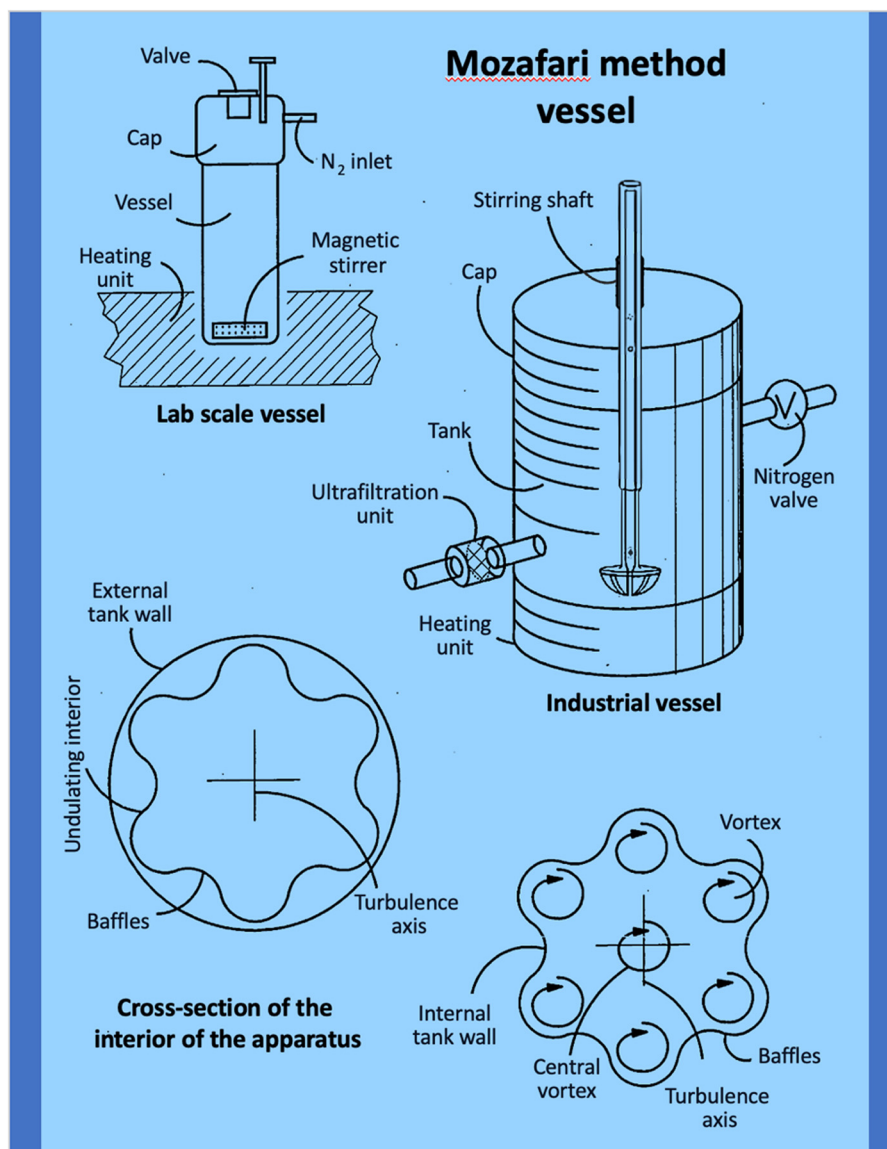
micro- and nano-sized carrier systems for the encapsulation and/or entrapment of bioactive compounds. The novel method is comprised of the following steps which are provided by the Mozafari vessel:

- (a) providing a complexation zone supplied with an aqueous medium containing the carrier material;
- (b) simultaneously stirring and heating the aqueous medium under an inert atmosphere;
- (c) adding the bioactive compound(s) to the aqueous medium while maintaining the complexation zone under the conditions of temperature effective to facilitate complexation of the bioactive material by the carrier material; and
- (d) recovering from the complexation zone a carrier complex of the bioactive compound(s) (Figure 2).

Further details of the apparatus are described in patents [33] and [34].

The enhanced efficacy of the apparatus – while maintaining the requirements of being completely a safe and ‘green technology’ – can be more perceived by comparing the stirring/mixing efficiency of a normal tank used in the pharmaceutical and other industries versus the Mozafari vessel as illustrated in Figure 3.

A small-scale prototype of the Mozafari vessel, with a capacity of 1Lt, is depicted in Figure 4.



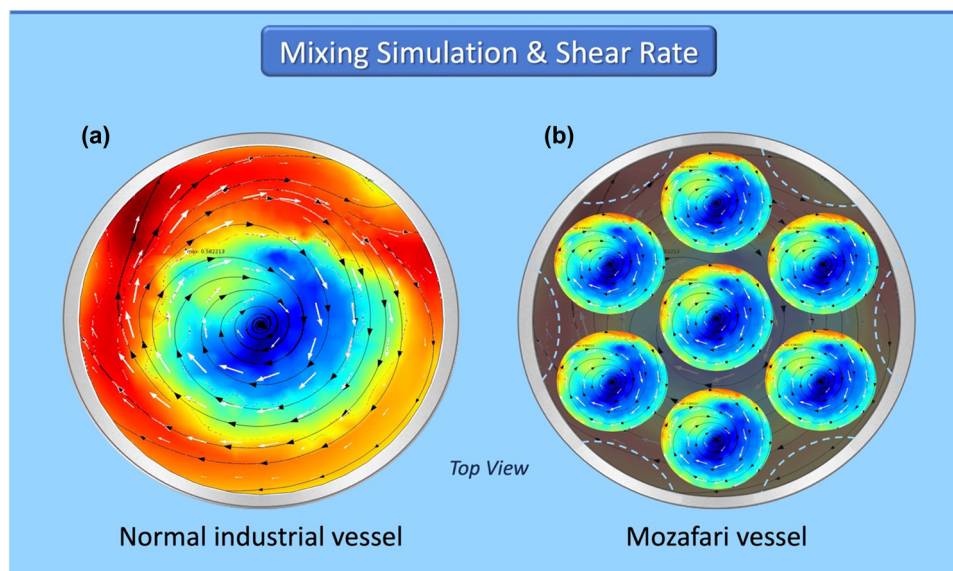
**Figure 2:** The specially designed vessel is used in the manufacture of micro- and nanoencapsulation systems by the Mozafari method. A cross-section of the apparatus, showing the baffled wall of the vessel, and multiple turbulences created by stirring during the manufacture process are demonstrated in the lower section of the figure. The improved efficiency of the methodology is believed to be due to these multiple turbulences which enable a single vessel to function as efficiently as seven vessels (the total number of turbulences) simultaneously.

## 5 Biomedical applications of lipid-based carriers

Lipid-based carrier systems can encapsulate both water-soluble and lipid-soluble compounds separately or simultaneously (e.g. when the synergistic effect is required) in addition to the amphiphilic molecules [25,26,35]. These vesicles are biocompatible and biodegradable and are able to provide sustained and controlled release. Their unique characteristics can positively affect drug pharmacokinetics and biodistribution. They are also good

candidates for enzyme replacement therapy and are used in antifungal, antiviral, and cancer therapy. They can be employed as carriers for small molecules (e.g. vitamins, minerals, or chemotherapeutic drugs) and for the encapsulation of large molecules such as cytokines and genetic material [24]. Lipidic systems are also used in radiopharmaceuticals, immunological products, cosmetics, cosmeceuticals, and dermatological formulations. Moreover, they can be employed for enzyme encapsulation and immobilization. They have unique emulsifying properties, can be used to stabilize emulsions, and are good wetting agents. Therefore,





**Figure 3:** Mixing simulation and shear rate illustration in (a) normal tank used in pharmaceutical and similar industries; (b) Mozafari vessel. The special design of the Mozafari vessel and the presence of six baffles in the tank are the reasons for the enhanced efficacy of this design in the green manufacture of homogenous and reproducible drug carriers with narrow size distributions.



**Figure 4:** A laboratory-scale prototype of a Mozafari vessel, made of pharmaceutical-grade stainless steel, with a volume of 1Lt.

they can coat the surface of crystals to make them hydrophilic [14,15,35].

Lipid vesicles are also used in the field of genetic engineering as gene and oligonucleotide carriers [24], in biology as models of cell membranes, and in the formulation of viral vaccines [15,36]. When the bioactive materials are encapsulated in the lipid carriers, they are protected against enzymes and other degrading agents in the body. The patient is also protected against the side effects of the encapsulated drugs. In the case of controlled or sustained release, drug release depends on the carrier ingredients, bilayer permeability, and the nature of the encapsulated or entrapped drug. The release of the drug also occurs as a result of lipid phase change in response to external stimuli such as variations in the pH or temperature. Lipidic carriers have also been used successfully for targeting their load to specific cells *in vitro* and *in vivo* [14,15]. As a result of their unique properties, including biocompatibility, versatility, and targetability, currently, there are several commercial and FDA-approved liposomal formulations in the clinical use for the treatment of different types of disease [37].

## 6 Synopsis

This entry aimed at providing clarifications about methods developed in our laboratory, which in some cases have

been subject to misinterpretations. As indicated in Table 1, the Mozafari method is a robust and simple technique that does not involve using organic solvents, detergents, high-shear-force procedures, and extreme pH values. The method can be used for the manufacture of different carrier systems including, but not limited to, phospholipid vesicles, tocosomes, niosomes, solid-lipid nanoparticles, and vesicular gels. In this method, heating and stirring of the aqueous lipid dispersion take place simultaneously. Temperature and mechanical agitation provide adequate energy for the formation of stable drug carriers. The particle size can be controlled by the phospholipid selection as well as the duration of the overall process. Bioactive agents (e.g. vaccine candidates, diagnostic agents, drugs, nutraceuticals, and genetic material) can be added at several stages, which provides versatility to the method, to allow the encapsulation of a vast variety of molecules and compounds. Accordingly, the drug can be added: (i) initially, along with the carrier ingredients and the aqueous medium; (ii) after the heating and agitation have been initiated; or (iii) after the termination of the heating and stirring step, i.e. after the carrier system has been formed. The last protocol is suitable for temperature-sensitive material. The method is fast, efficient, and completely suitable for the large-scale production of encapsulated compounds for pharmaceutical, cosmeceutical, nutraceutical, and biomedical industries. Future perspectives of the Mozafari method and the heating method are envisaged to include industrial-scale manufacture of FDA-approved targetable anticancer formulations, vaccines, and other medicinal and food supplement products. To achieve these goals, rationale and extensive clinical studies of the formulations prepared using the mentioned methods need to be performed in order to attest safety, efficacy, and reproducibility criteria.

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